51st Annual Meeting of the Brazilian Society for Biochemistry and Molecular Biology (SBBq) 46th Congress of Brazilian Biophysical Society (SBBf)/ Lafebs

Águas de Lindóia, SP, Brazil
September 5th to 8th, 2022

.

Ilustração da Capa: Alexandre Takashi

Welcome Letter

Welcome!

The 51st Annual Meeting of the Brazilian Society of Biochemistry and Molecular Biology (SBBq) will be co-organized with the 46th Congress of the Brazilian Society of Biophysics (SBBf)/Latin American Federation of Biophysical Societies (Lafebs), from September 5 to 8, 2022 in the Convention Center of the Majestic Hotel in Águas de Lindóia, SP.

The joint organizing committee is finalizing an exciting interdisciplinary program comprising 8 plenary lectures and 24 symposia with national and international world class scientists that will showcase the most recent advances and current challenges in a broad range of research topics in Biochemistry, Molecular Biology and Biophysics.

The annual meetings organized by SBBq and SBBf stand among the most traditional events in the Brazilian scientific community and for more than four decades have been a forum for scientific discussions that contribute to the advancement of fundamental knowledge regarding biochemical and biophysical phenomena and its applications, as well as for debates regarding science policy and science education that generate consensuses that over time have influenced public policies to the benefit of society.

After two years without being able to host in person meetings due to the Covid-19 pandemic, we look forward to see you in Águas de Lindóia in September!

Leda Q. Vieira
President of SBBq

Rosangela Itri
President of SBBf

SBBq Executive Committee

Board of Directors

Acting President:	Leda Q. Vieira (UFMG)
Past President:	Selma Jeronimo (UFRN)
Vice-President	Juliana Rangel Lopes Fietto (UFV)
General Secretary:	Walter R. Terra (USP-IQ)
First Secretary:	Eduardo M.R. Reis (USP-IQ)
Treasurer:	Aline Maria da Silva (USP-IQ)

Scientific Council

Alicia J. Kowaltowski (IQ-USP)
Ana Paula Valente (UFRJ)
Anibal E. Vercesi (Unicamp)
Hugo A. Armelin (Instituto Butantan)
Maria Ligia Macedo (UFMS)
Patrícia M. G. Paiva (UFPE)
Pedro L. de Oliveira (UFRJ)
Russolina Zingali (UFRJ)

Regional Representatives

Bahia	Maria de Fátima Dias Costa (UFBa)
Ceará	Marcio Viana Ramos (UFC)
Distrito Federal	Marcelo Valle de Souza (UnB)
Minas Gerais	
Paraná	Marcello Iacomini (UFPR)
Pernambuco	Vera Lúcia Menezes de Lima (UFPE)
Rio de Janeiro	Hatisaburo Masuda (UFRJ)
Rio Grande do Norte	Hugo Alexandre de Oliveira Rocha (UFRN)
Rio Grande do Sul	Carlos Alberto Saraiva Gonçalves (UFRGS)

Post Graduation in Biochemistry and Molecular Biology (PMBqBM)

Coordinator	Andrea Mara Macedo (UFMG)
Vice Coordinator	Flávia Meotti (IQ-USP)

Executive Secretariat

Cynthia Sayuri Bando	Marcelo Andrade
Arnaldo Casari	Marcia Braga

SBBF Executive Committee

Board of Directors

Acting President:	Rosangela Itri (IF-USP)
Vice-President	Celso Caruso (UFRJ)
Treasurer:	Leandro Barbosa (IQ-USP)
General Secretary:	Daniele ribeiro de Araujo (UFABC)
Scientific Director:	Adriana Fontes (UFPE)

SBBq and SBBF Scientific Committee

Leda Q. Vieira - President of SBBq
Rosangela Itri - President of SBBf
Antonio José da Costa Filho - FFCLRP-USP
Aline Maria da Silva - IQ-USP
Eduardo Reis - IQ-USP
Emanuel M. de Souza - UFPR
Eneida de Paula - Unicamp
Juliana Fietto - UFV
Leandro Barbosa - IF-USP
Maurício da Silva Baptista - IQ-USP
Pietro Ciancaglini - FFCLRP-USP
Richard C. Garratt - IFSC-USP
Sayuri Miyamoto - IQ-USP
Walter R. Terra - IQ-USP

CAPES

CNPq
Conselho Nacional de Desenvolvimento Científico e Tecnológico

FAPESP

Gold Sponsor

cytiva

Silver Sponsors

greiner BIO-ONE

MERCK

loccus

Bronze Sponsors

CRAL
SUPRINDO A SAÚDE DESDE 1977

ThermoFisher SCIENTIFIC

The support also provides by:

Asociación de Universidades GRUPO MONTEVIDEO

30 AÑOS

The Company of Biologists

Molecular Omics

RSC Chemical Biology

RSC Advances

Chemical Science

Nanoscale

Index

CF.01 - The function of matrix vesicles in physiological and pathological mineralization	6
CF.02. - Inflammasome activation in response to SARS-CoV-2 infection, from the intracellular biochemistry to the severe pulmonary inflammation	6
CF.03. - Bioenergetic Immune Exhaustion in Malaria	7
CF.04. - Geminivirus-Host Interactome: an immune hub cross-talking with antiviral, antibacterial immunity, and growth-promoting events	7
CF.06. - Purinergic signaling and innate immunity regulate hematopoiesis in "hormetic-dependent" manner	8
SP.01 - What is new about COVID-19: perspectives to a post-COVID	**9**
SP.01.01 - Different phenotypes of lung damage in COVID-19	9
SP.01.02 - Prothrombotic Vascular Dysfunction in COVID-19	9
SP.01.03 - Extrapulmonary infection in COVID-19 and its potential contribution to disease pathophysiology	10
SP.01.04 - RD&I Actions to Face the Covid-19 Pandemic	10
SP.02 - Nano/micro encapsulation of excipients with biological activity	**11**
SP.02.01 - Bioinspired materials for functional foods and antimicrobials	11
SP.02.02 - Advances and challenges to elucidate the mechanisms of action of polymeric nanopesticides	11
SP.02.03 - Micro/Nanotechnology Applied to Integrated Pest Management	12
SP.02.04 - Self-assembly investigation of amphiphilic peptides for pesticides biosensors development	12
SP.03 - Functions of Matrix Vesicles	**13**
SP.03.01 - TNAP activation by inflammation in vascular smooth muscle cells and release into extracellular vesicles: possible functions in atherosclerosis plaques	13
SP.03.02 - Vascular smooth muscle cells drive osteoblast-to-osteocyte transition via β-catenin signaling through exosome communication	13
SP.03.03 - Nanomaterials exposure, extracellular vesicles biogenesis and adverse cellular outcomes: a scoping review	14
SP.03.04 - Matrix eVesicles: exploiting the protein corona and its function in biomineralization	14
SP.04 - 25 Young Talent in Life Science Prize	**15**
SP.04.01 - Do astrocytes contribute to brain glucose metabolism?	15
SP.04.02 - Inflammasome activation in the pulmonary parenchyma defines two distinct profiles associated with cytokine storm and worsening of lung function in COVID-19 patients	15
SP.04.03 - PM2.5-Induced Cytotoxicity Exacerbates Foam Cells Formation By Macrophages	16
SP.04.04 - Extracellular Vesicles in Colorectal Cancer Cells: The Unconventional Release of LMWPTP and Impact on Human Fibroblast Biology	16
SP.04.05 - The Rho GTPase pathway as a potential chemosensitizer of TMZ and cisplatin-resistant GBM through a p53-dependent mechanism	17

SP.05 - Systems Biology and Biomarkers for Human Disorders	**18**
SP.05.01 - Molecular responses of astrocytes to brain injuries	18
SP.05.02 - Predisposition and potential indicators of alcohol use disorders	18
SP.05.03 - Metabolic factors associated with cognitive impairment in Alzheimer's disease	19
SP.05.04 - Imaging astrocyte metabolism	19
SP.06 - Carbohydrates: from isolation, to synthesis, dynamics and function	**20**
SP.06.01 - Synthesis of carbohydrates and glycoconjugates with biological applications	20
SP.06.02 - Natural Products from Brazilian Biodiversity, Small or Large Always Remarkable	20
SP.06.03 - Are accurate models for carbohydrates structure possible?	21
SP.06.04 - The function of heparan sulfate in the breast cancer	21
SP.07 - AUGM/Lafebs: COVID: from a Biophysical/Biochemical Perspective	**22**
SP.07.01 - Multiparatopic binders for SARS-Cov2 SPIKE: Best inhibitor ever, ever.	22
SP.07.02 - A Crystallographic Snapshot of SARS-CoV-2 Main Protease Maturation Process	22
SP.07.03 - Current and Upcoming Challenges in Infectious Disease Modeling: Lessons Learned After Two Ongoing Years of Molecular and Mathematical Modeling of SARS-Cov-2 and COVID-19	23
SP.07.04 - The moving frontiers in SARS-CoV-2 structural biology	23
SP.08 - Recent Advances in Molecular Entomology	**24**
SP.08.01 - Different strategies used by trypanosomes to develop in triatomines	24
SP.08.02 - The molecular basis of worker sterility in social bees – a glimpse into the evolution of altruism in social insects	24
SP.08.03 - The neglected virome of assassin bugs	25
SP.08.04 - Bad mosquito, good saliva: Aedes aegypti salivary molecules as a source of immunobiologics	25
SP.09 - Single-Cell Profiling: A New Frontier in Biology	**26**
SP.09.01 - High-Resolution Profile of Macrophage Subpopulations in the Tumor Microenvironment	26
SP.09.02 - Spatial single cell transcriptomics of the brain	26
SP.09.03 - Single cell transcriptomics and chromatin analysis illuminating gut microbiota-host interactions	27
SP.09.04 - Deciphering thermogenic adipose niche: cellular heterogeneity and communication	27
SP.10 - From Molecular Modeling to Drug Design	**28**
SP.10.01 - Normal Modes based new simulation techniques opens far-reaching possibilities in structural biology	28
SP.10.02 - Drug design and repurposing with DockThor VS web server focusing on SARS CoV 2 therapeutic targets and their non synonym variants	28
SP.10.03 - Engineering synthetic proteins for diagnostics and therapy of viral diseases	28
SP.10.04 - Integration of computational tools in the prospection of therapeutic targets and agents	29

SP.11 - AUGM/Lafebs: Novel approaches in Biophysics in South America: what young scientists are working on	**30**
SP.11.01 - Showing the way: RadA helicase mechanism during natural transformation in Streptococcus pneumoniae shown by CryoEM	30
SP.11.02 - New tools for the structural characterization of biomembrane systems	30
SP.11.03 - Structural Basis of Regulatory Protein-RNA Interactions	31
SP.11.04 - Biomechanics: The importance of evaluating nanomechanical properties in biological systems	31
SP.12 - Photobiology and Biophotonics	**32**
SP.12.01 - Multiphoton imaging on cancer biopsies	32
SP.12.02 - Photobiomodulation on genomic stability and telomere maintenance	32
SP.12.03 - The Correlation of Oxidative Stress Damage and Aging of Biomembrane: From Human Red Blood Cell to SH-SY5Y Neuroblastoma Cell	33
SP.12.04 - Deciphering biomembrane photodamage: Alkylation of hydrophilic sensitizers enhances the photo-induced oxidation of phospholipid membranes	33
SP.13 - From liquids to aggregates – the role of liquid-liquid phase separation in physiological and pathological states	**34**
SP.13.01 - Deconstructing virus condensation	34
SP.13.02 - An intrinsically disordered pathological prion variant Y145Stop converts into self-seeding amyloids via liquid-liquid phase separation	34
SP.13.03 - Phase separation of p53 precedes aggregation and is affected by oncogenic mutations and ligands	35
SP.13.04 - Molecular and cellular basis of hyperassembly and protein aggregation driven by a rare pathogenic mutation in DDX3X	35
SP.14 - Mitochondria, Metabolism and Beyond	**36**
SP.14.01 - Universal Mitochondrial Dysfunction During Space Travel	36
SP.14.02 - Macrophage immunometabolic regulation	36
SP.14.03 - High fat consumption and memory impairment: What is the role of metabolic changes in microglia?	37
SP.14.04 - Mitochondria and degenerative diseases: new therapeutic avenues targeting quality control.	37
SP.15 - Pathogenesis of Immune Based Disease	**38**
SP.15.01 - Bradykinin Liberation from High Molecular Weight Kininogen by Plasma Kallikrein on the Vessel Wall is Modulated by both C1 inhibitor and Prolylcarboxypeptidase	38
SP.15.02 - Chagasic Cardiomyopathy: A big BUG in heart electrophysiology.	39
SP.15.03 - Mechanisms of Neuroinflammation and Cognitive Impairment in Experimental Cerebral Malaria	39
SP.15.04 - Kinins as new players in cerebral malaria pathogenesis	40

SP.16 - Plant Signaling	**41**
SP.16.01 - How plants signal the time of the day	41
SP.16.02 - Signaling Between Plant Growth Controls and Beneficial Bacteria	41
SP.16.03 - Photoperiod interplays with age and gibberellin to modulate flowering in the day-neutral tomato	42
SP.17 - Microbiome/rhizosphere interactions	**43**
SP.17.01 - Unraveling the action of Nod factors in different Bradyrhizobium strains	43
SP.17.02 - Modulating drought stress response of maize by a synthetic bacterial community	43
SP.17.03 - Rhizosphere microbiome studies: an innovative tool towards sustainable agriculture	44
SP.17.04 - Bacterial and fungal interactions in leaves, rhizosphere and roots of soybean and maize based on co-occurrence networks	44
SP.18 – SBBf Young Scientist	**45**
SP.18.01 - Albumin Overload Impact Proximal Tubule Cells Through Hyper O-Glcnacylation	45
SP.18.02 - Longitudinal changes in astrocytes on a transgenic rat model of Alzheimer's Disease	45
SP.18.03 - Inflammasomes are activated in response to SARS-CoV-2 infection and are associated with COVID-19 severity in patients	46
SP.18.04 - Electrophysiological alterations in peripheral neurons induced by experimental Diabetes mellitus	46
SP.19 - Biointerfaces	**47**
SP.19.01 - Cationic Amino Acid Transporters: mechanistic insights from L- and D-enantiomer-generated currents	47
SP.19.02 - Insights from MD simulations of the □-opioid receptor embedded in a POPC membrane	47
SP.19.03 - Local effects of an alpha-helical peptide upon membranes	48
SP.19.04 - Design and characterization of nature-inspired lipid biointerfaces	48
SP.19.05 - Towards a better model of the plasma membrane: asymmetric lipid bilayer prepared by hemifusion	49
SP.20 - Impact of the pandemic on teaching and learning: what was and what remains?	**50**
SP.20.01 - Reflections on emergency remote teaching of Biochemistry at the Federal University of Viçosa - past, present, and future	50
SP.20.02 - More from the same: revisiting Jmol and R for Biochemistry learning during the coronavirus outbreak.	50
SP.20.03 - Biochemistry on screen – from classroom stages to personal computers and back.	50
SP.21 - APC (Article Processing Charge) - The Future of 1st world Science or the End of the 3rd World one?	**51**
SP.21.01 - Open Access' spider web and the empowering of Academia	51
SP.21.02 - Open Access publishing: old problems have been solved but challenging new ones are emerging	51
SP.21.03 - How to balance the costs of publication/journal subscription with an open and excellent science	52
SP.21.04 - Open Access in Latin America: too rich for waivers, too poor for article processing charges	52

SP.22 - Exploring the Conformational Dynamics and stability of proteins using Computational Simulation	**53**
SP.22.01 - Probing energy landscapes of intrinsically Disordered proteins	53
SP.22.02 - Biotin-painted proteins have thermodynamic stability switched by kinetic folding routes	53
SP.22.03 - Normal-mode-based enhanced sampling methods	54
SP.22.04 - Effect of phosphorylation on the structural dynamics and thermal stability of human Dopamine Transporter: a simulation study using Normal Modes, Molecular Dynamics and Markov State Model	54
SP.23- New Tools on Sirius Synchrotron Facility	**55**
SP.23.01 - CEDRO Beamline: Circular Dichroism Spectroscopy Boosted by the Synchrotron Radiation	55
SP.23.02 - Cateretê Beamline: Coherent Scattering Imaging Commissioning Updates	55
SP.23.03 - The reach of synchrotron infrared ultramicroscopy applied to the study of biosystems	56
SP.23.04 - The MANACA beamline at Sirius, structural biology at 4th Generation	56
SP.24 - Molecular and cellular mechanisms of tumor progression	**57**
SP.24.01 - Tumor extracellular Vesicles effects on endothelial cells	57
SP.24.02 - Development of time-resolved flow cytometry for the detection of NAD(P)H lifetime shifts: a tool for metabolic mapping of breast cancer	57
SP.24.03 - The role of alphavbeta3 integrin in tumor progression	58
SP.24.04 - The origins of heterogeneity in cancer cell fitness	58
SM.01 - Session 1: Biogenesis and functions of matrix vesicles	**59**
SM.01.01 - Osteoblast matrix vesicles: beyond matrix mineralization	59
SM.01.02 - Potential role of autophagy in the biochemical properties of matrix vesicles	59
SM.01.04 - Pyrophosphate regulators as key players for dental cementum formation and regeneration	60
SM.03 - Session 3: Functions/application of matrix vesicles	**61**
SM.03.01 - Roles of annexins in bone mineralization and vascular calcification	61
SM.03.02 - 3D printing of bioactive scaffolds based on starch/collagen-containing matrix vesicles	62
SM.03.03 - Effect of EVs released by osteoblasts on osteoclastogenesis and bone remodeling	62
SM.03.04 - Isolation and Characterization of Extracellular Vesicles Derived from Mesenchymal Stem Cells	63
SM.04 - LAFeBS-IUPAB Symposium	**64**
SM.04.01 - Giant hybrid polymer/lipid vesicles: Insights on phase separation and dynamics from advanced microscopy methodologies	64
SM.04.02 - Neutron studies in Bioscience: Opportunities and Challenges posed by the Argentine Neutron Beam Laboratory	64
SM.04.03 - The mechano-sensitive potassium channel and the K/Ca exchanger of the human erythrocyte: A new senescence hypothesis	65
SM.04.04 - To be or not to be: what is the function? The exquisite biophysics of the Golgi Reassembly and Stacking Proteins	65

Abstracts	67
Author’s Index	429
Keywords Index	449

Conferences

CF.01 - The function of matrix vesicles in physiological and pathological mineralization

Jose Luis Millan
Sanford Burnham Prebys, Human Genetics Program (La Jolla, CA, USA)

Hydroxyapatite (HAP) deposition in bones and teeth is a carefully orchestrated biochemical process aimed at balancing the concentrations of inorganic pyrophosphate (PPi), a potent calcification inhibitor, and the local concentrations of phosphate (Pi), a calcification promoter, to establish a proper PPi/Pi ratio to enable regulated tissue mineralization to take place. Mineralizing cells (chondrocytes, osteoblasts, odontoblasts, ameloblasts) bleb matrix vesicles (MVs) where the seed crystals of HA are initially formed before propagating onto the collagenous matrix. Current data are compatible with the following mechanistic pathway of physiological mineralization: MVs initiate mineral formation by accumulation of Pi generated intravesicularly by the action of PHOSPHO1 on phosphocholine and also via PiT-1-mediated incorporation of Pi generated extravesicularly by tissue-nonspecific alkaline phosphatase (TNAP) and/or ENPP1 using extracellular ATP, exported from the cells by the function of ANK and ABCC6, as substrate. The extravesicular propagation of mineral onto the collagenous matrix is mainly controlled by the PPiase activity of TNAP that restricts the concentration of PPi. Deficiency in TNAP leads to hypophosphatasia, a soft bones disease, while inactivating mutations in the ENPP1, ANK and ABCC6 genes underly the pathophysiology of ectopic calcification disorders, including generalized ectopic calcification of infancy (GACI), ankylosing spondylitis (ANK) and pseudoxanthoma elasticum (PXE), respectively. TNAP upregulation in the vasculature, either in the medial or intimal layers, leads to severe vascular calcification, and TNAP upregulation is part of the pathophysiology of GACI and PXE as well as of chronic kidney disease and atherosclerosis. These enzymes and transporters represent a druggable pathway for the development of novel treatments for disorders due to dysregulation in the PPi/Pi ratio. I will present preclinical data validating the use of TNAP as a biologic for the treatment of hypophosphatasia and a therapeutic target for the prevention of ectopic calcification.

Keywords: matrix vesicles, mineralization, Hydroxyapatite

CF.02. - Inflammasome activation in response to SARS-CoV-2 infection, from the intracellular biochemistry to the severe pulmonary inflammation

Dario Simões Zamboni [1]
[1]Departamento de Biologia Celular e Molecular e Bioagentes Patogenicos, Faculdade de Medicina de Ribeirao Preto, Universidade de Sao Paulo (Ribeirao Preto, SP, Brazil)

Severe cases of COVID-19 are characterized by a strong inflammatory process that may ultimately lead to organ failure and patient death. The NLRP3 inflammasome is a molecular platform that promotes inflammation via cleavage and activation of key inflammatory molecules including active caspase-1 (Casp1p20), IL-1β, and IL-18. We have demonstrated that the NLRP3 inflammasome is activated in response to SARS-CoV-2 infection and is active in COVID-19 patients. Studying moderate and severe COVID-19 patients, we found active NLRP3 inflammasome in PBMCs and tissues of postmortem patients upon autopsy. Inflammasome-derived products such as Casp1p20 and IL-18 in the sera correlated with the markers of COVID-19 severity, including IL-6 and LDH. Moreover, higher levels of IL-18 and Casp1p20 are associated with disease severity and poor clinical outcome. Data to be presented will highlight how the inflammasomes participate in the pathophysiology of the disease and a potential therapeutic target for COVID-19.

Keywords: SARS-CoV-2 , COVID-19, NLRP3

CF.03. - Bioenergetic Immune Exhaustion in Malaria

James Kazura [1]
[1]Department of Pathology, School of Medicine, Case Western Reserve University (Cleveland, OH, USA)

Unlike many childhood viral infections endemic in tropical areas of the world, protective immunity from malaria is non-sterilizing and develops slowly during childhood following repeated exposures to Plasmodium falciparum blood stage infection and febrile malaria illness. The molecular and biochemical mechanisms underlying the slow development of this “naturally acquired immunity” are not well understood. Understanding these mechanisms is important as this knowledge could reveal biological pathways that could be targeted to enhance the efficacy of existing and, in the future, more effective malaria vaccines. Our ongoing studies in sub-Saharan Africa and the tropical Pacific suggest that slow acquisition of malaria immunity is in large part due to Plasmodium falciparum induced depletion of selected host amino acids and other essential host nutrients that impair immune cell mitochondrial biogenesis and increased energy production that are required for generation of malaria memory B cells and plasma cells that produce long-lived P. falciparum antibodies. These data will be discussed in the context mouse malaria and bacterial sepsis models that allow for proof of causality.

Keywords: Bioenergetic , Malaria, Plasmodium falciparum

CF.04. - Geminivirus-Host Interactome: an immune hub cross-talking with antiviral, antibacterial immunity, and growth-promoting events

Elizabeth Pacheco Batista Fontes [1]
[1]Departamento de Bioquímica e Biologia Molecular., Universidade Federal de Viçosa (Viçosa, MG, Brazil)

All organisms must perceive and respond to changing growth conditions and environmental stimuli. Under acute adverse conditions, including heat shock, osmotic stress, and pathogen attack, the induced changes in energy-consuming cellular processes must be coordinated with the rate of energy-producing metabolic processes to prevent excessive stress built up and ensure cell survival. In-plant cells, photosynthesis, a chemical energy-producing process, must be coordinated with translation, the most energy-consuming process, under stress conditions. However, direct links between these major cellular processes have not been thoroughly investigated. In the last decade, we have made some progress toward deciphering a signaling pathway mediated by a leucine-rich repeat receptor-like kinase (NIK1), which has been identified through interaction with the geminivirus nuclear shuttle protein (NSP). We showed that NIK1 relays biotic signals to the RPL10/LIMYB signaling module to control translation as an antiviral immunity mechanism and negatively regulate antibacterial immunity. Currently, we further extended the characterization of this biotic stimuli-induced cell surface signaling pathway. We demonstrated that the NIK1/RPL10/LIMYB signaling circuit is also activated by abiotic stresses, including osmotic stress and high temperature, to control translation and photosynthesis coordinately. We have solid evidence to propose that NIK1 represents a signal-transducing hub activated by different stimulus-sensing transmembrane receptors to regulate growth-promoting events under biotic and abiotic stresses. Therefore, NIK1 may function as a coreceptor, relaying information from various sensing receptors towards growth control under different stresses.

Keywords: Geminivirus-Host , NIK1/RPL10/LIMYB , photosynthesis

CF.06. - Purinergic signaling and innate immunity regulate hematopoiesis in “hormetic-dependent” manner

Mariusz Z Ratajczak [1,2]
[1]Stem Cell Institute at James Graham Brown Cancer Center, University of Louisville (KY, USA),
[2]Laboratory of Regenerative Medicine Medical, University of Warsaw (Warsaw, Poland)

It is well known that hematopoietic and immune cells originate from a common hematopoietic/lymphopoietic stem cell what explains that these different cell types often share the same receptors and respond to similar factors. Moreover, the common goal of both lineages is to ensure tissue homeostasis under steady-state conditions, fight invading pathogens, and promote tissue repair. Accumulating evidence demonstrates that purinergic signaling and innate immunity modulate several aspects of hematopoiesis within the “hormetic zone” in which the biological response to low exposure to potential stressors generally is favorable and benefits hematopoietic stem/progenitor cells (HSPCs). Both purinergic signaling and innate immunity impact on hematopoiesis is pleiotropic and involves both the cellular arm, comprised of innate immunity cells, and the soluble arm, whose major component is the complement cascade (ComC). In addition, several mediators released by innate immunity cells, including extracellular nucleotides, inflammatory cytokines and small antimicrobial cationic peptides, affect hematopoiesis. Moreover, HSPCs and immune cells share several cell-surface pattern-recognition receptors (PRRs), such as Toll-like receptors (TLRs) and cytosol-expressed NOD, NOD-like, and RIG-I-like receptors and thus can be considered “pathogen sensors”. An important intracellular NOD-like receptor is Nlrp3 inflammasome that integrates purinergic signaling with proper activation of innate immunity. In addition, not only lymphocytes but also HSPCs express functional intracellular complement proteins, defined as complosome which poses challenging questions for further investigation of the intercellular ComC-mediated intracrine regulation of hematopoiesis and its interaction with purinergic signaling.
Keywords: Stem Cell, hematopoiesis , immune cells

Symposia

SP.01 - What is new about COVID-19: perspectives to a post-COVID

SP.01.01 - Different phenotypes of lung damage in COVID-19

Patricia Rieken Macedo Rocco [1]
[1]Laboratory of Pulmonary Investigation, Federal University of Rio de Janeiro (UFRJ) (RJ, Brazil)

Severe acute respiratory disease coronavirus 2 (SARS-CoV-2) is a coronavirus that has rapidly disseminated worldwide, causing the coronavirus disease 2019 (COVID-19) pandemic. Although initially described as a viral pneumonia, SARS-CoV-2 infection seems to present features of a multisystem disease, with impairment of several organs including heart, kidney, bowel, liver, and brain, as well as coagulation disorders. Therefore, we have proposed a new nomenclature, which includes the concept of multiple organ damage: multiple organ dysfunction in SARS-CoV-2 (MODS-CoV-2). The heterogeneous clinical manifestations in COVID-19 may be attributed to individual variation in expression not only of angiotensin-conversing enzyme 2 (ACE2) and type II transmembrane serine protease (TMPRSS2) receptors, but also of genes related to inflammation and immune responses. Accordingly, the excessive acute inflammatory responses seen in individuals with severe COVID-19 may lead to MODS and death. Based on chest computed tomography findings, COVID-19–induced lung impairment can be grouped into two main phenotypes: (1) multiple, focal, potently overperfused ground-glass opacities; and (2) typical moderate-to-severe ARDS, with alveolar edema and low compliance. As these phenotypes may be related to different pathological mechanisms and disease progression, personalized mechanical ventilation approaches should be implemented in order to allow more efficient clinical recovery for each individual.
Keywords: COVID-19, inflammation, phenotype, lung compliance

SP.01.02 - Prothrombotic Vascular Dysfunction in COVID-19

Alec Andrew Schmaier [1]
[1]Department of Cardiovascular Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School (Boston, MA, USA)

Severe COVID-19 is a respiratory illness characterized by microvascular thrombosis in the pulmonary vasculature. Vascular involvement leads to an array of complications ranging from thrombosis to pulmonary edema secondary to loss of barrier function. Constitutively, the vascular endothelial surface is anticoagulant, a property maintained at least in part via signaling through the cytoprotective Tie2 receptor. We have demonstrated that COVID-19 is associated with procoagulant endothelial dysfunction and alterations in the Tie2-angiopoietin axis. Plasma from patients with COVID-19 activates a thromboinflammatory gene expression pattern and promotes coagulation on the endothelial cell surface. Pharmacologic activation of Tie2 with the small molecule AKB-9778 (razuprotafib) reversed the prothrombotic state induced by COVID-19 plasma in primary endothelial cells and supports ongoing clinical trials investigating razuprotafib for moderate and severe COVID-19. Additionally, we have demonstrated that the SARS-CoV-2 accessory protein and cation channel ORF3a increases intracellular calcium concentration and phosphatidylserine externalization to augment procoagulant activity infected cells. Therefore, both systemic inflammatory effects and viral intrinsic mechanisms contribute to prothrombotic vascular dysfunction in COVID-19. We will review the current state of the evidence supporting endothelial dysfunction in COVID-19 with a focus on thrombosis and potential therapeutic targets
Keywords: COVID-19, Prothrombotic, SARS-CoV-2

SP.01.03 - Extrapulmonary infection in COVID-19 and its potential contribution to disease pathophysiology

Marcelo Alves da Silva Mori [1]
[1]Departamento de Bioquímica e Biologia Tecidual, Instituto de Biologia, UNICAMP (Campinas, SP, Brazil)

COVID-19 has been proven to be much more than an acute respiratory distress syndrome resulting from pulmonary infection by SARS-CoV-2. SARS-CoV-2 is capable of infecting many different cell types in addition to epithelial cells, where it elicits cell-specific responses that may contribute to disease pathogenesis. In my talk, I will describe our attempts to characterize the mechanism and impact of SARS-CoV-2 infection over non-epithelial cells such as adipocytes, immune cells, and cells from the central nervous system. I will also associate these extrapulmonary sites of infection with the pathophysiology of COVID-19 and its risk factors.
Keywords: COVID-19, SARS-CoV-2, respiratory distress syndrome

SP.01.04 - RD&I Actions to Face the Covid-19 Pandemic

Marcelo Marcos Morales [1]
[1]Ministério da Ciência, Tecnologia e Inovações, (Brasília, DF, Brazil)

At the beginning of the pandemic, the Ministry of Science, Technology and Innovations created the “Rede Virus MCTI”, a forum for scientific advice to assist in the definition of guidelines and priorities in the fight against Covid-19. From the establishment of priorities, investments were made in research that promoted the sequencing of the virus, the production of diagnostic tests with national technology, the repositioning of drugs and the development of vaccines against the disease, as well as studies on the economic and social impacts of the pandemic. Science and technology institutions spread throughout the national territory were mobilized in response to the pandemic. To support these initiatives, three public calls were launched for funding RD&I projects, for international cooperation and for the structuring of BSL-3 laboratories. Projects were also supported, through direct contracting, within the scope of the priorities defined by the Virus Network. The main results of the supported initiatives include the networks of virus's genetic sequencing, of wild animals’ surveillance and of wastewater monitoring, the development of supplies and diagnostic kits and the development of national vaccines against Covid-19. In the case of vaccines, ten national projects for its pre-clinical development were supported, which contemplated different strategies for vaccines divided into three broad areas: nucleic acid, chimeras and subunits (recombinant proteins, nanoparticles and VLPs). Five projects, whose Active Pharmaceutical Ingredient have been developed by Brazilian researchers, in national Science and Technology institutions and/or international partnership with technology transfer to national institutions, advanced to phase I and II clinical trials, also supported by MCTI. The ministry has also secured resources to support phase III clinical trials to complete the development of a national vaccine. All supported initiatives aim to give the country the autonomy and technological independence that have proved to be of vital importance in times of global health crises such as Covid-19.
Keywords: Covid-19, vaccine

SP.02 - Nano/micro encapsulation of excipients with biological activity

SP.02.01 - Bioinspired materials for functional foods and antimicrobials

Stefan Salentinig [1]
[1]Department of Chemistry, University of Fribourg (Switzerland)

Fluid-like colloidal structures including liquid crystals are key components in nature's own functional materials and important for a wide range of applications [1,2]. Recently, the self-assembly of nature's own complete diet, milk, into diverse liquid crystalline structures was discovered during its in vitro digestion. This was possible by the use of in operando small angle X-ray scattering and diffraction techniques at synchrotron sources, combined with advanced dynamic in vitro digestion models that simulate the conditions in the human digestive tract (Figure 1) [2,3]. The discovered prototypical natural nanomaterials have many implications for the design of novel adaptive materials including functional foods and antimicrobials [4,5]. They were blue-printed, for instance, to nano-architect delivery matrices for poorly water soluble bioactives for food applications and into antiviral as well as antibacterial materials. Further, the self-assembly of viruses in solution, and its implications for the design of advanced virus filtration materials is presented.[6] This material design was guided by highly contemporary experimental methods including time-resolved (grazing incidence) small angle X-ray scattering and diffraction, imaging ellipsometry, confocal Raman microscopy as well as cryogenic electron microscopy and NMR techniques. Additional biological assays were used to bridge the boundaries from the molecular and structural to the cellular level. The detailed insights into the dynamic self-assembly of biomolecules to functional supramolecular structures provide essential knowledge for the comprehensive design of advanced food materials. [1] Glatter O., Salentinig S. COCIS, 2020, 49, 82-93 [2] Lim S., Salentinig S. COCIS, 2021, 56, 101485 [3] Hempt C., Gontsarik M., Buerki-Thurnherr T., Hirsch C., Salentinig S. JCIS 2020, 574, 430-440. [4] Zabara M., Qun R., Amenitsch H., Salentinig S. ACS Applied Biomaterials, 2021, 6, 5295–5303. [5] Hong L., Gontsarik M., Amenitsch H., Salentinig S. Small, 2022, 2104211. [6] Watts S., Maniura-Weber K., Siqueira G., Salentinig S. Small, 2021, 30, 2100307
Keywords: SAXS, Antimicrobial nanomaterials, Food colloids

SP.02.02 - Advances and challenges to elucidate the mechanisms of action of polymeric nanopesticides

Leonardo Fernandes Fraceto
Institute of Science and Technology of Sorocaba, São Paulo State University - Unesp
Sorocaba - São Paulo – Brazil. leonardo@sorocaba.unesp.br

Studies have been demonstrating the potential in the delivery of agricultural ingredients using nanoparticles. Among a variety of ingredients that can be used for the production of nanoparticles containing active ingredients, polymeric matrices, proteins, lipids and carbohydrates have received a lot of attention as they are less impactful to the environment. However, there is still a great lack of studies that elucidate the mechanism of action of nanopesticides. In this sense, this lecture will present some advances used for the study of target strategies of nanopesticides and organisms, as well as the challenges to be overcome in order to elucidate the mechanisms of action of nanopesticides. Acknowledgements: FAPESP (2017/21004-5), CNPq and Capes.

SP.02.03 - Micro/Nanotechnology Applied to Integrated Pest Management

Jhones Luiz de Oliveira [1]
[1]Institute of Science and Technology of Sorocaba, São Paulo State University - Unesp (Sorocaba, SP, Brazil)

Over the last few decades, agriculture has suffered numerous changes, which have allowed a productivity increase. Much of these advances came with the use of compounds for pest control, as well as for crop nutrition and growth. However, due to its excessive use, numerous problems related to environmental contamination, toxicity to non-target organisms and also the development of resistance have emergerd. To address these challenges, numerous sustainable technological approaches are being explored, such as micro/nanotechnology. The use of these micro/nanotechnology based systems has been mainly focused for use as sustained release systems of agricultural active compounds and also microorganisms, as well as in plant improvement, soil management and precision agriculture. In the lecture we will provide an overview of the use of these systems related to the agricultural sector and their potential. We will also present an analysis of the main challenges, concerns and future perspectives for the use of these formulations in integrated pest management.

Keywords: micro/nanotechnology, Pest Management

SP.02.04 - Self-assembly investigation of amphiphilic peptides for pesticides biosensors development

Barbara Bianca Gerbelli [1,2], Pedro Leonidas Oseliero Filho[3,4], Heloísa Nunes Bordallo[3,5], Ian Willian Hamley[1], Wendel Andrade Alves[2]

[1]Department of Chemistry, University of Reading (Reading, United Kingdom), [2]Centro de Ciências Naturais e Humanas, Universidade Federal do ABC (São Paulo, SP, Brazil), [3]Niels Bohr Institute, University of Copenhagen (Copenhagen, Denmark), [4]Instituto de Física, Universidade de São Paulo (São Paulo, SP, Brazil), [5], European Spallation Source (Lund, Sweden)

Self-assembled structures obtained from organic molecules have shown great potential for applications in a wide range of domains. In this context, short peptides prove to be a particularly versatile class of organic building blocks for self-assembled materials. These species afford the biocompatibility and polymorphic richness typical of proteins while allowing synthetic availability and robustness typical of smaller molecules. At the nano-to-mesoscale, the architectures obtained from peptide units exhibit stability and a large variety of morphologies, the most common of which are nanotubes, nanoribbons, and nanowires. Here, we performed the synthesis of new lipopeptides that can be used as functional models of the enzyme acetylcholinesterase (AChE), a well-known pesticide biosensor. Our goal is to obtain different supramolecular organizations and investigate the correlation between structure and pesticides detection. To this end, scattering techniques (small-angle x-ray scattering – SAXS, and dynamic light scattering – DLS) were used to probe structural aspects and provide insights into the mechanisms involved in the self-assembly. Combined with direct space techniques such as calorimetry (isothermal titration calorimetry – ITC), spectroscopy (circular dichroism – CD, and fluoresce) and microscopy (transmission electron microscopy – TEM, and Cryo-TEM), we were able to also correlate the physicochemical mechanisms involved in the building of the nanostructures and their interaction with different pesticides. The satisfactory mimetic behavior of these new molecules used as model of AChE enzyme was confirmed by kinetic UV-Vis experiments of the Ellman hydrolysis reaction [3], a well-known method for the verification of AChE pesticide detection. [1] Wong, A.; Silva, T. A.; Caetano, F. R.; Bergamini, M. F.; Marcolino, L. H.; Fatibello, O.; Janegitz, B. C. (2017), C-Journal of Carbon Research, 3 (1). DOI: 10.3390/c3010008. [2] Liu, M.; Khan, A.; Wang, Z. F.; Liu, Y.; Yang, G. J.; Deng, Y.; He, N. Y. (2019), Biosensors & Bioelectronics, 130, 174-184. DOI: 10.1016/j.bios.2019.01.006. [3] Gerbelli, B. B. et al., in preparation.

Keywords: peptides , pesticides biosensors development, AChE

SP.03 - Functions of Matrix Vesicles

SP.03.01 - TNAP activation by inflammation in vascular smooth muscle cells and release into extracellular vesicles: possible functions in atherosclerosis plaques

Saida Mebarek [1], Rene Buchet[1], David Magne[1]
[1]Laboratory of MEM2, University of Lyon 1 (ICBMS UMR5246 , Villeurbanne, France)

Atherosclerosis is characterized by a multi-step process of chronic vascular inflammation and subsequent plaque formation. Inflammation is involved in all stages of the atherosclerotic process. During the formation of a lesion nidus, vascular smooth muscle cells (VSMCs) present in the media layer of the artery are exposed to modified cholesterol, cytokines and growth factors, and become activated. Indeed, VSMCs are able to adopt a number of phenotypes, including calcific (osteogenic, chondrocytic, and osteoclastic), adipogenic, and macrophagic phenotypes. VSMCs can release several types of extracellular vesicles (EVs) including exosomes, microvesicles (MVs)/microparticles and apoptotic bodies. Emerging evidence in the field suggests that these smooth muscle cell-derived EVs can contribute to intercellular communication during the development of atherosclerosis via the transfer of cellular contents such as protein and microRNA, which may prevent or promote disease progression depending on the context. Our objectives are to analyze the phenotype of VSMCs cells under inflammatory condition and to isolate and characterize the different type of EVs secreted by these cells. We used Human Coronary Artery Smooth Muscle Cells (HCASMCs). We observed that interleukin 1β (IL1β), proinflammatory cytokine induced a phenotypic switching of HCASMCs. Quantitative PCR and western blot revealed an increase in the expression of RUNX2, SOX9, OPG and TNAP in HCASMC cells cultured under inflammatory conditions, after seven, fourteen and twenty-one days. We isolated and characterized each class of microparticles produced by HCASMCs stimulated after fourtheen days by IL1β. We observed an enrichment of EVs in TNAP, suggesting that the increase in TNAP in EVs upon inflammation is a trigger mechanism to initiate calcification which could be sustained by calcification-induced inflammation and by enriched-TNAP EVs. (saida.mebarek@univ-lyon1.fr)
Keywords: inflammation, TNAP, atherosclerosis

SP.03.02 - Vascular smooth muscle cells drive osteoblast-to-osteocyte transition via β-catenin signaling through exosome communication

Célio J.C. Fernandes[1], Rodrigo A. Silva[2], Gwenny M. Fuhler[3], Maikel P. Peppelenbosch[3], Bram CJ van der Eerden[4], **Willian Fernando Zambuzzi*[1]**
[1]Bioassays and Cell Dynamics Lab, Dept. of Chemistry and Biochemistry, Bioscience Institute, UNESP (Botucatu, SP, Brazil), [2]School of Dentistry, University of Taubaté (Taubaté, SP, Brazil), [3]Department of Gastroenterology and Hepatology, University Medical Center Rotterdam's Gravendijkwal (Rotterdam, The Netherlands), [4]Department of Internal Medicine, Erasmus MC, University Medical Center Rotterdam's Gravendijkwal (Rotterdam, The Netherlands)

Blood vessel growth and osteogenesis in the skeletal system are coupled; however, fundamental aspects of vascular function in osteoblast-to-osteocyte transition have not been elucidated earlier. Our study demonstrates that vascular smooth muscle cells (VSMCs), but not endothelial cells, are sufficient to drive osteoblast-to-osteocyte transition. We also found that VSMC-derived exosomes loaded with transcript encoding proteins are related to osteocyte phenotype and Wnt/ß-catenin signaling members. In contrast, endothelial cell-derived exosomes delivered stimuli to mature osteoblast differentiation, up-reprogramming TGF gene family and osteogenic transcriptional factors, osterix and runx2. Importantly, VSMCs triggered better performance, which released exosome that presented less membrane zeta potential. Another important issue was the high level of ATP inside the exosomes, facilitating mechanisms of mineralization, since ATP is a substrate for alkaline phosphatase. Our findings identify a novel and hitherto unexpected role of vascular smooth muscle cells driving osteoblast-to-osteocyte transition and opens a new avenue to better understand bone-related diseases. *Correspondence to: w.zambuzzi@unesp.br
Keywords: mineralization, Beta-catenin, osteoblast-to-osteocyte

SP.03.03 - Nanomaterials exposure, extracellular vesicles biogenesis and adverse cellular outcomes: a scoping review

Jose Mauro Granjeiro [1]
[1]Departamento de Bioengenharia, INMETRO (Rio de Janeiro, Brasil)

The progressive increased use of nanomaterials (NMs) has awakened issues related to nanosafety and its potential toxic effects on human health. Emerging studies suggest that NMs alter cell communication by reshaping and altering the secretion of extracellular vesicles (EVs), leading to dysfunction in recipient cells. However, there is limited understanding of how the physicochemical characteristics of NMs alter the EVs' content and their consequent physiological functions. Therefore, this review explores the relevance of EVs in the nanotoxicology field. The current state of the art on how EVs are modulated by NM exposure and the possible regulation and modulation of signalling pathways and physiological responses were assessed in detail. This review followed the manual for reviewers produced by The Joanna Brigs Institute for Scoping Reviews and the PRISMA extension for Scoping Reviews (PRISMA-ScR): checklist and explanation. The research question, "Do NMs modulate cellular responses mediated by EVs?" was analyzed following the PECO model (P (Population) = EVs, E (Exposure)= NMs, C (Comparator) = EVs without exposure to NMs, O (Outcome) = Cellular responses/change in EVs) to help methodologically assess the association between exposure and outcome. For each theme in the PECO acronym, keywords were defined, organized, and researched in PubMed, Science Direct, Scopus, Web of Science, EMBASE, and Cochrane databases, up to September 30, 2021. In vitro, in vivo, ex vivo, and clinical studies that analyzed the effect of NMs on EVs biogenesis, cargo, and cellular responses were included in the analysis. The methodological quality assessment was conducted using the ToxRTool, ARRIVE guideline, Newcastle Ottawa and the EV-TRACK platform. The search in the referred databases identified 2944 articles. After applying the eligibility criteria and two-step screening, 18 articles were included in the final review. We observed that depending on the concentration and physicochemical characteristics, specific NMs promote a significant increase in EVs secretion as well as changes in their cargo, especially regarding the expression of proteins and miRNAs, which, in turn, were involved in biological processes that included cell communication, angiogenesis, and activation of the immune response, etc. Although further studies are necessary, this work suggests that molecular investigations on EVs induced upon NM exposure may become a potential tool for toxicological studies since they are widely accessible biomarkers that may bridge NMs exposure with the cellular response and pathological outcome.
Keywords: Nanomaterials, extracellular vesicles, biomarkers

SP.03.04 - Matrix eVesicles: exploiting the protein corona and its function in biomineralization

Heitor Gobbi Sebinelli [1], Luiz H.S. Andrilli[1], Marcos A.E. Cruz[1], Ana Paula Ramos[1], Maytê Bolean[1], José Luis Millán[2], Massimo Bottini[1,2,3], Pietro Ciancaglini[1]
[1]Department of Chemistry, Faculdade de Filosofia, Ciências e Letras (FFCLRP), University of São Paulo, (Ribeirão Preto, SP, Brazil), [2]Sanford Burnham Prebys, (La Jolla, CA, USA), [3]Department of Experimental Medicine, University of Rome Tor Vergata (Rome, Italy)

Hypertrophic chondrocytes, osteoblasts, and odontoblasts release a special class of extracellular vesicles called matrix vesicles (MVs) that are involved in the deposition of apatite minerals at collagenous sites of bone and dental calcification. During physiological and pathological calcifications, MVs are released by external budding of the apical microvilli, then bind to collagen fibrils and initiate biomineralization by virtue of their biochemical machinery. Current knowledge about MVs describes in its composition only the presence of "native" proteins, that is, the proteins are pre-arranged in one of the layers or in the lipid bilayer of the microvilli of the parental cells before the MVs are released. However, a recent study comparing the proteome of MVs and isolated apical microvilli from osteoblast lineages showed that several of the MV proteins do not originate from microvilli. This achievement suggests that extracellular space-derived proteins (ECS) can adsorb and form a crown of peripheral proteins on the surface of MVs known as the "protein corona". In vitro experiments showed that this crown can induce changes in physical properties of the MVs. Native vesicles have a monomodal distribution of the diameter with the peak centered in 200 nm, then, when depleted of protein corona MVs revealed a bimodal distribution behavior, with peaks centered in 200 and 300 nm. After protein re-adsorption, the bimodal behavior stands, but the peak centered in 200 nm increases. The zeta potential decreases with protein removal, and is partially recovered after incubation with the removed protein. As for TNAP activity, the physical changes caused by removal and re-adsorption of the protein corona showed some modulation of the enzymatic mineral propagation of the MVs. Although the removal of the protein lead to a slight increase of TNAP activity, the re-adsorption showed to be able to decrease it, revealing directly relatable to mineral propagation ability. **Keywords:** Matrix eVesicles, biomineralization, protein corona

SP.04 - 25 Young Talent in Life Science Prize

SP.04.01 - Do astrocytes contribute to brain glucose metabolism?

Andreia Silva da Rocha[1], Bruna Bellaver[1], Débora G. Souza[1], Guilherme Schu[1], Igor C. Fontana[1], Gianina T. Venturin[2], Samuel Greggio[2], Luiza S. Machado[1], Jaderson C. da Costa[2], Pedro Rosa-Neto[3], Luc Pellerin[4], Diogo O. Souza[1], Eduardo R. Zimmer[1]
[1]Graduate Program in Biological Sciences: Biochemistry, UFRGS (Porto Alegre, RS, Brazil), [2]Preclinical Research Center, Brain Institute (BraIns) of Rio Grande do Sul, PUC-RS (Porto Alegre, RS, Brazil), [3]Translational Neuroimaging Laboratory, McGill Centre for Studies in Aging, McGill University (Montreal, Quebec, Canada), [4]Inserm U1313, Université de Poitiers (Poitiers, France)

Advances in functional imaging allowed us to visualize brain glucose metabolism in vivo and non-invasively with [18F]fluoro-2-deoxyglucose positron emission tomography (FDG-PET) imaging. Since its development, 40 years ago, the FDG-PET signal source has been attributed to neuronal uptake, with hypometabolism being considered as a direct index of neuronal dysfunction or death. However, other brain cells are also metabolically active, including astrocytes. Based on the astrocyte-neuron lactate shuttle hypothesis, the activation of the glutamate transporter 1 (GLT-1) acts as a trigger for glucose uptake by astrocytes. With this in mind, we aimed to investigate the astrocytes role in brain glucose utilization by pharmacologically downregulating GLT-1 with clozapine (CLO), an antipsychotic drug. Adult male Wistar rats received clozapine in the drinking water for six weeks. Glucose brain metabolism was longitudinally accessed using FDG-microPET before and after the treatment. Glutamate uptake was evaluated in cortical brain slices. Immunocontent and expression of GLT-1 and GLAST were assessed on the cortical tissue and cultures. CLO treatment effects were also assessed in cortical astrocyte cultures (glucose and glutamate uptake) and in cortical neuronal cultures (glucose uptake). CLO markedly reduced in vivo brain glucose metabolism in several brain areas, especially in the cortex. Ex vivo analyses demonstrated decreased cortical glutamate transport along with GLT-1 mRNA and protein downregulation. In astrocyte cultures, CLO decreased GLT-1 density as well as glutamate and glucose uptake. By contrast, in cortical neuronal cultures, CLO did not affect glucose uptake. This work provides the first PET evidence that reduction of an astrocytic function, such as glutamate transport, can lower FDG-PET signal. These findings shed light on a potential important role played by astrocytes on brain glucose utilization, which supports further investigations to identify FDG-PET cellular origin and to reevaluate the interpretation of this important brain imaging technique. **Keywords:** metabolism, FDG-PET

SP.04.02 - Inflammasome activation in the pulmonary parenchyma defines two distinct profiles associated with cytokine storm and worsening of lung function in COVID-19 patients

Keyla Santos Guedes de Sá [1], Luana Alexandrina Amaral[1], Camila C.S. Caetano[1], Amanda de Matos Becerra[1], Sabrina Setembre Batah[2], Isadora Mafra de Oliveira[1], Macel Koenigkam-Santos[3], Ronaldo Martins[1], Eurico Arruda[1], Alexandre Todorovic Fabro[2], Dario Simões Zamboni[1]
[1]Dep de Biologia Celular e Molecular e Bioagentes Patogênicos, [2]Departamento de Patologia e Medicina Legal, [3]Dep de Imagens Médicas, Hematologia e Oncologia, Faculdade de Medicina de Ribeirão Preto (São Paulo, Brasil)

Inflammasome activation is associated with disease severity in patients infected with SARS-CoV-2 and influenza, but the specific cell types that drive inflammasome activation as well as the inflammatory profile associated with inflammation-mediated disease exacerbation is unknown. The aim of this project is understand the molecular mechanisms underlying the pathological processes that lead to death of COVID-19 and Influenza patients. Here, we assessed lung autopsy of 47 fatal COVID-19 patients and 12 fatal influenza patients and examined inflammatory profile, inflammasome activation and correlated with clinical and histopathological patient's conditions. We demonstrated the presence of more robust inflammasome activation in lethal cases of SARS-CoV-2 compared to Influenza and found a different profile of inflammasome-activating cells during these diseases. In COVID-19 patients, inflammasome activation is mostly mediated by macrophages and endothelial cells whereas in Influenza, type I and type II pneumocytes contribute more significantly. Analysis of gene expression allows classification of COVID-19 patients in two different clusters, cluster 1 (n=16 patients) died with higher viral loads and reduced inflammatory profile than oppose to cluster 2 (n=31). Illness time, mechanical ventilation time, pulmonary fibrosis, respiratory functions, histopathological status, and inflammasome activation differed in the two clusters. Our data reveal two distinct profiles in lethal cases of COVID19, indicating that the balance of viral replication and inflammasome-mediated pulmonary inflammation may lead to opposed clinical conditions, yet both lead to patient death. Understanding this process is critical for decisions concerning the higher efficacy of immune-mediated or antiviral-

mediated therapies for the treatment of critical cases of COVID-19. **Keywords:** COVID-19, Inflammasome, cytokine storm. **Supported by:** FAPESP

SP.04.03 - PM2.5-Induced Cytotoxicity Exacerbates Foam Cells Formation By Macrophages

Lílian Corrêa Costa-Beber [1], Hémelin Resende Farias[1], Rafael Kazmirczak Moraes[1], Jade de Oliveira[1], Fátima C.R. Guma[1]
[1]Departamento de Bioquímica, Universidade Federal do Rio Grande do Sul (RS, Brasil)

The exposure to fine particulate matter (PM0.1-2.5 µm), an air pollutant, enhances the susceptibility to atherosclerosis. The phagocytosis of oxidized LDL by macrophages is a fundamental trigger for foam cells formation. Besides, macrophages apoptosis aggravate the thrombus formation and inflammation. In this context, the 70 kDa-heat shock proteins (HSP70) are powerful anti-senescence chaperones, closely related to cell survival and anti-inflammatory signaling. We investigated if the atherogenic effect of PM2.5 could be related to an impairment in HSP signaling and cytotoxicity in macrophages. PM2.5 retained in glass fiber filters was partially extracted with PBS and further centrifuged at 1000g for 15 min. This solution (1 g filter/125 mL PBS) was diluted in DMEM 10% FBS ten times. We exposed RAW264.7 mouse macrophages cell line to PM2.5 for 48 h, and used PBS as a Control. Triglycerides intracellular accumulation was verified by AdipoRed staining; Cell death by Annexin and PI kit; cell proliferation by Ki-67 immune-content; and HSP70 levels by immunocytochemistry in flow cytometer. First, we established an *in vitro* model of foam cells, by exposing macrophages to PM2.5 for 48 h, and adding non-oxidized human LDL (50 µg/mL) at the last 24 h. As expected, the LDL induced an intracellular accumulation of lipids, which was exacerbated by the pollutant. Thus, we investigated if it could be related to an impairment in the apoptosis and proliferation. In fact, PM2.5 promoted macrophages apoptosis, whilst reduced the percentage of cells in proliferative phase. We also wondered if cytotoxicity could be related to an impairment in the HSP signaling. Curiously, the pollutant enhanced HSP70 levels, as an adaptive countermeasure to cytotoxicity. PM2.5-induced cytotoxicity exacerbates foam cells formation in macrophages, regardless of HSP70 levels.
Keywords: apoptosis, fine particulate matter, heat shock proteins
Supported by: CAPES-PROEX

SP.04.04 - Extracellular Vesicles in Colorectal Cancer Cells: The Unconventional Release of LMWPTP and Impact on Human Fibroblast Biology

Stefano Piatto Clerici [1], Sílvio Roberto Consonni[1], Carmen Veríssima Ferreira-Halder[1]
[1]Bioquímica e Biologia Tecidual, Universidade Estadual de Campinas (SP, Brasil)

Colorectal cancer (CRC) is in the top 10 cancers most prevalent worldwide. Low molecular weight protein tyrosine phosphatase (LMWPTP) participates in the metastatic process and resistance acquisition to chemotherapy in different types of tumors. In the context of CRC, LMWPTP is associated with malignant evolution and a less favorable prognosis. Research on tumor-derived extracellular vesicles (EVs) suggests its importance in mediating cell-to-cell communication and thus, potentially affecting cancer progression via multiple pathways. In the present study, we hypothesized that EVs derived from different CRC cell lines (HCT116 and HT29) differ in their ability to reprogram normal human fibroblasts through a process called tumor education. The EVs derived from CRC cell lines were isolated by a combination of ultrafiltration and polymeric precipitation, followed by characterization based on morphology (transmission electron microscopy), size and concentration (nanoparticle tracking analysis), and the presence or absence of EV and non-EV markers (immunoblotting's). The uptake of EVs (PKH26 labeled), migration, and molecular pathways were analyzed in human fibroblasts co-cultured with CRC-derived EVs. HT29 cells displayed a higher concentration of small extracellular vesicles (sEVs). The data suggested an unconventional way of LMWPTP secretion via HT29-derived sEVs in addition to that, the sEVs secreted by CRC cells can educate normal human fibroblasts to activate them in a state that confers migratory capacity.
Keywords: small extracellular vesicles, colorectal cancer, LMWPTP
Supported by: Sao Paulo Research Foundation (FAPESP) under grants 2018/03593-6 (SPC) and 2015/20412-7 (CVF-H).

SP.04.05 - The Rho GTPase pathway as a potential chemosensitizer of TMZ and cisplatin-resistant GBM through a p53-dependent mechanism

Yuli Thamires Magalhães [1], Fábio Luís Forti[1]
[1]Department of Biochemistry, University of São Paulo (São Paulo, Brasil)

Rho GTPases regulate several cellular processes related to tumor progression, and their expression and activity dictate invasiveness and aggressiveness in GBM. Despite the current aggressive therapies, effective treatments remain obscure. Here we proposed that Rho GTPase is acting on the genomic stability of GBM and their resistance to TMZ and cisplatin in a p53-dependent mechanism. Therefore we selected four GBM cell lines with different p53 status: U87-MG – wt-p53 – and T98G, U251-MG, U138-MG – distinct mut-p53 – and subjected them to Rho inhibition by C3 toxin and DNA damage by TMZ and cisplatin exposure. Preliminary bioinformatic analysis showed that prolonged exposure to cisplatin induces the transcription of Rho GTPases regulatory genes in U87-MG cells. Pulldown assays corroborated this analysis, showing an increase in Rho activity after cisplatin and TMZ exposure. IC50 for TMZ and cisplatin, as well as survival and proliferation, was decreased by Rho inhibition only in wt-p53 cells. HCR assay adapted to detect TMZ and cisplatin-induced DNA lesions showed that DNA repair capacity for both genotoxic drugs is diminished by Rho inhibition in wt-p53 cells. To verify the role of p53 in this mechanism, we submitted U87-MG to p53 knockdown, which prevent Rho inhibition-induced chemosensitivity and DNA repair impairment. Next, U87-MG cells were submitted to several regimens of TMZ or cisplatin treatments to obtain subpopulation of cells with different degrees of resistance to these drugs. The consecutive exposition to DNA damage leaded to enhanced p53 phosphorylation, p53 nuclear localization and F-actin induced polymerization and dynamics. Two clones of TMZ-resistant cells were selected, both showing significant increase in TMZ IC50 for survival, whereas the Rho inhibition reduced TMZ IC50 in both clones, significantly reversing the acquired resistance of these cells. These results strongly suggest a role of Rho in DNA repair and acquired resistance to TMZ showing that Rho pathway might be a fragile point against the effectiveness of usual therapies, being this effect clearly dependent on the p53 transcriptional activity.
Keywords: Glioblastoma chemoresistance, Rho GTPase, p53

SP.05 - Systems Biology and Biomarkers for Human Disorders

SP.05.01 - Molecular responses of astrocytes to brain injuries

Lina Maria Delgado Garcia[1], Tais Novaki Ribeiro[1], **Marimélia A. Porcionatto [1]**
[1]Neurobiology Lab, Department of Biochemistry, Escola Paulista de Medicina, Unifesp (São Paulo, SP, Brazil)

Acute injuries to the brain, such as stroke and traumatic brain injury (TBI), disrupt blood flow and compromise the integrity of the neurovascular unit, causing inefficient communication among its cellular components: neurons, astrocytes, and endothelial cells. The neurovascular unit is a multicellular structure whose primary function is to regulate vascular permeability and blood flow, allowing the supply of oxygen and glucose to brain cells. Disruption of the neurovascular unit leads to blood-brain barrier dysfunction, activation of inflammatory response, among other outcomes, including astrocyte reactivation. Using a mouse model of TBI, we described Notch1 activation, followed by upregulation of galectin 3 (Gal3) expression in reactive astrocytes. Galectins are an evolutionarily conserved group of proteins with carbohydrate-binding domains that participate in various biological processes, including proliferation, migration, and differentiation. Using proteomic analysis of Gal3 KO mouse brain tissue of animals submitted to a TBI model compared to control, not injured brain, we identified 915 proteins that were up or downregulated by TBI. Gene ontology analysis revealed the upregulation of proteins related to “glial response,” “vesicular transport,” and “apoptotic signaling,” and downregulation of proteins related to “neuronal morphogenesis” and “response to oxidative stress.” Identifying genes and proteins related to responses to brain injuries may lead to a better understanding of neuroinflammation and neurodegeneration processes, search for prognostic markers for brain injury, and new strategies to diminish brain damage

Keywords: traumatic brain injury, proteins, glial
Supported by: FAPESP, CAPES, CNPq

SP.05.02 - Predisposition and potential indicators of alcohol use disorders

Ana Carolina Luchiari [1]
[1]Laboratório de Peixes Ornamentais, Universidade Federal do Rio Grande do Norte (Natal, RN, Brazil)

Abusive consumption of alcohol and alcoholism represent major problems with enormous social and economic costs throughout the world. According to the World Health Organization (2018), there has been a 43.5% increase in alcohol consumption in Brazil in the last ten years. Despite alcohol abuse being a prerequisite for alcoholism, the susceptibility to developing this pathology differs between individuals. Alcohol acts on several systems, but the predominant deleterious action is seen on the Central Nervous System, where there are changes in neurotransmitter pathways that, in the long term, lead to drug dependence and associated pathologies. Although the literature on this subject is still limited, the characterization of an individual's behavioral profile, added to the genetic, physiological, and behavioral analysis in response to alcohol exposure suggests answers for a deeper understanding of the pathology and its consequences. Furthermore, the effects of alcohol at the early stages of development and its transgenerational effects are poorly understood. In this lecture, I will address the history of alcohol use by civilization, the development of research in search of answers and treatment alternatives, as well as the use of animal models that allow us to go beyond the behavioral study to characterize, diagnose and propose solutions for the alcohol abuse, as well as the future of research on this topic.

Keywords: alcoholism, alcohol

SP.05.03 - Metabolic factors associated with cognitive impairment in Alzheimer's disease

Mychael Vinícius da Costa Lourenço [1]
[1]Universidade Federal do Rio de Janeiro, UFRJ (RJ, Brazil)

Metabolic signals have emerged as key players regulating molecular pathways, neural circuits and cognition. Therefore, understanding metabolic defects may result in novel approaches to treat cognitive dysfunction and Alzheimer's disease (AD)-linked dementia. I will discuss experimental findings supporting that reductions in metabolic modulators, such as irisin or lipoxin, impairs memory in rodents. Furthermore, I will present evidence that changes in these metabolic mediators associate with impaired memory in humans affected by dementia. Overall, our results demonstrate the importance of adequate metabolic signaling to preserve cognition in rodents and humans and support further investigation on these mediators as potential biomarkers for cognitive impairment.
Keywords: Alzheimer's disease, Metabolic signals

SP.05.04 - Imaging astrocyte metabolism

Eduardo Rigon Zimmer [1]
[1]Department of Pharmacology, UFRGS (Porto Alegre, RS, Brazil)

Brain glucose utilization has been widely assessed in clinical settings with fluorine-18 fluoro- 2-deoxyglucose (FDG) positron emission tomography (PET) imaging. FDG-PET allows for differential diagnosis in brain disorders, such as Alzheimer's disease (AD). Its biological interpretation assumes that the FDG-PET signal is tightly connected to neuronal activity. However, the dynamics of glucose utilization by brain cells remain not fully understood. A complex interplay between neurons and astrocytes seems to coordinate brain energetics. Indeed, astrocytes take up large amounts of glucose and, thus, it is intuitive that they impact the FDG-PET signal. We recently demonstrated that glutamate transport via GLT-1, which is mainly located on astrocytes, acts as a trigger, signaling for FDG uptake in vivo. Our findings provide in vivo evidence that FDG-PET hypometabolism indicates more than just neuronal activity, with significant implications in its biological interpretation. In AD, for example, FDG-PET hypometabolism is considered a biomarker of neurodegeneration. However, astrocyte dysfunction may play a substantial role in FDG-PET signal changes in pathological scenarios.
Keywords: metabolism, neurodegeneration, FDG-PET

SP.06 - Carbohydrates: from isolation, to synthesis, dynamics and function

SP.06.01 - Synthesis of carbohydrates and glycoconjugates with biological applications

Vanessa Leiria Campo [1]
[1]Ciências Farmacêuticas, Centro Universitário Barão de Mauá de Ribeirão Preto (SP, Brasil)

This presentation will focus on the synthesis of carbohydrates and glycoconjugates as tools for development of new therapeutic and diagnostic strategies towards Chagas disease, cancer and dystroglycanopathies. Firstly, it will be presented general aspects of carbohydrate chemistry, involving protection/ deprotection reactions, anomeric functionalization and glycosylation reactions to get glycosyl-amino acids as building blocks for the synthesis of glycopeptides, followed by the solid phase and enzymatic reactions to get mucin-derived glycopeptides related to T. cruzi, cancer and α-dystroglycan. The use of CuI-catalyzed azide-alkyne 1,3-dipolar cycloaddition (CuAAC) reactions to get triazole-derived carbohydrates and glycoconjugates will also be shown. Relevant biological evaluations of the synthesized carbohydrates and glycoconjugates will be exhibited along the presentation, highlighting their applications as T. cruzi tran-salidase enzyme (TcTS) and galectin-3 inhibitors, as vaccines against Chagas disease and cancer, and as models to get monoclonal antibodies (mAbs) towards tumor cells and dystrophic muscle cells. Lastly, the startup GLYCOVAM, which is focused on the research and development of glycoconjugates applicable to the therapy and diagnosis of pathologies that affect animals and humans, will be presented.

Keywords: Carbohydrates, Synthesis, Biological activities
Supported by: FAPESP

SP.06.02 - Natural Products from Brazilian Biodiversity, Small or Large Always Remarkable

Vanderlan da Silva Bolzani [1]
[1]Dep. de Química Orgânica, Instituto de Química, Universidade Estadual Paulista (UNESP) (Araraquara, SP, Brazil)

The plant chemical diversity is fantastic, and natural product molecular structures is reflected in a large variety of biochemical reaction pathways, which is responsible for several classes of biologically active secondary metabolites. This metabolic complexity is fundamental for the communication, regulation and defense of the species, in the most diverse ecosystems and, particularly in extreme habitats, due to the fantastic chemical diversity of small and large molecules biosynthesized by plants, and other organisms of the terrestrial biodiversity. In Brazil we are facing several biomes and the Cerrado - an extremely environment, the second largest after the Amazon, due to its own characteristics, unique in the world, has a special vegetation for looking for natural products, which still chemically and pharmacologically understudied, as for example, peptides and polysaccharides. These macromolecules are accumulated in several plant species of this extremely environment and only a very few studies have been carried out with these plants, being therefore, a rich natural laboratory to be chemically and pharmacologically explored. It is also known that the metabolomic study of all primary and secondary metabolites (high and low molecular weight) is being indispensable for mapping all metabolites produced by a species, essential to understanding its survival in terrestrial and aquatic environments. Also, plant small or large metabolites are important supplies for the production of drugs, foods, cosmetics, fragrances, colorants, and agrochemicals, which support a vigorous bioeconomy in several countries.

Keywords: Natural Products, Brazilian Biodiversity, New Trends, Bioproducts
Supported by: CEPID-FAPESP, CNPq-INCT BioNat

SP.06.03 - Are accurate models for carbohydrates structure possible?

Hugo Verli [1]
[1]Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul (Porto Alegre, RS, Brazil)

While being the most abundant biomolecules in nature, carbohydrates possess unique features, including branching, diversity of linkages between monomers, high number of monomeric units, as well as configurational (like D or L residues and the anomeric effect) and conformational (like the pseudo rotational equilibrium) features. These molecules are also highly flexible and polar which, in turn, impose severe challenges for experimental characterization of their 3D structures. In a recent survey from the Protein Data Bank (PDB), we demonstrated that the experimental carbohydrate information known to date is highly limited in its amount, quality and diversity, bringing to computational techniques a major part of the responsibility to elucidate carbohydrates 3D structure and dynamics. In this context, our group has been dedicated to the development and application of methods and proceedings able to aid in the establishment of relations between structure / conformation with biological /pharmacological / pathological phenomena in nature, for both carbohydrates and glycoconjugates. We developed an accurate set of force field parameters for carbohydrates, glycoconjugates and glycosilated natural products, validated agains multiple sources of structural information and applied to diverse carbohydrate derivatives, including polysaccharides, glycolipids, semi-sinthetic compounds, chalcones, flavonoids, alkaloids and glycopeptides, among others, showing a wide range of biological activities. In association with tools developed in house to aid in the calibration to torsional potentials, obtain theoretical vicinal coupling constants for comparison to NMR data and analysis of solution ensembles of conformations, these works have been able to contribute in the understanding of the structural basis for carbohydrates biological functions, as well as aid other research groups with simple to use and free tools searching to build accurate structural models for carbohydrates and glycoconjugates.

Keywords: carbohydrates, Protein Data Bank, NMR

SP.06.04 - The function of heparan sulfate in the breast cancer

Maria Aparecida Da Silva Pinhal [1]
[1]Biochemistry Department, Universidade Federal de São Paulo (Unifesp) (SP, Brazil)

Heparan sulfates (HS) is a linear heteropolysaccharide ubiquitous in all species that display tissue organization. Heparan sulfate chains are attached to a core protein, forming proteoglycans involved in different biological functions such as cellular proliferation, migration, cell-matrix assembly, angiogenesis, inflammation and differentiation, and control exosome biogenesis. Heparan sulfate can be degraded by an endo-β- glucuronidase that cleaves β-D-glucuronyl(1→4)D-N-acetylated glucosamine which is overexpressed in cancer. Our group aims to evaluate the biological function of transmembrane heparan sulfate proteoglycans (syndecans) and heparanase in breast cancer. The results showed that besides HER2 levels, heparan sulfate shedding determines breast cancer cell susceptibility to the monoclonal antibody trastuzumab. Previous studies have demonstrated that serum or plasma from breast cancer patients increased expression of heparanase in circulating lymphocytes, and HS secreted by tumor cells carried by exosome seems critical to upregulate the expression of heparanase, suggesting a possible mechanism of crosstalk between tumor cells and circulating lymphocytes. We selected an HS-binding peptide using a phage display system. Circular Dichroism showed that the HS-binding peptide showed a higher affinity for heparin N- sulfated. HS-binding peptide inhibited the proliferation of endothelial cells by modulation of FGF-2 and decreased the tumor size using PDX (patient-derived tumor xenograft) cells from a patient with a triple-negative breast tumor. It was also verified that the silencing of syndecan-4 using shRNA in MDA-MB-231 cells promoted a decrease in response to paclitaxel which corroborates with in silico studies. The results confirm that heparan sulfate and syndecans represent key molecules in breast cancer development, mediating trastuzumab and paclitaxel response, being involved with angiogenesis, tumor formation, and tumor microenvironmental communication.

Keywords: Glycosaminoglycans, Proteoglycans, Neoplasms, Chemotherapy, Trastuzumab

SP.07 - AUGM/Lafebs: COVID: from a Biophysical/Biochemical Perspective

SP.07.01 - Multiparatopic binders for SARS-Cov2 SPIKE: Best inhibitor ever, ever.

Sergio Pantano [1]
[1]Biomolecular Simulations Group, Institut Pasteur de Montevideo (, URUGUAY)

High-affinity binders for the Spike protein of SARS-CoV-2 may have a diversity of uses from diagnostic devices to therapeutic strategies. However, single-domain binders may be extremely sensitive to spontaneously arising viral mutations. We have designed a self-associating thermostable polypeptide with the capacity of incorporating arbitrary binders fused at the N and C termini of a central multimerization domain. We generated different constructions incorporating designed mini proteins and nanobodies reported in the literature with the capacity to neutralize SARS-Cov2 entry. The multiparatopic oligomer maximizes avidity, inhibiting the binding of wt and mutated RBD to ACE2 at picomolar concentrations in-vitro and low nanomolar in intact viruses. The modular design allows to exchange the binding modules without compromising the multimeric structure. Therefore, it is possible to update the design by incorporating new miniproteins or nanobodies able to tackle continuously emerging escape variants.
Keywords: Protein scaffold, rational design, adaptable binder
Supported by: Institut Pasteur

SP.07.02 - A Crystallographic Snapshot of SARS-CoV-2 Main Protease Maturation Process

Gabriela Dias Noske[1], Aline Minali Nakamura[1], Victor Oliveira Gawriljuk[1], Rafaela Sachetto Fernandes[1], Gustavo M.A. Lima[2], Higor Vinicius Dias Rosa[1], Humberto D Pereira[1], Andrey F.Z. Nascimento[3], Marjorie Caroline Liberato Cavalcanti Freire[1], **Glaucius Oliva** [1], Andre Schutzer de Godoy[1]
[1]Institute of Physics of Sao Carlos, University of Sao Paulo (SP, Brazil), [2]BioMAX, MAX IV Laboratory (Lund, Sweden), [3]Brazilian Synchrotron Light Laboratory, (LNLS) (Campinas, SP, Brazil)

SARS-CoV-2 is the causative agent of COVID-19. The dimeric form of the viral Mpro is responsible for the cleavage of the viral polyprotein in 11 sites, including its own N and C- terminus. The lack of structural information for intermediary forms of Mpro is a setback for the understanding of this process. Herein, we used X-ray crystallography to characterize an immature form of the main protease, which revealed major conformational changes in the positioning of domain-three over the active site, hampering the dimerization and diminishing its activity. We propose that this form preludes the cis-cleavage of N-terminal residues. Using fragment screening, we probe new cavities in this form which can be used to guide therapeutic development. Furthermore, we characterized a serine site-directed mutant of the Mpro bound to its endogenous N and C-terminal residues during the formation of the tetramer. We suggest this form is a transitional state during the C-terminal trans-cleavage. This data sheds light in the structural modifications of the SARS-CoV-2 main protease during maturation, which can guide the development of new inhibitors.
Keywords: SARS-CoV-2, COVID-19

SP.07.03 - Current and Upcoming Challenges in Infectious Disease Modeling: Lessons Learned After Two Ongoing Years of Molecular and Mathematical Modeling of SARS-Cov-2 and COVID-19

Alvaro Olivera-Nappa [1]
[1]Dpt. of Chemical Engineering, Biotechnology and Materials, Centre for Biotechnology and Bioengineering, University of Chile (Metropolitan Area, Chile)

The last two-and-a-half years will remain branded with hot iron in our lives as the years of the COVID-19 pandemics, but also as a time when science advanced at a rhythm seldom witnessed before to tackle the unexpected consequences of SARS-CoV-2-related pathologies and death. We will present our research group's continued efforts to offer our experience and resources for a better understanding and management of the COVID-19 pandemics in Chile and the world, applying mathematical modeling to study the particularities of the viral spread. We first developed a mathematical way to use raw active-patient and death-toll official figures to readily compute the R_t spread index. Since crude official data could not be used to predict contagion, we modeled contagion delays, hidden and underreported cases, and asymptomatic disease to correctly predict impending waves by correlating model parameters with public-health interventions. Considering people's travels among neighboring Chilean regions, we demonstrated lack of real-time spatial information was the actual limit of predictive efforts. We also collaborated in describing a new successful SARS-CoV-2 variant from Punta Arenas, with one of the firstly described Spike-protein mutations. Molecular modeling collaboration with the University of Magallanes and the Pasteur Institute at Montevideo traced the increased infectivity back to a new mutation in the Spike protein, using molecular dynamics. With the MPI (Germany), we modeled the expected influence of the recently developed vaccines on the viral spread, and how vaccine allocation allowed a way out from non-pharmaceutical restrictions. Finally, we used Chilean genomic surveillance data to determine the spreading rate of variants, predict new waves, and find responsible causative agents of past waves. The future will undoubtedly bring developments with COVID-19, but also new infectious pandemics. We expect our efforts during the last two years will help confront them with powerful new insights to decrease their associated risk.
Keywords: COVID-19, Mathematical modeling, Epidemiological management
Supported by: Basal Project FB0001, ANID, Chile

SP.07.04 - The moving frontiers in SARS-CoV-2 structural biology

Francisco J. Barrantes [1]
[1]Lab. Molecular Neurobiology, BIOMED UCA-CONICET (C1107AAZ Buenos Aires, Argentina E-mail: francisco_barrantes@uca.edu.ar)

The COVID-19 pandemic at the end of 2019 met the health systems ill prepared to face a challenge of such magnitude[1,2]. However, some branches of science were not only ready but in excellent shape to tackle some basic aspects of the challenge. The basic sciences and in particular biophysics and structural molecular biology had an early incursion in the pandemic, solving the structure of the SARS-CoV-2 virus and individual protein molecules thereof in an unprecedentedly fast manner, thus providing the health system and the biotechnology sector with invaluable information on possible prophylactic or therapeutic targets to combat the viral disease [1, 3-4]. Cryo-electron microscopy (cryo-EM) was the main tool employed in this endeavor [5,6]. It must be stressed that such unique success could only be accomplished in such a short time because of the accumulated data on related Coronaviridae structures stemming mainly from X-ray diffraction studies in the preceding two decades [1,5]. New challenges in the form of variants of the original Wuhan strain have since appeared and structural biology has, once again, promptly responded. The talk will summarize the new advances and the state of the art in this frontier topic in Biomedicine. [1] Barrantes, F.J. While we wait for a vaccine: Why not think about available drugs
Keywords: SARS-CoV-2, COVID-19, Coronaviridae

SP.08 - Recent Advances in Molecular Entomology

SP.08.01 - Different strategies used by trypanosomes to develop in triatomines

Alessandra Aparecida Guarneri [1]
[1]Vector Behavior and Pathogen Interaction Group, Instituto René Rachou, Fiocruz (Belo Horizonte, MG, Brazil)

Triatomines are hematophagous insets that can be infected by Trypanosoma cruzi, the etiological agent of Chagas disease and by Trypanosoma rangeli, a protozoan that can infect humans but does not cause disease. As in mammals, the development of these parasites on their invertebrate hosts is also quite different. While T. cruzi develops exclusively in the intestinal tract of the insect and its infective forms are released in the feces, T. rangeli invades the hemocoel and salivary glands, where infective forms are produced and then, inoculated into the skin of the host during insect feeding. The development of these parasites promotes a series of physiological and behavioral alterations in the vector that will be discussed in view of parasite survival and transmission.
Keywords: Triatomines, Trypanosoma cruzi, Chagas Disease

SP.08.02 - The molecular basis of worker sterility in social bees – a glimpse into the evolution of altruism in social insects

Klaus Hartmann Hartfelder [1]
[1]Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo (SP, Brazil)

In the Origin of Species, Charles Darwin considered the sterility of the worker caste of social insects as a major challenge to his theory of evolution, because how and why could sterility be a trait that is favored by natural selection? Kin selection theory proposed by W.D. Hamilton in the mid 60s finally provided a theoretically consistent framework for the evolution of altruistic traits, such as the origin of a partially or fully sterile worker caste in social insects. But what are the molecular underpinnings of worker sterility? We address this question focusing on the honey bee as a genomic model organism. While an adult honey bee queen has very large ovaries, each consisting of over 15 ovariolar filaments, allowing her to lay over 1000 eggs per day, the ovaries of adult workers consist of only 2-20 ovarioles, and in the presence of the queen, these ovarioles are not activated. This major morphological and functional difference in the ovary phenotypes is due to massive programmed cell death in the ovaries of worker larvae. Over the last decade we could identify a long noncoding RNA, lncov1, and its interaction partner, Tudor-SN, as forming a regulatory module in the caste-specific development of the larval ovary, acting as a balance between cell death and survival. Besides its regulatory function in the larval ovary, we could then show that the lncov1/Tudor-SN module also plays a role in the activation of the ovary of adult honey bee workers, in response to the removal of the queen or the feeding a protein-rich diet. The evolutionary analysis of lncov1 then showed that this lncRNA is surprisingly conserved in the genomes of the social bees.
Keywords: bees, insects, lncRNA
Supported by: FAPESP 17/09128-0; CNPq 304139/2018-1

SP.08.03 - The neglected virome of assassin bugs

Attilio Pane [1]
[1]Institute of Biomedical Sciences, Federal University of Rio de Janeiro (Rio de Janeiro, Brasil)

Triatomine insects comprise hematophagous species, which have been firmly connected to the transmission of Trypanosoma cruzi, the causative agent of Chagas disease. Despite the medical relevance, the virome of these insects is still largely unexplored. We have recently described seven (+) single-strand RNA viruses (RpV1-7) infecting Rhodnius prolixus. We showed that the RpVs belong to the Iflaviridae, Permutotetraviridae and Solemoviridae and are vertically transmitted from the mothers to the progeny via transovarial transmission. Rhodnius appears to mount a RNAi-based antiviral response since we could detect 22-nucleotide viral siRNAs (vsiRNAs), but not viral piRNAs, in the insect ovary. The vsiRNAs are maternally deposited in the eggs, where they likely contribute to reducing the viral load and protecting the developing embryos. Interestingly, the RpVs display a broader range of hosts compared to the only other known triatomine virus, namely Triatoma virus, and are capable of infecting both mosquito cell lines as well as T. cruzi. Our results have begun to shed light on the complexity and host range restriction of the triatomine virome as well as its interaction with the insect hosts and the Chagas disease agent T. cruzi.

Keywords: Triatomine insects, Rhodnius prolixus, Chagas disease

SP.08.04 - Bad mosquito, good saliva: Aedes aegypti salivary molecules as a source of immunobiologics

Anderson de Sá Nunes [1]
[1]Department of Immunology, Institute of Biomedical Sciences, University of Sao Paulo (SP, Brazil)

Mosquito saliva is a source of bioactive molecules with anti-hemostatic, anti-inflammatory and immunomodulatory properties. The anti-hemostatic activities are well-characterized and include anticoagulant, antiplatelet and vasodilatory effects. Regarding the anti-inflammatory and immunomodulatory roles, in vitro studies have shown that mosquito salivary preparations are able to modulate the effector responses of immune cells such as mast cells, dendritic cells, macrophages and lymphocytes. In vivo models revealed that the salivary secretion deposited in the host tissue during mosquito blood feeding creates a permissive environment that is appropriate for infectivity, replication and dissemination of a number of pathogens transmitted by the mosquitoes. In fact, the ability of saliva to favor transmission of pathogens, a phenomenon originally termed saliva-assisted transmission (SAT), has an enormous impact on public health, affecting millions of people worldwide. Such effects are so noteworthy that important implications for mosquito-vertebrate host interactions are envisioned. Owing to their mechanism of action on the vertebrate immune system, an emerging and exciting area to be explored is the use of mosquito salivary bioactive molecules for the prevention/treatment of clinical conditions, such as disorders in hemostasis and the immune system. In the last few years, our group has been exploring the potential use of Aedes aegypti saliva and salivary components to treat and or prevent inflammatory/autoimmune diseases in mice by using experimental models of diseases such as multiples sclerosis, hepatitis and inflammatory bowel diseases, among others. Thus, despite the harm and annoyance caused by mosquitoes, we have been finding an interesting beneficial aspect of their salivary components, as a source of immunobiologics.

Keywords: Aedes aegypti, saliva, immunobiologics

SP.09 - Single-Cell Profiling: A New Frontier in Biology

SP.09.01 - High-Resolution Profile of Macrophage Subpopulations in the Tumor Microenvironment

Giovanna Resk Marklouf[1], Gabriela Rapozo Guimarães[1], Cristiane Esteves Teixeira[1], Marco Antônio Marques Pretti[1], Leandro de Oliveira Santos[1], Nayara Evelin Toledo[1], Nayara Tessarolo[1], João Ortigão Farias[1], Pedro Manoel Mendes de Moraes Vieiras[3], Marcelo Alves da Silva Mori[2], **Mariana Lima Boroni Martins** [1]

[1]Bioinformatics and Computational Biology Lab, Instituto Nacional de Cancer (RJ, Brasil), [2]Laboratório de Biologia do Envelhecimento (LaBE), Universidade de Campinas (SP, Brasil), [3]Departamento de Genética, Evolução, Microbiologia e Imunologia, Universidade de Campinas (SP, Brasil)

The tumor microenvironment (TME) comprises a set of normal cells, including stromal fibroblasts, endothelial cells, and immune cells, in addition to the extracellular matrix and malignant cells themselves. Myeloid-derived cells are one of the major components in TME, mainly represented by macrophages (Mø), which are highly plastic cells. Depending on their phenotype, they may be associated with an anti or pro-tumoral profile. The balance of Mø subpopulations in the TME can be decisive for patients' clinical outcomes in different cancer types. By using single-cell RNA-Seq (scRNA-seq), we aimed to characterize myeloid-derived cells subpopulations in different TME types and evaluate their clinical impact. scRNA-seq and clinical data from 142 subjects - healthy donors and patients with seven distinct cancer types - were collected from 13 public datasets. Data were subjected to a strict quality control pipeline, integrated using scVI, and clustered with the Leiden algorithm. Cell types were identified using canonical markers. Bulk RNA-seq data were deconvoluted to estimate the presence of these cells and correlated with clinical parameters. We obtained 408,276 high-quality integrated cells, including epithelial, malignant, immune, and stromal components. Regarding mononuclear phagocytes, we identified 6,212 cDCs, 10,321 monocytes, and 38,517 Møs, corresponding to 12, 10, and 13 states, respectively. Tumor-recruited and tissue-resident Mø subpopulations were deeply characterized. Three Mø subpopulations displayed remodeling phenotype with immunosuppressor activity and were associated with unfavorable outcomes in breast cancer while one subpopulation was expressing several genes from the antigen presentation pathway and was related to a favorable outcome. Integration of scRNA-seq data allowed us to transcriptionally characterize immune and non-immune cells in normal and tumor tissue. Regarding Mø, we have deeply characterized 13 subpopulations, including the clinical impact they can have on cancer patients, constituting an important target for future research in precision medicine.

Keywords: macrophages, scRNA-SEQ, tumo microenviroment
Supported by: FAPERJ

SP.09.02 - Spatial single cell transcriptomics of the brain

Helder Takashi Imoto Nakaya [1]
[1]Hospital Israelita Albert Einstein, (São Paulo, Brazil)

We will show in this seminar how spatially resolved single cell RNA sequencing data can provide important insights into brain functioning.

Keywords: Brain, cell, RNA

SP.09.03 - Single cell transcriptomics and chromatin analysis illuminating gut microbiota-host interactions

Vinicius Dias Nirello[1], Dieggo Rodrigues de Paula[1], Nathália Vitória Pereira Araújo[1], Mariane Font Fernandes[2], Marco Aurélio Ramirez Vinolo[2], **Patrick Daniel Varga-Weisz*[1,3,4]**

[1]International Laboratory for Microbiome Host Epigenetics, Department of Genetics, Evolution, Microbiology, and Immunology, Institute of Biology, University of Campinas (Campinas, SP, Brazil), [2]Laboratory of Immunoinflammation, Department of Genetics, Evolution, Microbiology, and Immunology, Institute of Biology, University of Campinas (Campinas, SP, Brazil), [3]São Paulo Chair of Excellence, International Laboratory for Microbiome Host Epigenetics, Department of Genetics, Evolution, Microbiology, and Immunology, Institute of Biology, University of Campinas (Campinas, SP, Brazil), [4]School of Life Sciences, University of Essex (Colchester, UK)

The gut microbiota can be considered a quasi-organ that contributes to the host metabolism in a profound way. A major contribution in this regard are short chain fatty acids (SCFAs), such as acetic, propionic and butyric acid, which are generated by the gut microbiota in the process of fermentation of complex carbohydrates [1]. These SCFAs affect metabolism of the intestinal epithelial cells but are also released into the blood stream, feeding distant organs. They have also signalling functions through activation of specific G protein coupled receptors and by modulating histone modifications, mainly histone acylations such as acetylation [2]. Recently, we have discovered alternative histone acylations in the gut epithelium, mainly crotonylation and found that these depend on the gut microbiota and are linked to SCFAs metabolism [3]. We find a significant correlation between changes in histone crotonylation over regulatory elements of genes linked to changes in microbiota-dependent gene expression in the colon epithelium. Therefore, histone crotonylation appears to be a mechanism of microbiota-host crosstalk. To illuminate these processes further we employ single cell transcriptomics, which can reveal cell type-specific changes in gene expression and identify co-regulated genes within a cell. We couple this with chromatin analysis such as ATAC-seq [4] and CUT&TAG [5] to illuminate how changes in gene expression are coupled to changes in histone modifications and chromatin accessibility. We combine these analyses with measurements of cellular acyl-CoA levels to canvas how histone modification and gene expression are tight together with cellular metabolic states. **Keywords:** Chromatin remodeling, histone modifications, epigenetics

SP.09.04 - Deciphering thermogenic adipose niche: cellular heterogeneity and communication

Yu-Hua Tseng [1]

[1]Joslin Diabetes Center and , Harvard Medical School (Boston, MA, USA)

The adipose tissue plays a crucial role in regulating energy homeostasis and is, therefore, a major contributor to the pathophysiology of obesity and metabolic diseases. Brown adipose tissue (BAT) and related beige fat specialize in thermogenic energy expenditure and are attractive targets for anti-obesity therapies. Thermogenic adipose tissue can rapidly respond to environmental challenges by changing cellular compositions and cell-cell communications. Such adaptation is essential to maintaining metabolic health. To dissect the complex cellular makeup of thermogenic fat, we performed single-cell RNA-sequencing analyses of BAT from mice housed at different temperatures. The results revealed a high degree of heterogeneity of thermogenic adipose niche and showed cold exposure-induced dynamic changes of cellular composition in BAT. Importantly, we identified a novel population of adipose progenitor cells (APCs), which was derived from the vascular smooth muscle (VSM) lineage and uniquely expressed Trpv1 (transient receptor potential cation channel subfamily V member 1). Lineage tracing studies revealed that the Trpv1pos VSM-APCs are a distinct population of adipocyte progenitors that contribute to brown and beige adipocyte pools in vivo. Cold exposure promotes the proliferation and differentiation of Trpv1pos progenitors into highly thermogenic brown adipocytes, providing an adaptive mechanism to enhance thermogenesis. In this talk, I will present our recent endeavors in understanding the functions and interactions among different cell types within the thermogenic adipose microenvironment.
Keywords: Diabetes, Brown adipose tissue, metabolic diseases

SP.10 - From Molecular Modeling to Drug Design

SP.10.01 - Normal Modes based new simulation techniques opens far-reaching possibilities in structural biology

David Perahia [1]
[1]Laboratoire de Biologie et Pharmacologie Appliquée (LBPA), Ecole Normale Supérieure Paris-Saclay (4 avenue des sciences, 91190, Gif-sur-Yvette, France)

In this presentation, I will provide an overview of techniques that have been developed based on vibrational normal modes (NM) of biological macromolecules that are now well established for their description of large-scale conformational motions. I will show how techniques combining NMs with molecular dynamics (MD) simulations allow going beyond the possibilities of one or the other. The constant methodological developments of such hybrid methods open up new areas of investigation and make it possible to reach structures of macromolecules or complexes appearing on the time scale beyond ms, to study their dynamical behavior, to search for allosteric and drug binding sites, to model large structures by cryo-electron microscopy at atomic-level with improved precision, etc. I will also give many illustrative examples.
Keywords: molecular dynamics, Normal Modes, structural biology

SP.10.02 - Drug design and repurposing with DockThor VS web server focusing on SARS CoV 2 therapeutic targets and their non synonym variants

Laurent Emmanuel Dardenne [1]
[1]Laboratório Nacional de Computação Cientifica, Coordenação De Modelagem Computacional (Petrópolis, RJ, Brazil)

Structure-based drug design (SBDD) methodologies are essential tools for the investigation of protein−ligand interactions, playing a crucial role in the rational development of new drugs. Molecular docking is one of the most used SBDD approaches and has been improved continuously through the development of more sophisticated and accurate strategies. The DockThor program is a protein−ligand docking method developed at the Brazilian National Laboratory for Scientific Computing freely available to the academic community via the DockThor-VS web server. In this talk, we will present the main methodological aspects and computational characteristics of the DockThor-VS virtual screening platform. We also present the recent developments implemented to permit high-throughput virtual screening drug repurposing experiments using curated libraries of currently available drugs on the market and curated structures of potential therapeutic targets from SARS-CoV-2. DockThor-VS is coupled to the Brazilian supercomputer Santos Dumont and available at https ://www.dockthor.lncc.br.
Keywords: Drug design, SARS CoV 2 , DockThor VS

SP.10.03 - Engineering synthetic proteins for diagnostics and therapy of viral diseases

Roberto Dias Lins Neto [1]
[1]Instituto Aggeu Magalhães, Fundação Oswaldo Cruz (Recife, PE, Brazil)

Protein engineering is a process to develop or tune synthetic proteins towards a desired molecular-level function. Using a combination of de novo design, molecular dynamics and machine learning algorithms, we have devised a protocol to design immunoreactive proteins inspired by their specific antigen-antibody interactions. The talk will showcase the in silico development and experimental validation of antigens for disease prognosis of Zika severity from serum samples of 4+ weeks pregnant women, as well as antibody mimetics capable of neutralizing Zika and SARS-CoV-2 infections in vitro.
Keywords: Protein engineering , Zika, SARS-CoV-2
Supported by: INOVA initiative from the Oswaldo Cruz Foundation, CNPq, FACEPE, LNCC, CuraZika Foundation, Instruct and CYTED/MICROBES.

SP.10.04 - Integration of computational tools in the prospection of therapeutic targets and agents

Pedro Henrique Monteiro Torres [1]
[1]Laboratório de Modelagem e Dinâmica Molecular, Instituto de Biofísica Carlos Chagas Filho Universidade Federal do Rio de Janeiro (RJ, Brazil)

Recent epidemic and pandemic events indicate that the current response to emerging infectious diseases is inadequate. On the other hand, advances in computational power and technologies for the simulation of biological systems began to allow large-scale predictive approaches to assist the development of new therapeutic compounds. For this purpose, we propose here the development, integration and application of computational tools, including a new paradigm for the early discovery of new drugs that will involve the continuous prospecting of targets and compounds with therapeutic potential. The prospection algorithm will rely on tools being developed by our group and will include proteome-scale structural modeling (ProtCHOIR), druggability analysis, AI-refined molecular docking (OCDocker), and metabolic network analysis.
Keywords: ProtCHOIR, OCDocker, computational tools

SP.11 - AUGM/Lafebs: Novel approaches in Biophysics in South America: what young scientists are working on

SP.11.01 - Showing the way: RadA helicase mechanism during natural transformation in Streptococcus pneumoniae shown by CryoEM

Leonardo Talachia Rosa [1,2], Emeline Vernhes[3], Patrice Pollard[3], Remi Fronzes[1]
[1]Institut Européen de Chimie et Biologie, (Pessac, France), [2]Dep de Bioquímica, Instituto de Química, Universidade de São Paulo (São Paulo, Brazil), [3]Laboratoire de Microbiologie et Génétique Moléculaires, (Toulouse, France)

Natural transformation is the process where bacteria uptake DNA from the environment and incorporate in their genome through homologous recombination. In Streptococcus pneumoniae, RadA is the helicase responsible for extending the homology region in the Displacement loop during the recombination event. RadA is composed of an N-terminal zinc finger, a central DnaB-like ATPase containing a conserved KNRFG motif, and a C-terminal Lon-protease-like domain. We here report the 3.15 Å cryoEM structure of RadA bound to DNA and ATP-y-S, using Single Particle Analysis (SPA-CryoEM). While the Lon hexamer remained in a planar axis, the ATPase domains were orchestrated in a helical rising conformation, shown to be crucial to promote cooperative binding between DNA and ATP: Each subunit contacts DNA through a flexible loop, 2 nucleotides above the adjacent subunit, following the DNA-B helix. On the ATP binding site, the helical rise is needed for correct positioning of the arginine finger and lysine piston on the gamma-phosphate, leading to proper positioning of the adjacent DNA binding loop. Site-directed mutations on the ATPase domains showed decreased affinity for DNA. Our results present RadA as a modular helicase, where the Lon-like domain functions as a loading and hexamerisation platform, while the flexible ATPase domain perform classical DnaB-like helicase activity.
Keywords: CryoEM, helicase, natural transformation
Supported by: Centre National de la Recherche Scientifique (CNRS)

SP.11.02 - New tools for the structural characterization of biomembrane systems

Rafael Gustavo Oliveira [1]
[1]Centro de Investigaciones en Química Biológica de Córdoba CIQUIBIC (CONICET), Departamento de Química Biológica Ranwel Caputto, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba (X5000HUA, Ciudad Universitaria, Córdoba, Argentina)

New emerging techniques will be allowing the experimental determination of structural parameters in membrane systems at the South Cone over the next years. First, the Laboratorio Argentino de Haces de Neutrones (LAHN) is planning for the second round of instrumentation a Neutron Reflectometer and two Small Angle Neutron Scattering instruments. I give an example (measured by our group at the Institute Laue Langevin, Grenoble) of the kind of experiments that will be performed at LAHN with lipid multilayers in humidity-temperature chambers. With this setup also mechanical parameters (like bending rigidity and compressibility) are obtained by analyzing the off-specular scattering. The neutron reflectometer also will allow the positioning of horizontal (liquid samples), then Langmuir troughs are planned for this instrument. SANS will allow for the same kind of measurements in isotropic systems, just like SAXS, but with contrast matching in scattering length density (analogous to electron density in X-ray experiments. Altogether, these efforts will cover different systems from monolayer to bi- and multi-layers, in a set of variable environmental conditions, like temperature, osmotic pressure, lateral pressure, chemical and isotopical composition, etc). Additionally, in our lab we have developed new measurements of quantitative reflectivity on Langmuir monolayers in a Brewster angle microscope (BAM). By contrast matching (as in neutron scattering) we experimentally determine the refractive index of the film, which combined with reflectivity allow us to calculate the thickness of the films. In order to evaluate what kind of thickness is actually measured, the results from BAM were compared to the ones determined by GIXOS (Grazing Incidence X-ray Off Specular Scattering). The last is a setup that allows the determination of the perpendicular structure of the monolayer at the Laboratorio Nacional de Luz Sincrotron (Campinas). The comparison of GIXOS and BAM thicknesses, permits us to conclude that the BAM setup measures the global thickness of the lipid monolayer. **Keywords:** biomembrane , GIXOS , BAM
Supported by: CONICET, SECyT (UNC) and FonCyT (Argentine) and FAPESP (Brazil) by grants. To the Laboratorio Nacional de Luz Síncrotron (Brasil), Hasylab (Germany), ESRF and ILL (Grenoble) by beamtimes.

SP.11.03 - Structural Basis of Regulatory Protein-RNA Interactions

Anderson de Sá Pinheiro [1]
[1]Departamento de Bioquímica, Instituto de Química, Universidade Federal do Rio de Janeiro (RJ, Brasil)

RNA-binding proteins (RBPs) play a central role in virtually all stages of post-transcriptional regulation of gene expression, ensuring homeostasis and determining cell phenotype. RBPs controll numerous processes related to RNA biology, including stability, transport/localization, degradation, quality control, splicing, polyadenylation, and translation. Almost 20% of human genetic diseases originate from a post-transcriptional misregulation of gene expression. Therefore, understanding the mechanisms employed by RBPs to specifically recognize RNA at the molecular level can help understanding these diseases and finding out new ways of treatment. In addition, many ribonucleoprotein complexes exert control over the cellular response to stress. Our group is also interested in uncovering the molecular mechanisms of abiotic stress reponse in plants, establishing a framework for the development of genetically improved stress-resistant crops. We study the structure, molecular recognition and dynamics of RBPs in solution using NMR spectroscopy combined with complementary biophysical and biochemical techniques. We determined the three-dimensional structure and characterized the internal dynamics and specificity of RNA interaction of the RRM1 domain of HuR, a major human post-transcriptional regulator. HuR-RRM1 uses aromatic and positively charged side chains positioned at its two central β-strands to recognize RNA. In addition, we showed that HuR-RRM1 dimerizes through and oppositely located α-helical interface. By using a hybrid method that integrates NMR-derived chemical shifts and molecular dynamics simulations, we statistically characterized the ensemble of conformations sampled by the two N-terminal RMM domains of HuR, showing that HuR-RRM12 populates a rather flat energetic surface in its free state. Furhtermore, we showed that the cold shock domain of the plant glycine-rich protein AtGRP2 exhibits a folded-unfolded equilibrium in its native state that is shifted toward the folded conformation upon RNA binding. Overall, these results help us to achieve a deeper understanding of the molecular principles that govern protein-RNA interaction.

Keywords: HuR, AtGRP2, RNA, structure, NMR
Supported by: CNPq, FAPERJ, CAPES, Brown University

SP.11.04 - Biomechanics: The importance of evaluating nanomechanical properties in biological systems

Luciana Magalhães Rabelo Alencar [1]
[1]Dept. of Physics, Biophysics and Nanosystems Laboratory, Federal University of Maranhão (São Luiz, MA)

It is a fact: numerous diseases, at the cellular scale, promote ultrastructural changes (i.e., roughness) in the cellular membrane on the outer or cytosolic face (due to changes in proteins or lipids) or the cytoskeleton arrangement. Another difference observed is in the cell nanomechanics (adhesion, elasticity, energy dissipation) related to the disease's development. Understanding and quantifying these mechanical markers allows a better understanding of pathologies and, when associated with therapies, especially for the application of nanostructured drugs, these markers can open new possibilities in early diagnosis, evaluation of drug interaction at the cellular scale, and pathogenesis. Scanning probe microscopy techniques, especially Atomic Force Microscopy (AFM), are ideal for the evaluation and quantification of these properties. This seminar will address the assessment of biomechanical and ultrastructural markers in cells, viral particles, and nanomaterials for biological applications via AFM.

Keywords: Biomechanics, Nanomechanical , Biological systems

SP.12 - Photobiology and Biophotonics

SP.12.01 - Multiphoton imaging on cancer biopsies

Ana Maria de Paula [1]
[1]Departmento de Física, University Federal de Minas Gerais (Belo Horizonte, Brazil)

We present microscopy images of biological tissue by second harmonic generation (SHG) and multiphoton excited fluorescence microscopy as a potential tool in helping with cancer diagnosis. Even today, the standard diagnosis for cancer is given by visual analysis of collected tissue samples, making the result dependent on the pathologist's experience. Then, it is important to develop techniques that help pathologists to make a diagnosis safely and efficiently. The non linear imaging allows to evaluate the tissue collagen and the cellular regions in the biopsies. We demonstrate a methodology to evaluate the changes caused by cancer in collagen and cellular parameters of histological biopsies using automated image analysis and machine learning techniques. The procedure allowed to distinguish between the healthy and cancerous tissue with an accuracy of around 90% for canine mammary cancer and for human prostate cancer. In addition the results for canine mammary gland carcinomas show that the measured tissue collagen parameters correlate with the clinical and pathological data, and the dogs survival times.
Keywords: Multiphoton, cancer , biopsies
Supported by: Fapemig, CNPq, and Capes

SP.12.02 - Photobiomodulation on genomic stability and telomere maintenance

Adenilson de Souza da Fonseca [1]
Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro (Rio de Janeiro, Brazil)

Photobiomodulation has been used for different therapeutic purposes, such as the reduction in inflammation, pain relief, and tissue regeneration. Despite therapeutic applications, there are doubts about the effects of photobiomodulation on DNA. In fact, it is not clear if non-ionizing radiations used for photobiomodulation are able to cause DNA lesions and/or induce DNA repair pathways, which can leave to genomic instability. Also, few data are available on photobiomodulation on telomeres and shelterin protein complex, which contributes to the maintenance of telomere length. Alterations of DNA repair pathways are involved in diseases, and genomic stability and telomere maintenance are crucial to cell and tissue homeostasis. Some results obtained in our laboratory have indicated that radiations emitted from low-power lasers, at conditions similar to those used for photobiomodulation, induce sublethal DNA lesions in bacteria. Such radiations modulate mRNA levels from DNA repair genes in eukaryotic cells, and in normal and injured biological tissues. Also, recent results indicate that photobiomodulation affects shelterin protein complex and telomere length. Taken together, our results suggest that DNA repair, genomic stability, and telomere maintenance are part of the photobiomodulation induced by radiations emitted from low-power therapeutic lasers.
Keywords: Photobiomodulation, DNA repair, genomic stability

SP.12.03 - The Correlation of Oxidative Stress Damage and Aging of Biomembrane: From Human Red Blood Cell to SH-SY5Y Neuroblastoma Cell

Koji Kinoshita [1,2]
[1]Department of Applied Physics, University of São Paulo (São Paulo, Brazil), [2]Institute of Molecular Medicine, University of Southern Denmark (Odense, Denmark)

Various types of photosensitizers have been widely used for photodynamic therapy (PDT) of cancer and skin infection/damage treatment over the last decades. However, the detailed mechanism behind changes to cell membrane mechanical properties under the photosensitizer-mediated oxidative stress is poorly understood. In our group, using human red blood cells (RBCs), the effects of oxidative stress of cis-porphyrin (CisDiMPyP) on the cell membrane have been investigated. Using the micropipette manipulation technique and the blight field microscopic imaging, we observed several different phenomena of RBC membrane, such as elongation, the increase of shear modulus from 6.7 x 10^{-6} to 8.2 x 10^{-6} N/m (700 s irradiation with 26 J/m^2 s irradiance), plastic-like deformation, wrinkle-like pattern formation, and hemolysis, depending on the degree of the oxidative stress. Interestingly, we also observed similar/same phenomena under the aging of RBCs. To describe these results of oxidative stress and aging, we consider that the damage to lipid membrane and cytoskeletal network proteins providing the cytoskeletal network reformation could be the important factors. Currently, to further understand oxidative damage, SH-SY5Y neuroblastoma cells with and without differentiation process were used to investigate a cell membrane's growth/extension under oxidative stress. We found that the oxidative stress caused, 1) the morphological change of non-differentiated SH-SY5Y cells, and 2) different behaviors of the cells' clustering for the differentiated SH-SY5Y cell under the development of tether network formation. Using the micropipette manipulation technique, the tether formation was also observed by pulling the N-cadherin on the membrane surface without the differentiation process. This suggests that the detachment of N-cadherin at the cytoskeletal network could be the key factor in the tether network development. We assume that the oxidatively damaged cell membrane caused not only the change of morphology and mechanical property but also the function of cell-cell interaction.

Keywords: photosensitizer, oxidative stress, biomembrane
Supported by: FAPESP, CNPJ 43.828.151/0001-45

SP.12.04 - Deciphering biomembrane photodamage: Alkylation of hydrophilic sensitizers enhances the photo-induced oxidation of phospholipid membranes

Andrés Héctor Thomas [1], Mariana Vignoni[1], Alexander Greer[2], Mauricio S. Baptista[3], Rosangela Itri[4]
[1]UNLP/CONICET, Instituto de Investigaciones Fisicoquímicas Teoricas y Aplicadas (Argentina), [2]City University of New York, Brooklyn, Brooklyn College (USA), [3]Universidade de São Paulo, Instituto de Química (Brazil), [4]Universidade de São Paulo, , Instituto de Física (Brazil)

Pterins are endogenous photosensitizers present in human skin and mainly act through type I mechanism. These processes are initiated by electron or hydrogen transfer from the substrate to the triplet excited state of the photosensitizer. All unsaturated lipids are well-known targets of oxidative damage, which can occur by photosensitized oxidation. In the case of vesicles dispersions, a hydrophilic photosensitizer will remain in the aqueous phase and the photosensitized oxidation of a target molecule in the membrane will be a dynamic process. On the other hand, if the photosensitizer is lipophilic, an association with a biomembrane is expected and, as the photosensitization is not limited by diffusion, the oxidation might be much faster. Pterin (Ptr) is hydrophilic, does not bind to phospholipid membranes and photoinduces the oxidation of polyunsaturated fatty acids of phospholipids present in large unilamellar vesicles (LUVs). In the search of better compounds that retain the photosensitizing properties of pterins and, at the same time, are able to bind to biomembranes, a series of decyl-pterin derivatives were synthesized and studied. Due to its photochemical properties, 4-(decyloxy)pteridin-2-amine (O-decyl-Ptr) was chosen for further studies using phospholipid membranes with various compositions. Conjugation of a decyl chain to the pterin moiety enables its facile intercalation in LUVs. Upon UVA irradiation lipid peroxidation photosensitized by O-decyl-Ptr leads to the formation of hydroxyl derivatives, hydroperoxides and hydroxyhydroperoxides. These photoproducts undergo a fast conversion into short-chain secondary products most likely due to further photosensitized processes. These short-chain oxidized lipids are responsible for destabilizing the phospholipid bilayer and promoting membrane leakage. The efficiency of photodamage, assessed in terms of oxidized products formation rate and membrane permeabilization, is much higher for O-decyl-Ptr than for free Ptr, which indicates that the intercalation of the alkyl-pterin to the membrane enhances the photosensitized reactions.

Keywords: biomembranes, photosensitization, pterins

SP.13 - From liquids to aggregates – the role of liquid-liquid phase separation in physiological and pathological states

SP.13.01 - Deconstructing virus condensation

Gonzalo de Prat Gay [1]
[1]Fundación Instituto Leloir, IIB-BA Conicet (Buenos Aires, Argentina)

Viruses have evolved precise mechanisms for using the cellular physiological pathways for their perpetuation. These virus-driven biochemical events must be separated in space and time from those of the host cell. In recent years, granular structures, known for over a century for rabies virus, were shown to host viral gene function and were named using terms such as viroplasms, replication sites, inclusion bodies, or viral factories (VFs). More recently, these VFs were shown to be liquid-like, sharing properties with membrane-less organelles driven by liquid-liquid phase separation (LLPS) in a process widely referred to as biomolecular condensation. Some of the best described examples of these structures come from negative stranded RNA viruses, where micrometer size VFs are formed toward the end of the infectious cycle. We here discuss some basic principles of LLPS in connection with several examples of VFs and propose a view, which integrates viral replication mechanisms with the biochemistry underlying liquid-like organelles. In this view, viral protein and RNA components gradually accumulate up to a critical point during infection where phase separation is triggered. This yields an increase in transcription that leads in turn to increased translation and a consequent growth of initially formed condensates. According to chemical principles behind phase separation, an increase in the concentration of components increases the size of the condensate. A positive feedback cycle would thus generate in which crucial components, in particular nucleoproteins and viral polymerases, reach their highest levels required for genome replication. Progress in understanding viral biomolecular condensation leads to exploration of novel therapeutics. Furthermore, it provides insights into the fundamentals of phase separation in the regulation of cellular gene function given that virus replication and transcription, in particular those requiring host polymerases, are governed by the same biochemical principles.

Keywords: Virus; Biophysics, Condensation, Phase separation

SP.13.02 - An intrinsically disordered pathological prion variant Y145Stop converts into self-seeding amyloids via liquid-liquid phase separation

Samrat Mukhopadhyay [1]
[1]Centre for Protein Science, Design and Engineering, Department of Biological Sciences; Department of Chemical Sciences. Indian Institute of Science Education and Research (IISER) (Mohali, Knowledge City, Sector 81, SAS Nagar, Mohali, Punjab 140306, India)

Biomolecular condensation via liquid-liquid phase separation of intrinsically disordered proteins/regions (IDPs/IDRs) along with other biomolecules is proposed to control critical cellular functions, whereas aberrant phase transitions are associated with a range of neurodegenerative diseases. Here, we show that a disease-associated stop codon mutation of the prion protein (PrP) at tyrosine 145 (Y145Stop), resulting in a truncated, highly disordered, N-terminal IDR, spontaneously phase-separates into dynamic liquid-like droplets. Phase separation of this highly positively charged N-terminal segment is promoted by the electrostatic screening and a multitude of weak, transient, multivalent, intermolecular interactions. Single-droplet Raman measurements, in conjunction with an array of bioinformatic, spectroscopic, microscopic, and mutagenesis studies, revealed a highly mobile internal organization within the liquid-like condensates. The phase behavior of Y145Stop is modulated by RNA. Lower RNA:protein ratios promote condensation at a low micromolar protein concentration under physiological conditions. At higher concentrations of RNA, phase separation is abolished. Upon aging, these highly dynamic liquid-like droplets gradually transform into ordered, β-rich, amyloid-like aggregates. These aggregates formed via phase transitions display an autocatalytic self-templating characteristic involving the recruitment and binding-induced conformational conversion of monomeric Y145Stop into amyloid fibrils. In contrast to this intrinsically disordered truncated variant, the wild-type full-length PrP exhibits a much lower propensity for both condensation and maturation into amyloids, hinting at a possible protective role of the C-terminal domain. Such an interplay of molecular factors in modulating the protein phase behavior might have much broader implications in cell physiology and disease.

Keywords: amyloid formation, biological phase transitions, intrinsically disordered proteins

SP.13.03 - Phase separation of p53 precedes aggregation and is affected by oncogenic mutations and ligands

Jerson Lima Da Silva [1]
[1]Institute of Medical Biochemistry Leopoldo de Meis, National Institute of Science and Technology for Structural Biology and Bioimaging, National Center of Nuclear Magnetic Resonance Jiri Jonas, Federal University of Rio de Janeiro Rio de Janeiro (RJ 21941-902, Brazil)

Mutant p53 tends to form aggregates with amyloid properties, especially amyloid oligomers inside the nucleus, which are believed to cause oncogenic gain-of-function (GoF). The mechanism of the formation of the aggregates in the nucleus remains uncertain. The present study demonstrated that the DNA-binding domain of p53 (p53C) underwent phase separation (PS) on the pathway to aggregation under various conditions. p53C phase separated in the presence of the crowding agent polyethylene glycol (PEG). Similarly, mutant p53C (M237I and R249S) underwent PS; however, the process evolved to a solid-like phase transition faster than that in the case of wild-type p53C. The data obtained by microscopy of live cells indicated that transfection of mutant full-length p53 into the cells tended to result in PS and phase transition (PT) in the nuclear compartments, which are likely the cause of the GoF effects. Fluorescence recovery after photobleaching (FRAP) experiments revealed liquid characteristics of the condensates in the nucleus. Mutant p53 tended to undergo gel- and solid-like phase transitions in the nucleus and in nuclear bodies demonstrated by slow and incomplete recovery of fluorescence after photobleaching. Polyanions, such as heparin and RNA, were able to modulate PS and PT in vitro. Heparin apparently stabilized the condensates in a gel-like state, and RNA apparently induced a solid-like state of the protein even in the absence of PEG. Conditions that destabilize p53C into a molten globule conformation also produced liquid droplets in the absence of crowding. The disordered transactivation domain (TAD) modulated both phase separation and amyloid aggregation. In summary, our data provide mechanistic insight into the formation of p53 condensates and conditions that may result in the formation of aggregated structures, such as mutant amyloid oligomers, in cancer. The pathway of mutant p53 from liquid droplets to gel-like and solid-like (amyloid) species may be a suitable target for anticancer therapy.
Keywords: p53; câncer, phase transitio, aggregation, amyloid

SP.13.04 - Molecular and cellular basis of hyperassembly and protein aggregation driven by a rare pathogenic mutation in DDX3X

Kleber Gomes Franchini [1,2]
[1]Brazilian Biosciences National Laboratory (LNBio), Brazilian Center for Research in Energy and Materials (CNPEM), (Campinas, SP, Brazil), [2]Department of Internal Medicine, School of Medicine, University of Campinas (Campinas, SP, Brazil)

Current studies estimate that 1-3% of females with unexplained intellectual disability (ID) present de novo splice site, nonsense, frameshift, or missense mutations in the DDX3X protein (DEAD-Box Helicase 3 X-Linked). However, the cellular and molecular mechanisms by which DDX3X mutations impair brain development are not fully comprehended. Here, we show that the ID-linked missense mutation L556S renders DDX3X prone to aggregation. By using a combination of biophysical assays and imaging approaches, we demonstrate that this mutant assembles solid-like condensates and amyloid-like fibrils. Although we observed greatly reduced expression of the mutant allele in a patient who exhibits skewed X inactivation, this appears to be enough to sequestrate healthy proteins into solid-like ectopic granules, compromising cell function. Therefore, our data suggest ID-linked DDX3X L556S mutation as a disorder arising from protein misfolding and aggregation.
Keywords: Biophysics, Molecular biology, Neuroscience

SP.14 - Mitochondria, Metabolism and Beyond

SP.14.01 - Universal Mitochondrial Dysfunction During Space Travel

Afshin Beheshti [1]
[1]NASA, Ames Research Center (California, USA)

Determining the biological impact of spaceflight through novel approaches is essential to reduce the health risks to astronauts for long-term space missions. The current established health risks due to spaceflight are only reflecting known symptomatic and physiologic responses and do not reflect early onset of other potential diseases. There are many unknown variables which still need to be identified to fully understand the health impacts due to the environmental factors in space. One method to uncover potential novel biological mechanisms responsible for health risks in astronauts is by utilizing NASA's GeneLab platform (genelab.nasa.gov). GeneLab is a public repository that hosts multiple omics datasets generated from space biology experiments that include experiments flown in space, simulated cosmic radiation experiments, and simulated microgravity experiments. Utilizing GeneLab, a comprehensive multi-omics approach was implemented correlating transcriptomics, proteomics, metabolomics, and methylation analysis. We found that cells have stronger overall biological response than the tissues to spaceflight, with mitochondrial activity and innate immunity pathways being heavily impacted. NASA Twin Study results are consistent with a specific alteration in mitochondrial ATP production. Our results indicate that the space environment can directly induce mitochondrial damage, with mitochondrial dysfunctions being a cause for chronic inflammation and both being involved in the development of metabolic disorders that cause changes in lipid metabolism. We also found biological changes occurring during spaceflight with cell cycle, circadian rhythm and olfactory activity pathways can also influence and be influenced by alterations on mitochondrial activity. In conclusion we discovered that mitochondrial dysfunction is a key driver in biological response to spaceflight and potentially can lead to health risks.

Keywords: Mitochondria, omics

SP.14.02 - Macrophage immunometabolic regulation

Pedro Manoel Mendes de Moraes Vieira [1]
[1]Departamento de Genética, Evolução, Microbiologia e Imunologia, Instituto de Biologia, Unicamp (Campinas, SP, Brazil)

The cellular metabolism of macrophages is finely regulated according to the energetic and metabolic requirements to perform their functions. The hypoxia-induced factor 1 alpha subunit (HIF-1α) has been shown to be an important regulator of the proinflammatory profile and metabolic adaptation of macrophages. Murine macrophages stimulated with LPS and IFN-γ have reduced mitochondrial respiration and expression of components of the electron transport chain (ETC), which are regulated by HIF-1α in a mechanism dependent on transcriptional control of the enzymes aconitate decarboxylase 1 (responsible for converting aconitate into itaconate) and nitric oxide synthase 2 (responsible by the production of nitric oxide). Also, HIF-1α plays a role on SARS-CoV-2 infection. Monocytes infected with SARS-CoV-2 have high production of mitochondrial reactive oxygen species (mtROS), which leads to activation of HIF-1α and subsequent glycolysis. Moreover, during systemic low-grade inflammation, such as diet-induced obesity, free fatty acids induce the activation of the HIF-1α pathway, leading to mitochondrial fission and the production of pro-inflammatory cytokines in a mechanism dependent of BCL2 Interacting Protein 3 (BNIP3). Thus, inflammatory stimuli that induce the activation of HIF-1α and its target genes is key to control mitochondrial activity and the subsequent metabolic adaptation of macrophages and their inflammatory profile.

Keywords: Macrophage , immunometabolic , mitochondria

SP.14.03 - High fat consumption and memory impairment: What is the role of metabolic changes in microglia?

Andreza Fabro de Bem [1]
[1]Departamento de Ciências Fisiológicas, Universidade de Brasília, Instituto de Ciências Biológicas (Brasília, DF)

The ingestion of high amounts of fat is not only a risk factor for metabolic diseases, but also impacts different brain functions, particularly those related to learning and memory processes. Recent data from our group shows that a high-fat diet quickly leads to memory impairment and mitochondrial dysfunction in metabolic peripheral tissues as well in the hippocampus. Cognitive impairment and neurometabolic changes persist and intensify with the advance of obesity. Although we know that neuroinflammation and neurovascular dysfunction are associated with hippocampal damage, the causal relationship and the time-course of these events upon a HFD routine are not yet well established. We are working on the hypothesis that a high-fat diet rapidly induces a metabolic shift in microglia, a population of phagocytes residing in the central nervous system, leading to a disruption of synaptic homeostasis. These cells are very responsive to peripheral signals, such as circulating fatty acids, that can modulate the microglial response leading to neuroinflammation and neuronal damage. Thus, it is imperative to understand the initial mechanisms that trigger diet-induced hippocampal dysfunction, even before the onset of obesity itself. Despite recent contributions to microglia activation in obesity, it is unclear whether metabolic changes in these cells have a key role in neuronal dysfunction that leads to cognitive damage. Understanding the triggering mechanisms of hippocampal damage induced by a high-fat diet will enable the application of efficient therapeutic approaches in the prevention and treatment of brain disorders that occur in obesity. To test our hypothesis, we are using a set of pharmacological and chemogenetic (microglia activation and inhibition) approaches to investigate the role of microglial activity in triggering high-fat diet-induced hippocampal dysfunction.
Keywords: microglia, metabolic diseases, mitochondria
05059 - Mitochondria, Metabolism and Beyond

SP.14.04 - Mitochondria and degenerative diseases: new therapeutic avenues targeting quality control.

Julio Cesar Batista Ferreira [1]
[1]Dep de Anatomia, ICB - USP (São Paulo, SP)

Disruption of mitochondrial function is a common feature of inherited mitochondrial diseases (mitochondriopathies) and many other infectious and non-infectious diseases including viral, bacterial and protozoan infections, inflammatory and chronic pain, neurodegeneration, diabetes, obesity and cardiovascular diseases. Mitochondria therefore become an attractive target for developing new therapies. I will describe critical mechanisms involved in the maintenance of mitochondrial functionality and discuss strategies used to identify and validate mitochondrial targets in different diseases. I will also highlight some of our most recent preclinical and clinical findings using molecules targeting mitochondrial bioenergetics, morphology, number, content and detoxification systems in common pathologies.
Keywords: Mitochondria , degenerative diseases, mitochondriopathies

SP.15 - Pathogenesis of Immune Based Disease

SP.15.01 - Bradykinin Liberation from High Molecular Weight Kininogen by Plasma Kallikrein on the Vessel Wall is Modulated by both C1 inhibitor and Prolylcarboxypeptidase

A.A. Merkulova[1], S. B. Abdalian[1], G.L. Forbes[1], P. Zeng[1], A.S. Pinheiro[1], **Alvin H Schmaier [1,2]**
[1]Department of Medicine, Hematology/Oncology, Case Western Reserve University, (Cleveland, OH, USA),
[2]Department of Medicine, Hematology/Oncology, University Hospitals Cleveland Medical Center (Cleveland, OH, USA)

What regulates local bradykinin (BK) formation on endothelium and what is the proximal mechanism for constitutive BK formation in the intravascular compartment? Prolylcarboxy- peptidase (PRCP) is a serine protease that activates prekallikrein (PK) to plasma kallikrein (PKa) with a 7 nM Km. We have previously shown that PRCP activates prekallikrein to plasma kallikrein when bound to cultured endothelial cells (EC). We propose that proplylcarboxypeptidase (PRCP) contributes to the constitutive formation of BK in the intravascular compartment. In the present investigation we examine the ability of PRCP to cleave high molecular weight kininogen (HK) and liberate BK in the presence of C1 inhibitor (C1INH). These studies examine the relative influence of the simultaneous presence of variable concentrations of C1INH and PRCP on HK cleavage and BK liberation from activating PK on EC membranes. The present investigation is performed with cultured microvascular endothelial cells. The physical relationship of HK, PK, C1INH, and PRCP on these cells is shown by confocal microscopy. PK activation and BK liberation is measured by chromogenic assay and ELISA, respectively. Changes in structure of EC bound HK and factor XII (FXII) are shown by immunoblot. EC expression of PRCP is modulated by siRNA knockdowns and cell transfection, respectively. On cultured human microvascular endothelial cells (EC), PK, C1INH, PRCP, and high molecular weight kininogen (HK), in decreasing order, colocalizes with ICAM on non-permeabilized EC. Further, PK and HK, PK and PRCP, PK and C1INH, and PRCP-C1INH colocalize on EC as well. Thus, all the components to generate BK associate on EC. he degree of EC PK activation in the presence of HK is modulated by the ambient C1INH concentration. C1INH modulates PK activation in a concentration-dependent fashion from 20 nM (23% inhibition) to 620 nM (73% inhibition). The specificity of PK activation on EC is as follows. Takeda's plasma kallikrein inhibitor Mab M202-H03 or rEPI-KAL2, a small recombinant protein, completely blocks PKa formation on EC at 0.1 μM (IC50=10 nM) or 0.3 μM (IC50=25 nM), respectively. A PRCP inhibitor (Calbiochem) or chloroquine completely inhibits PKa formation at 100 μM (IC50=10 μM) or 3 μM (IC50= 1.5 μM). In the absence of C1INH, forming PKa on EC cleaves HK completely to a 65 kDa H-chain and 46 kDa L-chain in 60 min. Alternatively, in the presence of 2 μM C1INH, only 50% of the HK becomes cHK. The degree of BK formation on EC also is modulated by the ambient C1INH concentration. C1INH concentrations from 0.5 to 2.5 μM decrease the concentration of BK formed by PKa formation on EC from 2.5 nM to 1 nM, respectively. Factor XII (FXII) does not activate when incubated with EC for 1 h. However, if FXII is incubated with EC in the presence of HK and PK, FXII becomes αFXIIa. This reaction is blocked by antipain (a PKa inhibitor), cysteine (a PRCP inhibitor), or C1INH. PRCP is another regulator of PK activation on EC. Studies examined PK activation on EC in the absence and presence of PRCP siRNA knockdowns or transfection in the absence or presence of C1INH. Using siRNA to PRCP, we observe almost complete absence of PRCP antigen and a 69% reduction in relative PKa formation on EC. If PRCP is reduced by its siRNA, the inhibitory effect of C1INH, 0.25 to 1 μM, on relative PKa formation is magnified from 49 to 82% in normal cells to 76 to 93%, respectively, in the knocked down cells. Alternatively, if cells are transfected with rPRCP to increase their PK activating ability by 35%, the inhibitory effect of C1INH, 0.25 to 1 μM, on relative PKa formation is reduced from 36 to 93% in the absence of the transfection to 0 to 55%, respectively, in the transfected cells. These combined studies imply that the local level of BK formed on EC is modulated in part by the local concentrations of C1INH, HK, PK and PRCP. These pathways are independent of activated factor XII and contact activation and maybe important for the pathogenesis of the hereditary angioedemas. In situations where vessel membrane PRCP and/or PK-HK are more highly expressed, patients with lowered C1INH levels, e.g., Types I and II hereditary angioedema, will be more susceptible to increased local BK formation leading to angioedema without contact activation

Keywords: Bradykinin, Plasma Kallikrein, Prolylcarboxypeptidase

SP.15.02 - Chagasic Cardiomyopathy: A big BUG in heart electrophysiology.

Jader Dos Santos Cruz [1]
[1]ICB, Universidade Federal de Minas Gerais (Belo Horizonte, MG, Brazil)

Chagas disease (CD), caused by the parasite Trypanosoma cruzi, is a neglected disease that induces heart failure and arrhythmias in approximately 30% of patients during the chronic phase of the disease. There are several substrates for arrhythmias in the heart. Some of them involve changes in the electrical properties of cardiomyocytes, the working cells of the heart. In our study we evaluate the potential involvement of Na+/Ca2+ exchanger (NCX) in the arrhythmic phenotype of cardiomyocytes isolated from mice infected with Trypanosoma cruzi, between 180- and 200- days post-infection, which is considered the chronic phase of CD in this animal model. In our study we found several arrhythmogenic membrane potential oscillations during action potential measurements, in rest and using a protocol to simulate a pause after a tachycardia. Using pharmacological approach, we determine that NCX significantly contributed to the arrhythmogenic phenomena observed. Thus, in our study we demonstrate that NCX may be relevant to the cellular arrhythmogenic profile observed in cardiomyocytes during the chronic phase of experimental CD and blocking NCX may be a new therapeutic strategy to treat arrhythmias in this condition.
Keywords: Chagas disease, Trypanosoma cruzi, Na+/Ca2+

SP.15.03 - Mechanisms of Neuroinflammation and Cognitive Impairment in Experimental Cerebral Malaria

Hugo Caire de Castro Faria Neto [1]
[1]Dep. Imunofarmacologia, FIOCRUZ (Rio de Janeiro, Brazil)

Malaria is caused by Plasmodium infection and remains a serious public health problem worldwide, despite control efforts. Malaria can progress to severe forms, affecting multiple organs, including the brain, causing cerebral malaria (CM). CM is the most severe neurological complication of malaria, and cognitive and behavior deficits are commonly reported in surviving patients. The number of deaths from malaria has been reducing in recent years and as a consequence neurological sequelae have been more evident. Neurological damages in malaria might be related to the neuroinflammation, characterized by glia cell activation, neuronal apoptosis and changes in the blood-brain barrier (BBB) integrity. The neurovascular unit (NVU) is responsible for maintaining the homeostasis of the BBB. Endothelial and pericytes cells in the cerebral microvasculature and neural cells, as astrocytes, neurons and microglia, compose the NVU. The NVU can be disturbed by parasite metabolic products, such as heme and hemozoin, or cytokines that can promote activation of endothelial and glial cells and lead to increased BBB permeability and subsequently neurodegeneration. We will show new experimental data to corroborate the roles of neuroinflammation, microglia activation and changes in NVU as central players in cognitive dysfunction after CM.
Keywords: Malaria, Neuroinflammation , Plasmodium

SP.15.04 - Kinins as new players in cerebral malaria pathogenesis

Ana Acacia Pinheiro Caruso Neves [1]
[1]Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brazil)

The Kallikrein-Kinin System (KKS) has been associated to inflammatory and immunogenic responses in the peripheral and central nervous system, however little is known about the role of its proinflammatory arm in the pathogenesis of neglected parasitic diseases, such as malaria. Plasmodium falciparum (Pf) is the etiologic agent of the disease in humans, and it is responsible for the most severe forms of malaria, including cerebral malaria (CM). CM occurs in 1% of infected patients (2 to 3 million cases annually), with high fatality rate (15–20% of cases). The dynamics of CM pathology is not fully understood, but it includes parasite sequestration to brain endothelial cells, endothelial activation and recruitment and activation of leukocytes and platelets, forming a positive loop in local pro-inflammatory response. This complex phenomenon culminates in endothelial apoptosis and blood-brain barrier breakdown. In this context, we will present data showing the influence of Plasmodium falciparum infected erythrocytes (Pf-iRBC) conditioned medium in parasite sequestration and BBB integrity, pointing out kinins as new players in cerebral malaria pathogenesis.

Keywords: malaria, Kallikrein-Kinin System, Plasmodium falciparum

SP.16 - Plant Signaling

SP.16.01 - How plants signal the time of the day

Carlos Takeshi Hotta [1]
[1]Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo (São Paulo, Brazil)

Introduction The circadian clock is a signalling network that allows plants to keep track of the time of the day. Plants with a working circadian clock grow more and better. However, circadian rhyyhms must be synchronized with environmental rhythms to work correctly. Sugarcane is a high-density crop in which individuals shade each other as they develop, which generates distinct microenvironments in different parts of the field. Objectives As microenvironments have different environmental rhythms from the whole environment, we hypothesized that microenvironment rhythms due to shading evoke metabolic and transcriptional rhythms changes. Materials and Methods Sugarcane leaves were harvested every 2 h for 26 h in 4-months and 9-months old plants. Transcriptional rhythms were measured by using Agilent custom oligo arrays or RT-qPCR. Metabolic rhythms were measured using an Agilent 7890 gas chromatograph coupled to a Leco Pegasus 2 time-of-flight mass spectrometer. Results and Discussion Transcription levels of ScLHY, a circadian clock gene, a peak near dawn in 4-months old sugarcane. However, the ScLHY peak is later in 9-months old plants. These phase changes can also be seen in metabolite rhythms and other genes' transcriptional levels. We hypothesized that microenvironment changes due to shading evoke these phase changes. We tested this hypothesis by building a wooden wall in the field and testing the phase of circadian clock genes. Conclusions We suggest that the microenvironments caused by shading lead the plant circadian clock to detect dawn hours later than sunrise.

Keywords: sugarcane, rhythms, circadian clock
Supported by: FAPESP 19/0853-4 and Serrapilheira Institute (Serra-1708-16001)

SP.16.02 - Signaling Between Plant Growth Controls and Beneficial Bacteria

Adriana Silva Hemerly [1]
[1]Laboratório de Biologia Molecular de Plantas, Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro (Rio de Janeiro, RJ, Brazil)

In a growing population, producing enough food has become a challenge in the face of the dramatic increase in climate changes. Plants, during their evolution as sessile organisms, developed countless mechanisms to better adapt to the environment and its fluctuations. One important way is through modulation of their body and their forms, by perceiving environmental stimulus, followed by activation of signaling cascades and gene expression responses, that end in an accurate control of plant growth. One of the best-reported outcomes of plant association with endophytic beneficial bacteria is the promotion of plant growth by direct and indirect mechanisms, as well as increase in tolerance against biotic and abiotic stresses. Bioinoculants of associative and endophytic nitrogen-fixing bacteria lead to positive results on increase in plant growth and productivity of economically important crops. Our group has been studying plant genes involved in the establishment of this beneficial type of association with nitrogen-fixing bacteria. Integrated differential transcriptomes were generated, providing an overview of sugarcane and maize expression profiles during plant-bacteria association. Functional analyses of plant genes are being performed. The data suggest that an important control of the efficiency of the association is already set in the early stages of plant-bacterium recognition, when specific plant genotypes sense the environment and regulate several plant signaling pathways involved in microorganism recognition, plant defense, nitrogen metabolism and cell division controls. Several of the genetic controls and expression profiles might possibly be used as tools for optimization of plant growth and response to bioinoculants, presenting a sustainable alternative to the use of chemical fertilizers, with positive economic and environmental impacts on agriculture.

Keywords: Plant , gene expression, nitrogen-fixing
Supported by: FAPERJ, CNPq, FINEP, CAPES

SP.16.03 - Photoperiod interplays with age and gibberellin to modulate flowering in the day-neutral tomato

Mateus H. Vicente[1], Lazaro E.P. Péres[2], Federico Valverde[3], **Fabio Tebaldi Silveira Nogueira** [1]
[1]Laboratory of Molecular Genetics of Plant Development, Department of Biological Sciences (LCB), Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ), University of Sao Paulo (USP) (Piracicaba, SP, Brazil), [2]Laboratory of Hormonal Control of Plant Development, Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ), University of Sao Paulo (USP) (Piracicaba, SP, Brazil), [3]Instituto De Bioquimica Vegetal Y Fotosintesis, (Sevilla, Spain)

Distinct environments and latitudes limited human agricultural activities due to the photoperiodic response and its strong impact on flowering time and yield of crops. CONSTANS (CO) is a crucial regulator in the photoperiodic pathway in several species, including Arabidopsis, in which CO activates the florigen FLOWERING LOCUS T (FT) to promote flowering under long day conditions. Cultivated tomato (Solanum lycopersicum) is considered a neutral-day plant, but its wild relatives show delay in flowering under long day conditions, which is associated with the high activity of SELF-PRUNING 5G (SP5G). Here, we isolated tomato CONSTANS1 (SlCO1) and showed that SlCO1 induces the activity of SP5G, which in turn represses the expression of the tomato FT homolog, SINGLE FLOWER TRUSS (SFT). Our data also suggest that tomato photoperiodic pathway is negatively and positively regulated by the gibberellin (GA)-inhibitor DELLA and PHYTOCHROME B1 (PHYB1) proteins, respectively. Analyzes of a series of tomato double mutants indicate interactions at genetic level among the photoperiodic- (SlCO1/SP5G), age- (microRNA156/SPLs), and GA-associated flowering pathways. Importantly, at molecular level, DELLA interferes with SlCO1-dependent activation of SP5G, while SP5G inhibits miR156/SPL-dependent activation of SFT. We are currently identifying novel common targets of the photoperiodic-, age-, and GA-associated flowering pathways by RNAseq. Understanding the photoperiodic responses and its interaction with gibberellin and age flowering pathways may be an important tool to guide tomato and other crops breeding programs.

Keywords: photoperiodic, day-neutral tomato

SP.17 - Microbiome/rhizosphere interactions

SP.17.01 - Unraveling the action of Nod factors in different Bradyrhizobium strains

Luciane Maria Pereira Passaglia [1]
[1]Genética, Departamento de Genética and Programa de Pós-graduação em Genética e Biologia Molecular, Instituto de Biociências, Universidade Federal do Rio Grande do Sul (UFRGS) (RS, Brasil)

The Biological Nitrogen Fixation, one of the most important ecosystem processes carried out by diazotrophic bacteria, brings several benefits to agriculture, with unquestionable environmental and economic gains. Among the most used bacteria as inoculants, those of the genus Bradyrhizobium stand out due to their symbiotic association with soybean [Glycine max (L.) Merril], plant with the highest agricultural production in the world. The symbiotic relationship between plants and bacteria depends on the efficiency of signal exchange, which starts with the release of flavonoid molecules by the host plants. Flavonoids bound by the regulatory protein NodD lead to the transcription of bacterial nodulation genes (nod, nol, and noe) and, consequently, to the synthesis of Nod Factor (NFs), by the compatible bacterium strain. In this work, we investigated the relationship between elite strains of Bradyrhizobium and soybean, regarding their NFs production and inoculation performance. For this, the genomes of the four Bradyrhizobium strains currently used as inoculants for soybean in Brazil, SEMIA 587, SEMIA 5019, SEMIA 5079, and SEMIA 5080, were sequenced and analyzed to identify genes related to their NFs production and decoration. The NFs set of each strain was purified, identified, and used in an inoculation experiment with different soybean varieties. The results indicate different responses in bacterial activity through the presence of NFs in the interaction with the plant. Strains SEMIA 5019 and SEMIA 5080 presented the best results when inoculated together with their respective NFs for several parameters and soybean varieties. For SEMIA 587 and SEMIA 5079, however, the presence of their respective NFs did not improve the results for some soybean varieties. As far as we know, this work is the first to identify so many NFs in the Bradyrhizobium strains and test their influence on the interaction between inoculant and host plant.
Keywords: Bradyrhizobium, soybean, symbiosis
Supported by: CAPES and CNPq

SP.17.02 - Modulating drought stress response of maize by a synthetic bacterial community

Rafael Soares Correa de Souza [1]
[1]SYMBIOMICS, (Florianópolis, SC, Brasil)

Plant perception and responses to environmental stresses are known to encompass a complex set of mechanisms in which the microbiome is involved. Knowledge about plant physiological responses is therefore critical for understanding the contribution of the microbiome to plant resilience. However, as plant growth is a dynamic process, a major hurdle is to find appropriate tools to effectively measure temporal variations of different plant physiological parameters. Here, we used a noninvasive real-time phenotyping platform in a one-to-one (plant–sensors) set up to investigate the impact of a synthetic community (SynCom) harboring plant-beneficial bacteria on the physiology and response of three commercial maize hybrids to drought stress. SynCom inoculation significantly reduced yield loss and modulated vital physiological traits. SynCom-inoculated plants displayed lower leaf temperature, reduced turgor loss under severe drought stress and a faster recovery upon rehydration, likely as a result of sap flow modulation and better water usage. Microbiome profiling revealed that SynCom bacterial members were able to robustly colonize mature plants and recruit soil/seed-borne beneficial microbes. The high-resolution temporal data allowed us to record instant plant responses to daily environmental fluctuations, thus revealing the impact of the microbiome in modulating maize physiology, resilience to drought, and crop productivity.
Keywords: Plant , synthetic community (SynCom), Microbiome

SP.17.03 - Rhizosphere microbiome studies: an innovative tool towards sustainable agriculture

Vania Carla Silva Pankievicz [1,2]
[1]Biochemistry, Universidade Federal do Paraná (PR, Brasil), [2]na, GoGenetic (PR, Brasil)

Uncovering the depths of soil microbial communities is the new frontier for regenerative agriculture. It is not enough just to know who is present, but what these microorganisms mean; and to go further, how to use this information to boost productivity and defend the crop in a sustainable way. The microbiome is the key tool for understanding and using nature for the longevity of land use. In this lecture we will talk about numerous applications and interpretations of these results.

Keywords: microbiome, root, nitrogen
Supported by: CAPES

SP.17.04 - Bacterial and fungal interactions in leaves, rhizosphere and roots of soybean and maize based on co-occurrence networks

Guillermo Uceda-Campos[1], Maike Rossmann[1], Andrew Maltez Thomas[1], Suzana Sato Guima[1], Layla Farage Martins[1], Aline Maria da Silva[1], **João Carlos Setubal** [1]
[1]Departamento de Bioquímica, Instituto de Química, USP (SP, Brazil)

In this talk I will present results from a survey of bacterial and fungal communities associated with the rhizosphere, root endosphere, leaf endosphere, and soil of soybean (Glycine max) and maize (Zea mays), in samples from eight cultivated fields in four states in Brazil, using 16S rRNA gene and ITS amplicon high-throughput sequencing. Based on this data we obtained microbial cores in terms of Amplicon Sequence Variants for all compartments and for each plant, a total of 16 core sets. In addition we carried out a co-occurrence analysis of bacterial and fungal genera, in each plant and each compartment, which resulted in dozens of predicted interactions between members of these two groups.

Keywords: endosphere, Glycine max, Zea mays

SP.18 – SBBf Young Scientist

SP.18.01 - Albumin Overload Impact Proximal Tubule Cells Through Hyper O-Glcnacylation

Rodrigo Pacheco da Silva de Aguiar [1], Diogo de Barros Peruchetti[1], Maria-Paz Marzolo[2], Ana Acacia de Sá Pinheiro[1,3], Wagner Barbosa Dias[1], Celso Caruso Neves[1,3,4]

[1]Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil (Rio de Janeiro, Brazil), [2]Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile (Santiago, Chile), [3] Rio de Janeiro Innovation Network in Nanosystems for Health, Rio de Janeiro Innovation Network in Nanosystems for Health (Rio de Janeiro, Brazil), [4]National Institute of Science and Technology for Regenerative Medicine, National Institute of Science and Technology for Regenerative Medicine (Rio de Janeiro, Brazil)

Urinary protein loss, a condition called proteinuria, is a hallmark of chronic kidney disease, CKD, progression. Proteinuria reflects protein overload at the proximal tubule epithelial cells, PTECs, possibly participating in CKD progression. Identifying the molecular mechanisms mediating this process is essential for the development of new treatments. Our group has previously demonstrated an association among essential hypertension and tubular proteinuria with development of tubule-interstitial injury. The molecular mechanism involved a hyper O-GlcNAcylation in PTECs, but the trigger of this process is unknown. Here, we aimed to study the possible role of PT albumin overload as a trigger for dysregulated O-GlcNAcylation and its impact in PT albumin endocytosis. LLC-PK1 cells, a well characterized PTECs, were incubated with 20 mg/mL albumin mimicking PT albumin overload. LLC-PK1 cells transfected with megalin constructs tagged with hemagglutinin (mMeg-HA) were used to study the traffic and expression of megalin, a receptor involved in PT albumin endocytosis. Albumin endocytosis was assessed by albumin-FITC uptake. Surface megalin expression was determined by confocal microscopy. O-GlcNAcylation were also evaluated in 2 different animal models: 1) subclinical acute kidney injury, subAKI; 2) Adriamycin-induced nephropathy (CEUA-045/17). Initially, we observed that the incubation of LLC-PK1 cells with Thiamet G (5 µM), an O-GlcNAcylation enhancer, promoted an inhibition in albumin endocytosis and reduced surface megalin expression. Importantly, 20 mg/mL albumin induced time-dependent hyper O-GlcNAcylation, reduced albumin endocytosis and decreased megalin surface expression. Co-treatment with OSM-1, an inhibitor of O-GlcNAcylation, blocked the deleterious effects of albumin overload. Importantly, subAKI and adriamycin-induced nephropathy mice models showed increased renal cortex O-GlcNAcylation correlated with decreased proximal tubule albumin endocytosis and reduced megalin expression. In conclusion, our results indicate that albumin overload impairs PT protein reabsorption through an increase in O-GlcNAcylation. This mechanism may be involved in the progression of proteinuria induced by albumin overload. **Keywords:** Albumin overload, Megalin, O-GlcNAcylation. **Supported by:** FAPERJ, CAPES and CNPq

SP.18.02 - Longitudinal changes in astrocytes on a transgenic rat model of Alzheimer's Disease

Andreia Silva da Rocha [1]

[1]Biochemistry, Universidade Federal do Rio Grande do Sul (Rio Grande do Sul, Brazil), [2]Psychiatry, University of Pittsburgh (Pennsylvania, United States), [3]Neurology and Neurosurgery, McGill University (Quebec, Canada), [4]Neurobiology and Behavior, University of California - Irvine (California, United States), [5]Pharmacology, Universidade Federal do Rio Grande do Sul (Rio Grande do Sul, Brazil)

Imaging and fluid biomarkers have helped to reconceptualize Alzheimer's Disease(AD) as a continuum. However, the biological understanding of these biomarkers remains under investigation. In this context, animal models that mimic the AD dynamic cascade present high translational value and can be used to refine our interpretation of biomarker findings. In light of this, we longitudinally evaluated the TgF344-AD rat, a model which presents age-dependent amyloid pathology and tau-tangle like deposits. TgF344-AD rats and wild-type littermates were longitudinally evaluated at 3, 6, 9, and 12 months of age. Rats underwent [18F]FDG-microPET scans, behavioral tasks (Y-maze and novel objective recognition) and CSF samplings. CSF glial and immune markers were measured by multiplex ELISA. A cross-sectional cohort was used to measure cortical glutamate uptake (ex-vivo slices) and to verify, by immunohistochemistry, amyloid plaque and tau deposits at the same time points. Further examination of astrocyte proteins immunocontent was conducted at 9mo. At 3mo, 6mo and 12mo no changes in [18F]FDG metabolism were found. At 9mo, we identified a significant cortical hypermetabolism in the TgF344-AD and a decline in their alternance performance in the Y-maze task. CSF GFAP levels were elevated at 6mo and 9mo while CSF S100B was decreased at 9mo. Additionally, the cortical glutamate uptake and GFAP cortical immunocontent were increased at 9mo and 12mo. The recognition memory and CSF inflammatory markers (sTREM2, IL-6, TNF-α, IL-10) were not altered in any of the ages evaluated. Our results suggest that the TgF344-AD model presents an early cortical glucose hypermetabolism, biomarker evidence of reactive astrogliosis, and spatial memory impairment. At the same age, we identified abnormalities in astrocyte glutamate uptake in their cortex. Due to the critical role of astrocytes in brain glucose handling, our findings suggest that astrocyte reactivity could be driving glucose hypermetabolism and memory impairment at this initial stage of disease. **Keywords:** Alzheimer's Disease, Transgenic rat model , Astrocytes. **Supported by:** CAPES, CnPq, FAPERGS, Serrapilheira, Alzheimer's Association

SP.18.03 - Inflammasomes are activated in response to SARS-CoV-2 infection and are associated with COVID-19 severity in patients

Tamara Silva Rodrigues [1], Keyla Sá[1], Camila Silva Caetano[1], Adriene Yumi Ishimoto[1], Leticia Almeida[1], Amanda Becerra[1], Samuel dos Santos Oliveira[1], Letícia de Souza Lopes[1], Ricardo Cardoso Castro[2], Sabrina Setembre Batah[1], Ronaldo Bragança Martins Júnior[1], Eurico Arruda[1], Dario Simões Zamboni[1]
[1]Departamento de Biologia Celular e Molecular e Bioagentes Patogênicos, Universidade de São Paulo (São Paulo, Brasil), [2]Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo (São Paulo, Brasil)

Severe cases of COVID-19 are characterized by a strong inflammatory process that may ultimately lead to organ failure and patient death. The aim of this work was to investigate inflammasome activation and its role in COVID-19 pathophysiology. IL-1β by ELISA, Caspase-Glo 1 luminescent assay and LDH were assessed in the supernantant from infected monocytes. NLRP3 and ASC puncta formation were observed by immunofluorescence in infected monocytes as well as in PBMCs from COVID-19 patients. FAM-YVAD assay was used to stain for active intracellular caspase-1 in PBMCs. IL-18 and caspase-1p20 were also measured by ELISA in patients sera. We found that SARS-CoV-2 infection triggers caspase-1 activation and IL-1β production. We also observed that SARS-CoV-2 triggers LDH release in monocytes in a process that is independent of priming and requires viable SARS-CoV-2. Next, we measured the formation of NLRP3 and ASC puncta and found that SARS-CoV-2 triggers puncta formation in a process that requires viable virus. Using the FAMYVAD assay, we found that on the day of hospitalization, the PBMCs from patients showed a higher percentage of FAMYVAD+ cells compared with healthy controls. Moreover, microscopy observation of these cells allows clear visualization of NLRP3 and ASC puncta in PBMCs, indicating active inflammasomes in cells from COVID-19 patients. Studying moderate and severe COVID-19 patients, we performed immunohistochemistry and immunofluorescence assays and found active NLRP3 inflammasome in PBMCs and tissues of postmortem patients upon autopsy. Inflammasome-derived products such as Casp1p20 and IL-18 in the sera correlated with the markers of COVID-19 severity, including IL-6 and LDH. Moreover, higher levels of IL-18 and Casp1p20 are associated with disease severity and poor clinical outcome. Our results suggest that inflammasomes participate in the pathophysiology of the disease, indicating that these platforms might be a marker of disease severity and a potential therapeutic target for COVID-19.
Keywords: Inflammasomes, SARS-CoV-2, COVID-19. **Supported by:** FAPESP

SP.18.04 - Electrophysiological alterations in peripheral neurons induced by experimental Diabetes mellitus

Francisco Walber Ferreira Da Silva [1,2], K.S. Silva-Alves[2], A.N. Coelho-de-Souza[3], J.H. Leal-Cardoso[2]
[1]Curso de Engenharia Civil, Centro de Ciências Exatas e Tecnologia, Universidade Estadual Vale do Acaraú (Sobral, CE, Brasil), [2]Laboratório de Eletrofisiologia, Instituto Superior de Ciências Biomédicas, Universidade Estadual do Ceará (Fortaleza, CE, Brasil), [3]Laboratório de Fisiologia Experimental, Instituto Superior de Ciências Biomédicas, UECE (Fortaleza, CE, Brasil).

Superior cervical ganglia (SCG) is an autonomic nervous system component that innervates the neck, head and also innervates the heart and pineal gland. The neuronal membrane of these cells could be modeled by an electric circuit, in which ion channels are presented as resistors (or conductance), the ion driving force as batteries and plasmatic membrane as a capacitor. This membrane analog could be used to study the electrical properties of neuronal membrane and allow a neuronal classification based on the passive electrical properties of these cells. Thus, the objective of this work was the use of electrical membrane analog to classify the neurons of superior cervical ganglia of rats. To achieve this objective, first we modeled the SCG neuronal membrane using a simple resistor-capacitor circuit and its equations were solved in a C program. In sequence, experimental data from SCG neurons were acquired and analyzed. The experimental electrophysiological data (resting potential, E_m; input resistance, R_{in}; and time decay, τ_m) were inserted in the program to obtain IxV plots and neuronal membrane data. With experimental and simulated IxV plots, we made a digital subtraction of the signals and the resultant signal analyzed. The total number of analyzed cell were 77 and data are expressed as mean ± S.E.M. To compare groups, one-way ANOVA test were used followed by Holm-Sidak ($P < 0.05$). In an initial visual inspection of IxV plot, we categorized 2 types of cells: one that exhibited a rectification of voltage trace, named with rectification (WR) and the other that does not exhibits this rectification (NR). After the subtraction of the signals, we found another group of cells that presented a negative deflection (ND) on the voltage trace. Thus, three neuronal groups were found: WR, NR and ND. When comparing the electrophysiological data between groups, there was no difference in E_m and R_{in}. However, the time decay were statistical different between groups, with mean values of 13.2 ± 1.0 (n = 50), 6.7 ± 0.8 (n = 17) and 13.2 ± 0.8 ms (n = 10) for NR, WR and ND groups, respectively. For active parameters, there was no statistical difference between groups in rheobase, but for all parameters related to the action potential (amplitude, duration and maximum ascendant and descendant inclination) there was statistical difference between NR and WR. The use of membrane electrical analog allows a classification of superior cervical ganglion neurons based on the passive membrane electrical properties. This approach could be used to improve the SCG neuronal classification in physiological and pathological states.
Keywords: Diabetes mellitus. **Supported by:** This research was funded by CNPq, FUNCAP, ISCB-UECE

SP.19 - Biointerfaces

SP.19.01 - Cationic Amino Acid Transporters: mechanistic insights from L- and D-enantiomer-generated currents

Ruben Daniel Peluffo [1]
[1]Biological Sciences, CENUR Litoral Norte, Univ. de la Republica (Salto, Uruguay)

Cationic amino acid transporters are highly selective for L-enantiomers such as L-arginine (L-Arg). Because of this stereoselectivity, little is known about the interaction of these transporters with D-isomers. To study whether these compounds provide information on the molecular mechanism of transport, inward currents activated by L-Arg with low apparent affinity were measured in whole-cell voltage-clamped cardiomyocytes as a function of extracellular L-Arg and D-Arg concentrations. D-Arg inhibited L-Arg currents in a membrane potential (VM)-dependent competitive manner, indicating the presence of D-Arg binding sites in the carrier. Accordingly, D-Arg-dependent charge movements were also detected in these cells. Analysis of steady-state currents showed that L- and D-Arg binding reactions dissipate a similar small fraction of the membrane electric field. Since D-Arg is not transported, these results suggest that enantiomer recognition does not occur at the amino acid binding site but rather at conformational transitions that prepare or participate in amino acid translocation. Simulations of the VM dependence of maximal current levels with a four-state alternating model suggest that the outward movement of a negative charge in the unliganded transporter participates in the transport process. Consequently, inward translocation of the L-enantiomer-bound complex might be an electroneutral process.
Keywords: Arginine, Currents, Transport

SP.19.02 - Insights from MD simulations of the μ-opioid receptor embedded in a POPC membrane

Diniz Maciel de Sena Junior [1]
[1]Departamento de Química Biológica, Universidade Regional do Cariri (URCA) (Cariri, Ceará)

Roughly one third of FDA approved drugs target a G-protein coupled receptor (GPCR), a large class of membrane proteins that transduce external stimuli to the interior of the cell. The μ-opioid receptor (MOR) is a class A GPCR that mediates the pain signaling process and is, thus, the main target of morphine. The inactive and active conformation of the MOR have been solved by X-ray diffraction of the crystallized murine receptor, which shares 94% sequence identity with the corresponding human protein. In this talk, insights on receptor conformation obtained from all atom molecular dynamics simulations will be presented. The base system is comprised of the receptor (in active and inactive forms) embedded in a 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) membrane, surrounded by 0.15 M aqueous NaCl solution. Classical and enhanced sampling molecular dynamics simulations were performed using agonists (BU72, morphine), a partial agonist (naloxone benzoylhydrazone) and an antagonist (naloxone) in the binding site, as well as the apo receptor. Molecular switches and interhelical distances were used to assess the receptor´s conformational states. In addition, water molecules flux inside the transmembrane helix bundle and interactions with membrane lipid molecules were observed. These features can be used to better understand the receptor activation mechanism and help the design of safer and more effective drugs.
Keywords: GPCR, opioids, molecular dynamics

SP.19.03 - Local effects of an alpha-helical peptide upon membranes

Filipe da Silva Lima [1]
[1]UFPE, (Recife, PE, Brazil)

BP100, a short antimicrobial peptide, produces membrane perturbations that depend on lipid structure and charge, salts presence, and peptide/lipid molar ratios. As membrane perturbation mechanisms are not fully understood, the atomic scale nature of peptide/membrane interactions requires a close-up view analysis, which can be done by using Molecular Dynamics (MD) simulations. We performed MD simulations of BP100 adsorbed at the bilayer interface with varying composition and, in agreement with experimental results, our simulations showed that, when buried into the membrane, BP100 causes a decrease in lipid lateral diffusion and lipid acyl-chain order parameters and sharp local membrane thinning. These effects were most pronounced on the closest lipids in direct contact with the membrane-bound peptide. In DPPG and anionic-aggregate-containing DPPC/DPPG membranes, peptide flip (rotation of its non-polar facet towards the membrane interior) induced marked negative membrane curvature and enhanced the water residence half-life time in the lipid hydrophobic core and transmembrane water transport in the direction of the peptide. These results further elucidate the consequences of the initial interaction of cationic alpha-helical antimicrobial peptides with membranes.
Keywords: membranes, Molecular Dynamics, DPPC/DPPG

SP.19.04 - Design and characterization of nature-inspired lipid biointerfaces

Rafael Vasconcelos de Melo Freire [1], Eliane Haenni[1], Linda Hong[1], Mark Gontsarik[1], Stefan Salentinig[1]
[1]Dept of Chemistry, University of Fribourg (, Switzerland)

Lipid-based colloids have applications in fields such as food, pharmaceutical, and biotechnology and are responsible for a variety of important biological functions. They play a major role in processes such as lipid digestion and nutrient delivery, which occur in dynamic environments where parameters such as composition, pH and salinity are in constant change and can influence the lipids' self-assemblies and induce colloidal transformations. The connection between structure of oil/water biointerfaces to their function, as well as how we can tune these interfaces to achieve desired properties is yet to be fully elucidated. In this study, we investigate the responsiveness of structural and interfacial properties of triolein/oleic acid/water system. Triolein being a common triacylglycerol in food and oleic acid being a lipolysis product of triolein substrate. We explored pH as a trigger of oil-water interfacial tension and low energy emulsification. Small angle X-ray scattering, spinning drop tensiometry, multi-angle dynamic light scattering, electrophoretic mobility analysis and cryogenic electron microscopy are used to study stimuli-responsive interfacial tension, nanostructural transformations and particle size/stability. Lipid composition and ionic strength were found to influence the apparent protonation constant of oleic acid. Increasing pH progressively from 6.1 to 9.5 led to nanostructural changes in the system, from emulsions droplets to emulsified microemulsions, liposomes and finally lamellar stacks. Increasing pH above apparent pKa values also led to better emulsion stability and significant decrease of interfacial tension, allowing usage of low energy dispersion methods. These results indicate that this food-relevant system can be tuned by pH, ionic strength and lipid composition to control nanostructure, stability, particle size and required emulsification energy. The findings from this study can improve the design of functional food emulsions, with properties such as a tailored digestion rate or nutrients prone to degradation.
Keywords: food colloids, lipid self-assemblies, interfacial tension

SP.19.05 - Towards a better model of the plasma membrane: asymmetric lipid bilayer prepared by hemifusion

Thais Azevedo Enoki [1]
[1]Department of Molecular Biology and Genetics, Cornell University (Ithaca, NY, USA)

With the advance of new methods to prepare asymmetric lipid bilayers, a large effort has been applied to understand the complex behavior of the plasma membrane and inter-leaflet coupling between its asymmetric leaflets. This physical barrier designed to protect the cell is composed of two leaflets of different lipid composition, which individually may have very different phase behavior. Here, we describe a new method to prepare asymmetric giant unilamellar vesicles (aGUVs), where we control the hemifusion of giant unilamellar vesicles (GUVs) and a supported lipid bilayer (SLB) to engineer asymmetric bilayers. We used different fluorescent dyes to monitor the inner and the outer leaflets of the unsupported aGUVs. We confirmed that almost all new exchanged lipids in the aGUVs are found in the outer leaflet of these asymmetric vesicles. In addition, we test the stability of the aGUVs formed by hemifusion in preserving a trapped fluorescent dye during the procedure. To model the plasma membrane, we prepare aGUVs that have one leaflet that phase separates into liquid disordered (Ld) and liquid ordered (Lo) phases, whereas the apposed leaflet has a lipid composition that would form a single fluid phase in an ordinary symmetric vesicle. To access the physical and chemical properties of the asymmetric bilayer, we monitored the partition coefficients of fluorescent probes in each individual leaflet, and the generalized polarization of C-Laurdan, a fluorescent sensor for the lipid packing/order of its surroundings. For aGUVs having high percentage of lipid exchange (> 70%) the dye partition indicates induced ordered domains. Moreover, we observed different cases where an ordered domain can induce order in the apposed leaflet. Further investigation of C-Laurdan spectra suggests that the induced ordered domains are enriched in cholesterol. This result brings a significant implication for the plasma membrane: The cytoplasmic leaflet may have “raft-like” domains induced by the exoplasmic leaflet.

Keywords: membrane, vesicles

SP.20 - Impact of the pandemic on teaching and learning: what was and what remains?

SP.20.01 - Reflections on emergency remote teaching of Biochemistry at the Federal University of Viçosa - past, present, and future

Juliana Lopes Rangel Fietto [1]
[1]Biochemistry and Molecular Biology Department, Universidade Federal de Viçosa (Viçosa, MG, Brasil)

The Covid19 pandemic drastically affected the teaching and learning process in Brazilian Universities. From an essentially face-to-face teaching process and methodologies, we changed to fully remote teaching. Each institution had to adapt to this new scenario, with several challenges, from limited or low-quality internet access, to the absence of training and adequate materials for remote teaching. In this work, it will be approached the experience of the Federal University of Viçosa regarding the adaptations to remote teaching, from teacher training to hiring internet for students in need. In addition, it will also present the experience in the teaching of basics biochemistry of subjects provided to different graduation courses and about specific biochemistry teaching for Biochemistry Bachelor´s graduation course. We will see the vision of students and teachers that lead us to reflect on the past, the present context, and the future of Biochemistry Teaching in a globalized world subject to new future pandemics.
Keywords: remote teaching , education

SP.20.02 - More from the same: revisiting Jmol and R for Biochemistry learning during the coronavirus outbreak.

José Maurício Schneedorf Ferreir d Silva [1]
[1]Laboratório de Bioquímica, Universidade Federal de Alfenas (Minas Gerais, Brasil)

The Covid-19 pandemic has brought to light the inherent difficulties of distance teaching and learning. In an almost instantaneous way, the entire staff of educational players of all levels, both stimulators and depreciators of distance learning, found themselves at the mercy of ICTs during the academic period, including those related to Biochemistry subjects. As a whole, Biochemistry comprises the structure and function of biomolecules, quantitative relationships, and metabolism. Besides the learning objects in the area, two well-established environments stand out together for remote learning due to their versatility, portability, flexibility, gratuity, expandability, and a steep learning curve: Jmol (structure and function; Jmol development team) and R (quantitative relationships; R Core Team).
Keywords: Education, Covid-19, distance teaching

SP.20.03 - Biochemistry on screen – from classroom stages to personal computers and back.

Miguel Augusto Rico Botas Castanho [1]
[1]Universidade de Lisboa, (Lisbon, Portugal)

Biochemistry matters are frequently sources of inspiration for artists, whether in paintings, literature or films. This has been deeply explored as a didactic tool in a recent book [1]. As an add on, selected film footage can be used to teach biochemistry in an appealing and engaging way. The usage of the footage videos is a appropriate approach for remote as well as in-presence teaching/learning. Footage videos are therefore suitable didactic tools to maintain (and improve) in the transition from the remote teaching/learning of the pandemic period to the in-presence classroom teaching/learning of the post-pandemic period. Footage of documentaries on the biographies of the Brazilian singer Raúl Seixas [2] and the Portuguese marathoner Francisco Lázaro [3] will be presented as illustrative examples.
Keywords: Education, didactic tool

SP.21 - APC (Article Processing Charge) - The Future of 1st world Science or the End of the 3rd World one?

SP.21.01 - Open Access' spider web and the empowering of Academia

Daniel Prieto Mena [1]
[1]Instituto de Investigaciones Biologicas Clemente Estable (Av. Italia 3318, Montevideo 11600, Montevideo, Uruguay)

Science is a central element of culture. Its progress and the benefits derived from it are enshrined in the Universal Declaration of Human Rights. The nature of scientific knowledge is collective and depends on communication for its construction. The more accessible this knowledge is, the faster and more efficiently science will advance, benefiting all of society. However, its communication generates millionaire profits for a powerful editorial oligopoly. Since the dawn of the Internet, alternatives to the limitations imposed by publishers that require payment of reading fees have emerged. Open Access eliminates payments for reading, democratizing access to scientific knowledge and bringing it to the entire society. Today, many public and private agencies require that the results of the projects they fund be published in Open Access. Publishers, for their part, have accommodated their business plans to this trend by imposing a new barrier to knowledge: the publication fee. There are alternatives such as the return of scientific communication to the academy, but they require our active participation and the coordination of efforts of the scientific community beyond borders as never before.
Keywords: Open Access

SP.21.02 - Open Access publishing: old problems have been solved but challenging new ones are emerging

Adeilton Alves Brandão [1]
[1]Editor in chief of Memorias do Instituto Oswaldo Cruz, Instituto Oswaldo Cruz (Rio de Janeiro, Brazil)

The advent of Open Access has solved the problem of "not being able" to read a scientific article due to lack of funds to subscribe a journal, and has also reduced the "latest scientific information" gap between the high and low income regions. But new problems have arisen, e. g., 1) the elevated cost for publishing in Open Access journals that use the Article Processing Charge; 2) the emergence of the "predatory journals", i. e. journals created only for money making purpose with no rigor or good publishing practice. The "high prestige" journals now offer to authors the option of publishing under Open Access. This "hybrid model" is generating "side effects" for the Open Access movement: a) major publishers are skyrocketing the prices for publishing an article; and b) they are helping to build a new wall in science that separates scientists who can pay to publish an open access article from those who cannot. To help solving these problems the funding agencies and research organizations should create “publishing platforms” or "institutional Journals", which might operate under the open access model. The publishing cost could be covered either by the funding agency or the institutional publisher, that is, authors would not pay any fee for publication in these platforms/institutional journals. If scientists have to pay for publishing open access articles they should spend only a minimal fraction of their scarce resources and must not "comply" to any standard or metrics defined by the interests of the publishing industry.
Keywords: Open Access, Article Processing Charge, publishing practice

SP.21.03 - How to balance the costs of publication/journal subscription with an open and excellent science

Jerson Lima Da Silva [1]
[1]Instituto de Bioquímica Médica Leopoldo de Meis, Instituto Nacional de Biologia Estrutural e Bioimagem, Centro Nacional de Ressonância Magnética Nuclear Jiri Jonas, Universidade Federal do Rio de Janeiro (Rio de Janeiro, RJ, Brazil)

The main goal of science is to spread its advance to the people in the whole world. Science is mostly supported by funds from public agencies or non-profit organizations. The main drive for the open science movement is the argument if the research has already been funded why to pay to have access to the articles. Brazil pioneered with the SciELO (Scientific Electronic Library Online) created in 1997 that includes a cooperative electronic publishing model of open access journals. In the last 20 years, many journals have made their article open access or become fully open, such as the Journal of Biological Chemistry (JBC). JBC, one of the most important journals in Biochemistry, was established in 1905 and since 1925, it is published by the American Society for Biochemistry and Molecular Biology. In January 2021, JBC turned into gold open access. The same open access movement has been taken by several journals. Open access journal implies thar researchers must pay article-processing charges (APCs), which may dissuade authors from using this option, especially in developing countries. The lecture will focus on how to balance the costs of publication with open science.
Keywords: article-processing charges, Open access Journal, open science

SP.21.04 - Open Access in Latin America: too rich for waivers, too poor for article processing charges

Alicia Juliana Kowaltowski [1]
[1]Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo (São Paulo, Brazil)

One of the unquestionable arguments in favor of the open access movement is to make the scientific endeavor more global and inclusive. However, a recent review1 demonstrates that open access articles display lower author geographical diversity, as article processing charges (APCs) are a barrier for scientists from developing countries. Additionally, most Latin American authors are not eligible for waivers or discounts within open access-promoting Plan S. While we can ask for waivers individually, in practice we find this ineffective and demeaning. As a result, much of the Global South still prefers to publish their results behind paywalls, but this option may not be available soon, as Plan S will require all signatory agencies to ensure authors publish in open access-only journals by December 31st, 2024. As a result, most prestigious journals have agreed to transition to fully open access within less than two years. Given this and the lack of caps on APC values, many of us in the Global South will no longer have affordable and inclusive publication venues to divulge our work alongside scientists in the Global North. Solutions for this pressing situation include (i) focusing less on immediate open access implementation and more on fair pricing policies by editorial companies; (ii) organizing joint negotiations between agencies in the Global South and major editorial companies to promote fair bargains in the transition from subscription models to open access deals, (iii) demanding caps and fair pricing control in the academic editorial market, and (iv) stipulating that Plan S and major editorial companies incorporate upper middle-income countries like Brazil within pre-determined APC waiver or significant (at least 50%) discount programs
Keywords: Open Access

SP.22 - Exploring the Conformational Dynamics and stability of proteins using Computational Simulation

SP.22.01 - Probing energy landscapes of intrinsically Disordered proteins

Vitor Barbanti Pereira Leite [1]
[1]Instituto de Biociências, Letras e Ciências Exatas, UNESP (São José do Rio Preto, SP, Brazil)

Intrinsically disordered proteins (IDPs) lack a rigid 3D structure. Their disordered nature and correspondingly large structural fluctuations lead to a complex high-dimensional phase structural space. Because of the lack of a reference conformation, their energy landscape representation in terms of reaction coordinates presents a significant challenge. In this presentation, we analyze IDP atomistic trajectories, simulated using AWSEM forcefield, and the energy landscapes were elucidated using the energy landscape visualization method (ELViM),1 a reaction-coordinate-free approach suited to explore frustrated energy landscapes. We investigate two well-known IDPs. The first IDP is the Prostate-Associated Gene 4 (PAGE4), a transcriptional coactivator that potentiates the oncogene c-Jun. Two kinases, namely HIPK1 and CLK2, phosphorylate PAGE4 generate phosphorylated variants at different serine/threonine residues with opposing functions. ELViM allows us to identify and compare the population distributions of different conformational ensembles of PAGE4 phosphoforms using the same effective phase space. The predominant conformational ensembles explain PAGE4 phosphoforms functional mechanisms in agreement with experimental observations. In the second system, we examine ensembles of amyloid-β monomer variants their propensities to form fibers. The amyloid-β (Aβ) monomer, an intrinsically disordered peptide, is produced by the cleavage of the amyloid precursor protein, leading to Aβ-40 and Aβ-42 as major products. These two isoforms generate pathological aggregates, whose accumulation correlates with Alzheimer's disease (AD). Experiments have shown that even though the natural abundance of Aβ-42 is smaller than that for Aβ-40, Aβ-42 is more aggregation-prone compared to Aβ-40. Moreover, several single-point mutations are associated with early-onset forms of AD. ELViM is shown to distinguish the monomer ensembles of variants that rapidly form fibers from those that do not form fibers as readily. The results shed light on the potential of ELViM to probe IDPs. [1] A.B. Oliveira Jr. et al, J. Chem. Theory Comput., 15, 6482-6490 (2019).
Keywords: Intrinsically disordered proteins, ELViM, PAGE4

SP.22.02 - Biotin-painted proteins have thermodynamic stability switched by kinetic folding routes

Ronaldo Junio de Oliveira [1]
[1]Laboratório de Biofísica Teórica, Departamento de Física, Instituto de Ciências Exatas, Naturais e Educação, Universidade Federal do Triângulo Minei (Uberaba, MG, Brazil)

Biotin-labeled proteins are widely used as tools to study protein-protein interactions and proximity in living cells. Proteomic methods broadly employ proximity-labeling technologies based on protein biotinylation in order to investigate the transient encounters of biomolecules in subcellular compartments. Biotinylation is a post-translation modification (PTM) in which the biotin molecule is attached to lysine or tyrosine residues. So far, biotin-based technologies have proved to be effective instruments as affinity and proximity tags. However, the influence of biotinylation on aspects such as folding, binding, mobility, thermostability, and kinetics need to be investigated. It was selected a couple of proteins to test the influence of biotinylation on thermodynamic and kinetic properties. Apo (without biotin) and holo (biotinylated) protein structures were used separately to generate all-atom structure-based model simulations in a wide range of temperatures. The proteins had their estimated thermodynamic stability changed by altering their energy landscape upon biotinylation. In all cases, after comparison between the apo and holo simulations, it was observed changes on the free-energy profiles and folding routes. In addition, the energetic barriers were altered with the density of states clearly showing changes in the transition state. This study suggests that analysis of large-scale datasets of biotinylation-based proximity experiments might consider possible alterations in thermostability and folding mechanisms imposed by the attached biotin labels.

Keywords: Biotin-painted, thermodynamic, Biotinylation

SP.22.03 - Normal-mode-based enhanced sampling methods

Paulo Ricardo Batista [1]
[1]PROCC - Programa de Computação Científica, Fiocruz (Rio de Janeiro, RJ)

Proteins are involved in all vital functions, participating in physiological and pathological processes. Their structures are intimately related to function. However, proteins are dynamic entities and need to be flexible to i. undergo conformational changes; ii. interact with biological partners; and iii. perform their functions. Molecular dynamics (MD)-based simulations are commonly applied to study protein dynamics. MD main limitation relies on its difficulty to sample biological relevant rare events, which usually occur in prohibitive time scales. On the other hand, normal modes successfully describe intrinsic slow motions and experimentally-observed protein conformational changes. Our group recently developed hybrid simulation approaches that allow one to explore slow dynamics processes while using MD and normal modes. The studied targets are involved in several pathologic processes or biotechnological applications.
Keywords: Normal-mode-based, Proteins, Molecular dynamics

SP.22.04 - Effect of phosphorylation on the structural dynamics and thermal stability of human Dopamine Transporter: a simulation study using Normal Modes, Molecular Dynamics and Markov State Model

Ana Ligia Scott [1]
[1]Laboratório de Biofísica e Biologia Computacional, Universidade Federal do ABC (São Pauo, Brasil)

Human Dopamine Transporter (hDAT) plays an important role in the modulation of the dopamine Influx/Efflux and it may be involved in the mechanism of certain neurodegenerative diseases such as Parkinson's disease. Macromolecules adopt many conformations in solution dependent on their structure and shape, which determine their dynamics and function. Considering this context, several studies have reported importante metasates for Dopamine transport: outward-facing open state (OFo), the outward-facing closed state (OFc), the holo-occluded state closed (holo), and the inward-facing open state (IFo). Experimental assays showed that different phosphorylation conditions can affect the rate of dopamine absorption. This work presents a protocol using hybrid simulation methods to study the conformational dynamics and stability of hDAT states under different phosphorylation conditions. With this protocol we accomplished the conformational space sampling of hDAT, determined the free energy profile, identified the metastates and the transition probability between them for each phosphorylation condition. We also present results correlating the different phosphorylation conditions with collective movements changes and the stability of the metastates described in the literature. The results suggested that the phosphorylation condition called here NP-333 (same as P-22 but SER333 is not phosphorylated) was the one that most affected the dopamine transport, corroborating the experimental results. Additionally, our results showed that just one phosphorylation could alter intrinsic protein motions affecting the sampling of one or some important metastates for dopamine transport. In this sense, modification in phosphorylation leads to changes in protein movements and conformational dynamics, affecting the stability of the metastates and the transition between them and, consequently, in the transport of dopamine.
Keywords: phosphorylation, simulation study, Normal Modes

SP.23- New Tools on Sirius Synchrotron Facility

SP.23.01 - CEDRO Beamline: Circular Dichroism Spectroscopy Boosted by the Synchrotron Radiation

Juliana Sakamoto Yoneda on behalf of CEDRO team
Brazilian Synchrotron Light Laboratory – LNLS/CNPEM, Campinas, Brazil.
email: juliana.yoneda@lnls.br

CEDRO is the beamline dedicated to Circular Dichroism spectroscopy, in the ultraviolet region, at Sirius. This spectroscopy is based on the differential absorption of circularly polarized light to left and right by optically active entities, i.e., chiral materials. It is a valuable tool for analyzing the structure of chiral molecules, including biomolecules such as proteins, nucleic acids, and carbohydrates. The replacement of conventional lamps by the synchrotron radiation provides many advantages for this spectroscopy (now called SRCD – synchrotron radiation circular dichroism), due to the higher intensity and allowance of recording CD spectra in an extended energy range [1]. The purpose of CEDRO is offering the best setup to study biological materials especially those which are difficult to be analyzed in a conventional instrument (for instance, membrane proteins which are relevant drug targets and intrinsically disordered proteins that exhibit diverse important functions). In this talk, the beamline layout and current status will be presented, as well as the future developments to meet different user requirements. **References:** [1] BA Wallace. The role of circular dichroism spectroscopy in the era of integrative structural biology. Current Opinion in Structural Biology. 2019; 58:191–196.

SP.23.02 - Cateretê Beamline: Coherent Scattering Imaging Commissioning Updates

Carla Cristina Polo [1]
[1]Laboratório Nacional de Luz Síncrotron (LNLS), Centro Nacional de Pesquisa em Energia e Materiais (São Paulo, Brazil)

Cateretê is the coherent X-ray scattering dedicated beamline, at Sirius. During the initial commissioning phase, we are focusing on the developments of imaging experiments, which can provide high resolution 3D data. Along the talk, the focus will be on the scientific results obtained since May 2021, related to material and biological sciences, and challenges to be overcome. Finally, we will show the beamlines capabilities which turn the end station competitive in the biological coherent imaging world and the future technical developments to meet and the user community demands
Keywords: ptychography, nano-tomography, x-rays

SP.23.03 - The reach of synchrotron infrared ultramicroscopy applied to the study of biosystems

Raul de Oliveira Freitas [1]
[1]Brazilian Synchrotron Light Laboratory - LNLS, CNPEM (Campinas, SP, Brazil)

Infrared spectroscopy (FTIR) is an established and non-destructive analytical tool sensitive to vibrational signatures of materials which are directly related to their molecular composition. Due to the intrinsically unambiguous vibrational identity of molecules, FTIR can access chemical composition of materials as a label-free modality. In the last two decades, many scientific questions in diverse fields, ranging from materials to biological sciences, were raised related to the chemical heterogeneity of materials at the sub-micron scale, lengths usually not accessible to classical FTIR due to the Abbe limit for IR light. Hence, new analytical modalities were developed in order to overcome those conceptual limits enabling chemical analysis at the nanoscale. This is the case for scattering Scanning Near-field Optical Microscopy (s-SNOM), an ultramicroscopy modality that allows IR spectroscopy beyond the diffraction limit, reaching c.a. 25 nm spatial resolution. In this presentation, basic aspects of this technique will be displayed when combined with IR synchrotron, enabling broadband IR nanospectroscopy of multidisciplinary materials. This modality is available in one of the Sirius experimental stations, the IMBUIA beamline. Examples of applications in biophysics and perspectives for this community will be exposed as well as guidelines for using this open-user installation.

Keywords: Infrared spectroscopy, Ultramicroscopy , synchrotron

SP.23.04 - The MANACA beamline at Sirius, structural biology at 4th Generation

Andrey Fabricio Ziem Nascimento [1]
[1]Brazilian Synchrotron Light Laboratory , LNLS/CNPEM (Campinas, , Brazil)

MANACA (MAcromolecular micro and Nano Serial CrystAllography) is the first macro-molecular crystallography beamline at Sirius, optimised for high flux, micro-beam size and small beam divergence (0.44 mrad). The focus is optimized to 10 x 7 um (HxV) at sample position, but the beam size can be adjusted from 100 x 80 um to 10 x 7 um al-lowing match the beam to the crystal size. Additionally, the beam can also be cut to achieve smaller sizes (e.g. 5x5 um). The photon flux at sample is ~3x1012 ph/s/100mA at 12.7 keV and energy range from 5 to 20 keV. The experimental station has an auto-matic sample changer that can mount-unmont a sample in less than 1 min, allowing the data collection of more than 30 samples per shift. Setups for serial crystallography data collection and analyses, as well as automation procedures, are being prepared [1]. The great beam characteristics provided by Sirius [2] and the high stability and preci-sion of the optics and experimental station allows the diffraction of challenging sam-ples such as viruses (and other big unit cell crystals), membrane proteins and com-plexes, which commonly yield small crystals. The energy range and beamline setup allow native SAD phasing, reducing the necessity of additional experiments to solve new structures. The experiment control uses a user-friendly graphical interface (MXCuBE) [3], and automatic data processing (from data reduction to initial modelling) is available. The MANACA beamline is also prepared for remote access and have al-ready performed remote experiments with international users. In this talk, I will show the current status of MANACA beamline and its potential to help the structural biology community to answer their scientific questions. Also, the last developments on phasing (native SAD), multi-crystal, serial and room-temperature data collection will be shown. References: [1]Nascimento, A.F.Z. et al. Launch of the Manacá Beamline at Sirius: First Protein Crystallography Structures and New Opportunities for Pharmaceutical Development Using Synchrotrons. Synchrotron Radiation News 34, 3–10 (2021). [2] Liu, L., Milas, N., Mukai, A. H. C., Resende, X. R. & Sá, F. H. de. The Sirius project. J Synchrotron Rad 21, 904–911 (2014). [3] Oscarsson, M. et al. MXCuBE2: the dawn of MXCuBE Collaboration. J Synchrotron Rad 26, 393–405 (2019).

Keywords: macromolecular crystallography, native SAD, structural biology

SP.24 - Molecular and cellular mechanisms of tumor progression

SP.24.01 - Tumor extracellular Vesicles effects on endothelial cells

Vanessa Morais Freitas [1]
[1]Tumor Microenvironment Lab, Department of Cell and Developmental Biology, Biomedical Sciences Institute, University of Sao Paulo (SP, Brazil)

Extracellular Vesicles (EVs) influences tumor microenvironment through modulation of cell-cell communication. Our focus in this moment is how EVs modulates endothelial cells behavior. First, we observed increase in length and sprouting number of endothelial cells treated with EVs from breast tumor cells MDA-MB-231. After, using single cell RNA sequencing we analyzed transcriptomes changes on co-cultured fibroblasts and endothelial cells treated or not with tumor-derived EVs. Beyond others, we observed increase in member of chemokine family on both cell types. We also detected that EVs enhance endothelial cells activation causing increase in lymphocyte and tumor cells adhesion. Based on this we conclude that EVs are essential players to tumor success modulating in vitro tumor angiogenesis and also tumor cell adhesion to endothelial cell.

Keywords: Extracellular Vesicles, Tumor, endothelial cells

SP.24.02 - Development of time-resolved flow cytometry for the detection of NAD(P)H lifetime shifts: a tool for metabolic mapping of breast cancer

Jessica P Houston [1]
[1]Department of Chemical and Materials Engineering, New Mexico State University (Las Cruces, NM, USA)

Autofluorescence is a natural indicator of intracellular metabolic changes and can be readily tracked using flow cytometry. The main metabolite that contributes to this signal is reduced nicotinamide adenine dinucleotide phosphate (NAD(P)H). NAD(P)H is not only a strong intrinsic fluorophore but also exhibits unique optical characteristics that make its presence a reliable metric of metabolic pathways within cells. NAD(P)H excites at 340 nm and emits at 460 nm. Additionally, the average decay time of the emission NAD(P)H varies depending on the interaction of NAD(P)H with enzymes that are involved in oxidative phosphorylation and glycolysis. With measurable fluorescence lifetime shifts of NAD(P)H, predictions of the metabolic state of the cell can be made. In an enzyme-bound state, NAD(P)H experiences an autofluorescence lifetime lengthening relative to the unbound state. Additionally, the bound state correlates to when cells are predominantly undergoing oxidative phosphorylation as opposed to glycolysis for energy generation. Using a simple time-resolved flow cytometer we are able to make autofluorescence measurements of MCF-7 breast cancer cells and calculate the fluorescence lifetime, which we can relate to metabolic changes within this cell line. Tamoxifen is an estrogen modulator that is used in the treatment of breast cancer, yet patients can develop resistance. Thus, knowledge of how resistance occurs is essential. Therefore, in this work we are developing screening methods that seek to answer the questions: do MCF-7 cells that are homeostatic have a baseline metabolic state that differs from MCF-7 cells that have been treated with tamoxifen?; how does the metabolic profile of MCF-7 cells that are resistant to tamoxifen treatment shift when compared to non-resistant cells?; and which energy generation pathway is favored in each respective cell type? This project provides a link between the metabolism of cells under different conditions with a unique lifetime-dependent screening tool. Having the ability to measure lifetime shifts in large numbers of cells could lead to a holistic understanding of how treatment affects cell growth and when onset of drug resistance occurs. Ultimately the impact could be significant in terms of the breadth of treatment option for patients.

Keywords: NAD(P)H , breast cancer, cytometry

SP.24.03 - The role of alphavbeta3 integrin in tumor progression

Heloisa Sobreiro Selistre De Araujo [1]
[1]Dep. Ciências Fisiológicas, Universidade Federal de São Carlos (São Carlos, SP, Brazil)

Cell adhesion to the extracellular matrix is essential for the maintenance of tissue integrity; curiously, it is also a critical process for cell motility and tumor cell invasion. Therefore, understanding the molecular mechanisms of cell adhesion and migration are of relevance for human health and disease. Integrins are the main adhesion receptors and key components of the cell migration machinery, being strongly implicated in each step of cancer progression and the establishment of metastasis. Altered integrin profile is frequently detected in tumors and in the tumor endothelium, contributing to a pro-angiogenic microenvironment. Despite the key role of integrins in many steps of metastatic cascade, therapeutic targeting of these receptors remains a challenge. The alphavbeta3 integrin, the vitronectin receptor, is critically involved in tumor angiogenesis and in malignant cell dissemination. Disintegrins are small proteins found in some snake venoms that bind to integrins with high affinity, blocking downstream integrin signaling and function. We have used a recombinant RGD disintegrin, DisBa-01, from Bothrops alternatus venom as a tool to study alphavbeta3 integrin function in several in vitro models of cell migration and metastasis. DisBa-01 has high affinity to alphavbeta3 integrin (KD = 1.6 x 10-7M) and inhibits cell migration and directionality, angiogenesis and melanoma metastasis. Inhibition of alphavbeta3 integrin decreases CD63 expression in breast cancer cells, a small extracellular vesicles (SEVs) marker, indicating a key role for alphavbeta3 integrin in cell-cell communication through SEVs. In vitro assays performed in hypoxia (1% O2) demonstrated that alphavbeta3 integrin role in cell motility depends on oxygen levels and higher inhibitor concentrations may be necessary to achieve the same inhibitory effect as in normoxia. These versatile responses add more complexity to the role of αvβ3 integrin during tumor progression.
Keywords: cell adhesion, cell migration, integrins, tumor microenvironment, disintegrins, angiogenesis
Supported by: FAPESP, CNPq and CAPES

SP.24.04 - The origins of heterogeneity in cancer cell fitness

Guido Lenz [1]
[1]Dep de Biofísica, Universidade Federal do Rio Grande do Sul (Porto Alegre, RS, Brasil)

A central paradigm of cancer biology is the large degree of heterogeneity in multiple features of cancer cells. The origin of this heterogeneity is genetic in large part, but can also stem from non-genetic sources. Cell fitness defined by the number of live descendants after a given time is a key feature of cancer cells. Cancer cell lines, primary glioblastoma cell cultures and non-tumoral cells generated heterogeneity in cell fitness during the formation of colonies. This generation of fitness was increased in the presence of chemotherapeutic agents in vitro at therapeutically relevant conditions. Generation of cell cycle heterogeneity was mediated both by asymmetric mitosis and changes over time of ERK signaling whereas heterogeneity in response to chemotherapeutic agents was mediated by diverse levels of DNA double strand breaks. A combination of histone deacetylase and DNA-methyltransferase inhibitors present during the formation of colonies stabilized their fitness for at least four generations. Collectively, these results support the understanding that cancer cell fitness is dynamic and its modulation is a fundamental aspect to be considered in comprehending cancer cell biology and its response to therapeutic interventions.
Keywords: cell cycle, cancer, DNA

SM.01 - Session 1: Biogenesis and functions of matrix vesicles

SM.01.01 - Osteoblast matrix vesicles: beyond matrix mineralization

Rene Buchet [1], Agnieszka Strzelecka-Kiliszek[2], Rene Buchet[1], Slawomir Pikula[2], Saida Mebarek[1]
[1]University of Lyon, UCBL, CNRS, ICBMS UMR 5246, IMBL (F-69622 Lyon, France), [2]Laboratory of Biochemistry of Lipids, Nencki Institute of Experimental Biology, Polish Academy of Sciences (3 Pasteur Street, 02-093 Warsaw, Poland)

Matrix vesicles (MVs) are released from mineral-competent osteoblasts. They originate from the apical region of microvilli-like plasma membrane. MVs are vesicles of 100-300 nm diameter, have high alkaline phosphatase activity and can initiate the formation of apatite in their lumen. They are entirely distinct from exosomes, which have negligible alkaline phosphatase activity and do not mineralize well. In this talk, we will discuss the methods of extractions of MVs, and their lipid and protein compositions. Several pieces of experimental evidence, including electron microscopy coupled with X-ray and infrared spectroscopy which indicate that MVs initiate apatite in the lumen of MVs will be presented. Sources of phosphate to initiate apatite inside MVs will be discussed, more specifically the phosphatide, which is the product of the hydrolysis of phospholipase D (PLD) and polyphosphate. Our findings indicate that PLD activity, especially which of PLD1 isoform, may enhance the mineralization process in osteoblastic cells. Nonetheless, the lack of PLD1 or PLD2 do not seem to significantly affect bone formation in adult mice. Although polyphosphate was found to be present in MVs and in osteoblasts, it was unclear which enzymes can control the production and the hydrolysis of polyphosphate. Finally, we will present future directions for the research on the functions of MVs released from osteoblasts.
Keywords: mineralization, Osteoblast matrix vesicles, membrane
Supported by: HC Partnership Program POLONIUM 2021/2022 from NAWA to ASK and SM and the statutory funds of the NIEB PAS.

SM.01.02 - Potential role of autophagy in the biochemical properties of matrix vesicles

Massimo Bottini [1]
[1]Department of Experimental Medicine, University of Rome Tor Vergata (Italy)

Autophagy is a highly conserved degradative pathway exploited by cells to produce energy for survival under starvation or as a response to cytotoxic insults. Recent studies have hypothesized that mineralization-competent cells exploit autophagic mechanisms to transport CaP minerals from the mitochondria to the inner leaflet of the plasma membrane, from which, the minerals are released entrapped by a special class of extracellular vesicles, named matrix vesicles. Matrix vesicles harbor the complete biochemical machinery to locally produce phosphate ions and remove mineralization inhibitors (i.e., pyrophosphate) to enable the maturation of CaP minerals to apatite. If autophagy regulates the biogenesis and biological functions of matrix vesicles is still unknown. Herein we tackle this question by assessing the mineralization ability of osteoblasts cultured in an osteogenic media in the presence and in the absence of chloroquine, an autophagy inhibitor. Alizarin Red-S assay suggested that chloroquine negatively affected the ability of the cells to mineralize. We also found that chloroquine did not affect the number of matrix vesicles released by the osteoblasts, however the vesicles released by chloroquine-treated cells display a defective mineral precursor in their lumen, as suggested by atomic force microscopy and transmission electron microscopy, as well as ability to mineralize, compared to the vesicles released by untreated cells. Proteomic and lipidomic analyses were used to assess possible molecular mechanisms mediating the role of autophagy in the ability of matrix vesicles to mineralize. In conclusion, our study strongly suggests a role of autophagy in the assembly of the matrix vesicles' biochemical machinery.
Keywords: Matrix vesicles, Autophagy, Biochemical machinery.
Supported by: University of Rome Tor Vergata

SM.01.04 - Pyrophosphate regulators as key players for dental cementum formation and regeneration

Francisco Nociti [1]
[1]Faculdade de Odontologia de Piracicaba, UNICAMP (SP, Brasil)

Modulators of the mineralization inhibitor pyrophosphate (PPi) are critical toward maintaining mineralized tissue homeostasis. Extracellular PPi concentration is determined by several key regulators, including: tissue-nonspecific alkaline phosphatase (ALPL gene; TNAP protein), an enzyme that promotes mineralization by PPi hydrolysis; the progressive ankylosis protein (ANKH; ANK), a membrane protein that mediates intracellular to extracellular PPi transport; and ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1; ENPP1), an enzyme that produces adenosine monophosphate (AMP) and PPi from adenosine triphosphate (ATP). Loss of function of any of these regulators leads to dysregulated mineralization [1]. The oral cavity is home to four mineralized tissues, the only such place in the body. The bulk of teeth is composed of dentin, which extends to the tooth root. Enamel covers the coronal portion of the tooth, and cementum is found along the root surface. The coronal 2/3 of cementum consists of the acellular cementum, while the apical 1/3 is composed of the cellular cementum. The periodontal complex includes cementum and alveolar bone, joined into a functional unit by the periodontal ligament (PDL). Periodontal disease can progressively destroy all periodontal tissues. Current therapies to promote their repair are unpredictable, particularly in regards to the acellular cementum, which is the key tissue anchoring the PDL to the tooth root. Insights into the developmental biology of cementum may provide novel approaches to promote its regeneration and the return of periodontal function. Previous work has revealed that acellular cementum is exceptionally sensitive to alterations in PPi metabolism. Loss-of-function mutations in ALPL (the disease hypophosphatasia, HPP; OMIM#241500, 241510, 146300) increase systemic PPi levels and inhibit cementogenesis, phenocopied by the Alpl knockout (Alpl KO) mouse model of HPP [2,3]. Loss-of- function mutations in ENPP1 (the disease generalized arterial calcification in infancy, GACI; OMIM#208000) decrease systemic PPi levels and promote rapid cementogenesis and expanded cementum, evident in humans and Enpp1 mutant mice [4]. Loss-of-function mutations in ANKH (the disease craniometaphyseal dysplasia, CMD; OMIM#123000) also decrease systemic PPi, and Ank−KO mice exhibit a hypercementosis phenotype remarkably similar to Enpp1 loss-of-function mice [3,5]. Pyrophosphate (PPi) is a physiologically important regulator of mineralization, with systemic and local concentrations determined by TNAP, ANK, and ENPP1, among other factors. As discussed above, acellular cementum matrix deposition rate is inversely proportional to the extracellular levels of PPi, with reduced cementum in Alpl KO mice (increased PPi), and excess cementum in Ank KO mice (decreased PPi). Interestingly, genetic correction of PPi by deletion of the Ank allele in Alpl, Ank dKO mice ameliorated Alpl KO dentoalveolar mineralization defects, particularly restoring acellular cementum and associated PDL attachment and function. In addition, simultaneous ablation of both Ank and Enpp1 did not expand acellular cementum growth beyond that observed in single KO mice, but also did not result in ankylosis, in part due to increased presence of osteoclasts that modeled alveolar bone to allow for the expanding cementum layer. While these mouse models provide insights into cementum formation during development, we are now suggesting for the first time, that pharmacologic manipulation of PPi levels may regulate cementum growth / regeneration. This concept provides new insights into how PPi developmentally regulates cementum, additionally supporting interventions targeting PPi metabolism as novel therapeutic approaches. Our studies support modulation of PPi as a novel therapeutic approach to protect, repair, or regenerate acellular cementum [6]. We have shown previously in a proof-of principle study of healing after periodontal fenestration that reduced PPi in Ank KO mice encouraged increased regeneration of cementum compared to WT mice [7]. We also extended those findings by reporting that administration of ENPP1-Fc relatively corrected PPi levels and attenuated acellular cementogenesis, demonstrating that the tissue is amenable to PPi modulation throughout life. Notably, ankylosis was ameliorated in Enpp1 mutants dosed with ENPP1-Fc, showing potential for this approach to control ectopic calcification in periodontal tissues. This is in line with previous studies where administration of etidronate profoundly affected cementogenesis [8-11]. However, to create a positive

environment for cementogenesis, PPi levels should be targeted for reduction by antagonizing ANK/ANKH or ENPP1 function, or by increasing TNAP function. An engineered, mineral-targeted form of TNAP (asfotase alfa) is currently used in enzyme replacement therapy for HPP, and we have shown that early administration in Alpl KO mice can completely prevent cementum defects [12-13]. Clinically, a recent study has demonstrated that early enzyme replacement therapy for HPP by asfotase alfa will lead to a significant lower number of lost teeth, indirectly indicating that asfotase alfa therapy may revert the effects of HPP on dental cementum [14]. Further studies should now be planned to determine whether increasing TNAP or modulating activities of other PPi regulators have therapeutic potential to improve cementum repair/regeneration and restore periodontal function. As part of this panel, we will address these questions by presenting our work on this field.
Keywords: mineralization, Pyrophosphate , dental cementum

SM.03 - Session 3: Functions/application of matrix vesicles

SM.03.01 - Roles of annexins in bone mineralization and vascular calcification

Agnieszka Strzelecka-Kiliszek [1], J. Mroczek[1,2], L. Weremiejczyk[1], S. Pikula[1]
[1]Laboratory of Biochemistry of Lipids, Nencki Institute of Experimental Biology, Polish Academy of Sciences (3 Pasteur Street, 02-093 Warsaw, Poland), [2]Faculty of Chemistry, University of Warsaw (1 Pasteur Street, 02-093 Warsaw, Poland)

Whereas aging is mainly associated with a decrease in bone mineralization (BM) causing osteoporosis or bone cancers, paradoxically it is also linked to an increase in vascular calcification (VC) leading to atherosclerosis. The extracellular vesicles (EVs) secreted by vascular smooth muscle cells (VSMCs) are similar to the matrix vesicles (MVs) secreted by osteoblasts (OBs). Studying both types of vesicles and their potency as therapeutics may help to develop our knowledge about intercellular communication. In this research, special attention is given to two families of proteins crucial for vesicles function: tissue-nonspecific alkaline phosphatases (TNAPs) and phospholipid-binding annexins (AnxAs) responsible for P ions or Ca ions turnover, respectively. We will discuss the role of AnxAs in vesicles formation, intravesicular ions transport, TNAP activation and apatite release. Most likely outcome for industrial perspectives is identification of biomarkers of pathological mechanism leading to trans-differentiation of VSMCs into mineralization competent cells during VC and compare it with the physiological mechanism of apatite production by OBs during BM. Recently published observations suggested the effects of flavonoids on both processes and one of the promising and frequently studied flavonoid is apigenin. Our findings indicate that osteogenic factors enhanced the expression of AnxAs in VSMCs comparing to OBs. It was accompanied with increased TNAP activity measured by ELISA assay and mineralization ability tested by AR-S/CPC assay, suggesting AnxAs as potential biomarkers of pathological mechanism. Their level was reduced by treatment with already known TNAP (Lev, MLS) and Ca channels (K201) inhibitors. Overexpressed AnxA6 accumulated in cytoplasm of resting cells, whereas in stimulated cells, it bound to the vesicular structures. AnxA6 channel activity was observed by BLM technique depending on the applied voltage. Addition of proteoliposomes containing AnxA6 to cell cultures increased their mineral content, TNAP activity and co-localization of AnxA6 with TNAP. All of these phenomena were prevented by apigenin, which stronger than inhibitors affected the mineralization ability of the cells by disturbing TNAP and AnxA6 anchoring into membranes, as examined by FM analysis of whole cells stained with fluorophores or TEM-TOMO analysis of vesicular fractions stained with gold particles. This flavonoid also changed both the structure and the ion composition of produced apatite, as showed using TEM-EDX ion microanalysis and mapping. Summarizing, the cooperation of TNAP and AnxAs in vesicles secreted by mineralization competent cells as well as by trans-differentiated cells is crucial in development of bones and in calcification of vascular system.
Keywords: bone mineralization, vascular calcification, TNAP and AnxAs
Supported by: HC Partnership Program POLONIUM 2021/2022 from NAWA to ASK, ERA-CVD/MICROEXPLORATION/4/2018 grant from NCRD to SP and the statutory funds of the NIEB PAS.

SM.03.02 - 3D printing of bioactive scaffolds based on starch/collagen-containing matrix vesicles

Maryanne de Melo[1], Ramos Ana Paula[1], Pietro Ciancaglini[1], **Bianca Chieregato Maniglia [1]**
[1]Department of Chemistry, University of São Paulo - Ribeirão Preto (SP, Brazil)

The role of bone tissue engineering in the field of regenerative medicine has been one of the main research topics in recent years. In this context, the production of bone scaffolds via 3D printing can lead to materials with improved properties and customized structures. Besides that, matrix extracellular vesicles (MVs) have been explored as a new therapeutic strategy to improve bone tissue regeneration. Adding all these innovations, this presentation will address our study involving the production of three-dimensional printed starch/collagen scaffolds containing MVs isolated from preosteoblast cells. The use of starch and collagen for 3D printed structures has already been reported. Starch is a cheap, renewable, and biocompatible source, while collagen is the main protein found in the bone organic phase. However, these biomaterials when used isolated present poor rheological properties, high swelling, highly hydrophilic character, low mechanical strength, and instability for long-term application. To overcome these obstacles, the mixture of starch and collagen was used to produce biomaterials with superior properties. Also, the addition of MVs improved the bioactivity of these 3D-printed scaffolds, supporting the mineralization process. To overcome these obstacles, the mixture of starch and collagen was used to produce biomaterials with superior properties. Also, the addition of MVs improved the bioactivity of these 3D-printed scaffolds, supporting the mineralization process. In summary, this presentation will show the results obtained in this study and also a brief discussion about the contribution of the 3D printing technology, the use of alternative sources for biomaterials production, and the potential of MVs in improving bone regeneration.
Keywords: 3D bio-printing, bioactive scaffolds, starch/collagen
Supported by: FAPESP (20/08727-0 and 21/05947-2)

SM.03.03 - Effect of EVs released by osteoblasts on osteoclastogenesis and bone remodeling

Keteryne Rodrigues da Silva [1], Gabriela Guaraldo Campos Tótoli[2], Pietro Ciancaglini[3], Sandra Yasuyo Fukada[1]
[1]Ciências Biomoleculares, Faculdade de Ciências Farmacêuticas de Ribeirão Preto (São Paulo, Brasil),
[2]Departamento de Biologia Básica e Oral, Faculdade de Odontologia de Ribeirão Preto (São Paulo, Brasil),
[3]Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto (São Paulo, Brasil)

Bone remodeling is a metabolic process that occurs to maintain the structural integrity of the skeleton. A balance occurs between bone matrix resorption and synthesis, involving an orchestrated activity of specialized cells such as osteoblasts and osteoclasts. Osteoblasts are differentiated cells originating from mesenchymal stem cells, the main function is the production of bone matrix, whereas osteoclasts originate from hematopoietic stem cells and act on bone resorption. An imbalance between osteoclast and/or osteoblast activity leads to dynamic changes in bone tissue, observed in several osteolytic pathologies. It is well described that there is an intense communication between cells. Recent studies have shown that cellular communication can occur through cell fragments called extracellular vesicles (EVs). Among the EVs, exosomes stand out and are characterized by having 40 to 160 nM diameter and carrying organelles derived from their parent cells such as proteins, messenger RNAs (mRNAs), microRNAs (miRNAs), and DNA fragments. In cancer, exosomes play an important role in the tumor context, being able to regulate the processes of metastasis and drug resistance. Osteosarcoma (OS) is the most common bone tumor in children and adolescents. The malignant transformation of osteoprogenitors that will rise to osteosarcoma is defined by the production of an extensive and incompletely mineralized matrix. The identification of changes in the normal function of osteoblasts, as well as their EVs, may provide new therapeutic approaches for OS patients. The fact that exosomes coordinate bidirectional and balanced signaling of osteoclasts and osteoblasts, opens up the possibility to limit pathological bone destruction and the knowledge of these mechanisms may lead to the development of therapeutic agents that can interrupt osteoclastogenic functions and prevent bone loss. Our study aims to evaluate the effect of EVs released by osteoblasts on the formation and activation of osteoclasts in vitro and in vivo models of osteosarcoma.
Keywords: EVs, exosome, osteoblast. **Supported by:** FAPESP

SM.03.04 - Isolation and Characterization of Extracellular Vesicles Derived from Mesenchymal Stem Cells

Gabriela Guaraldo Campos Tótoli [1], Gileade Freitas[1], Heitor Sebinelli[2], Luiz Henrique Andrilli [2], Robson Diego Calixto [1], Letícia Adolpho [1], Helena Lopes[1], Pietro Ciancaglini[2], Adalberto Rosa[1]
[1]Departamento de Biologia Básica e Oral , Bone Research Lab, Faculdade de Odontologia de Ribeirão Preto, Universidade de São Paulo, Brasil (SP, Brasil), [2]Departamento de Química , Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Brasil (SP, Brasil)

Extracellular vesicles (EVs) perform various functions, as they are part of sophisticated mechanisms of communication between cells. The aim of this study was to isolate and characterize extracellular vesicles secreted by mesenchymal stem cells (MSCs) under two conditions of cell growth: culture medium without the presence of fetal bovine serum (FBS-) or with fetal bovine serum free of EVs by ultracentrifugation (FBS+). For this, MSCs were immortalized by the overexpression of hTERT, generating iMSCs, which were characterized by morphology, gene expression of hTERT, proliferation, panel expression of surface markers and potential for osteoblastic differentiation. For the isolation of EVs, we cultivated iMSCs in growth medium until they reached 80% confluence, when the medium was removed, the cultures, washed 3 times with phosphate buffered saline (PBS) and cultivated for another 24 hours in FBS+ or FBS-. At the end of this period, the medium was collected, concentrated by ultrafiltration and the EVs obtained by ultracentrifugation at 110,000g for 2 hours, stored at -80°C, and characterized by size, stability (zeta potential), quantity and morphology, through the analysis of dynamic light scattering, nanoparticle tracking, atomic force microscopy and transmission electron microscopy. Our results showed that the MSCs can be immortalized by the overexpression of hTERT and the iMSCs maintain characteristics of MSCs by their proliferation, expression of markers and differentiation osteoblastic and mineralized matrix formation. The iMSCs cultivated under two different conditions secreted EVs with a diameter between 40 and 300nm, compatible with exosomes and microvesicles, being stable before and after freezing and with concentrations between 3.33 – 3.62e+10 particles/ml. In morphological analyses, the EVs obtained in FBS- or FBS+ presented spherical morphology with smooth and homogeneous surfaces. Independently the presence or not of FBS, the methodology developed was effective for isolation and obtaining EVs derived from iMSCs.

Keywords: Extracellular Vesicles, Bone Regeneration , Mesenchymal Stem Cells
Supported by: FAPESP - Fundação de Amparo à Pesquisa do Estado de São Paulo

SM.04 - LAFeBS-IUPAB Symposium

SM.04.01 - Giant hybrid polymer/lipid vesicles: Insights on phase separation and dynamics from advanced microscopy methodologies

Fábio Fernandes[1,2,3], T. Tuyen Dao[1], **Manuel José Estevez Prieto** [1,2]
[1]iBB-Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa (Av. Rovisco Pais, 1049-001 Lisboa, Portugal), [2]Associate Laboratory i4HB, Institute for Health and Bioeconomy at Instituto Superior Técnico, Universidade de Lisboa (Av. Rovisco Pais, 1049-001 Lisboa, Portugal), [3]Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa (1049-001 Lisbon, Portugal)

Recently, hybrid polymer/lipid vesicles were introduced e.g. for drug delivery comprising bimolecular recognition, and the aim is to blend into a single entity the advantages of both different and well established systems, liposomes and polymersomes (vesicles of pure polymer), allowing a fine tuning of stability, bifunctionality and permeability. A comprehensive study of phase separation and domain formation in Giant Hybrid Unilamellar Vesicles (GHUV) was carried out, using several types of polymers and lipids, considering both architecture (grafted, triblock), and molar mass variations. The micro-domains were directly visualized by confocal imaging using suitable labeled polymers and lipids, and FRET-FLIM was used for the detection of nano-domains below microscopy resolution. The sizes, stability and morphology of the observed phase separation are as predictable, dependent on the lipid/molar fraction, but other factors such as the line tension at the domain boundaries, which is fine tuned by the molar mass and polymer architecture, are ruling factors. The lateral diffusion of lipid within polymer chains was determined by FRAP, and it was concluded that no progressive (intermediate) effect is induced by the lipid, the presence of lipid domains playing a clear role.
Keywords: hybrid polymer, lipid vesicles, phase separation
Supported by: FCT (Portugal)- Fundação projects UIDB/04565/2020 and UIDP/04565/2020 (iBB) and LA/P/0140/2020 (i4HB), and IDS-FunMat (EU). The authors also acknowledge funding from the Portuguese Platform of Bioimaging (PPBI-POCI-01-0145-FEDER-022122).

SM.04.02 - Neutron studies in Bioscience: Opportunities and Challenges posed by the Argentine Neutron Beam Laboratory

Gonzalo Agustín Rumi [1], P.Y. Steinberg[2]
[1]Comission Nacional de Energıa Atomica, Laboratorio Argentino de Haces de Neutrones (Buenos Aires, Argentina), [2]Comission Nacional de Energıa Atomica, Gerencia Quimica (Buenos Aires, Argentina)

The Argentine Neutron Beam Laboratory (LAHN – Laboratorio Argentino de Haces de Neutrones) is a facility dedicated to the study and characterization of material systems by means of state-of-the-art neutron techniques. It will make use of the neutron beams provided by RA–10, a 30 MW open-pool reactor currently being built by the National Atomic Energy Commission (CNEA) in Centro Atomico Ezeiza, near Buenos Aires city, Argentina. LAHN aims to become a hub for interdisciplinary research, providing world–class tools for characterization and scientific research to a wide and international community of users. The techniques available include neutron imaging and tomography, diffraction optimized for strain mapping, polarized neutron reflectometry and small angle scattering. Properties like charge neutrality, spin angular momentum and wavelengths in the range of 0.1– 2 nm make thermal and cold neutrons a valuable probe for non-destructive investigation in a variety of systems. In fact, neutrons provide unique advantages in comparison with other kinds of radiation, such as X-rays, including higher penetration depths, a scattering length which varies non-monotonically with the atomic number, sensitivity to local magnetic fields and the possibility to achieve contrast– matching by deuteration. A wide array of techniques has been developed to take advantage of these unique capabilities. These are applied across diverse research fields, such as materials science, cultural heritage, nanotechnology and biological systems, to name only a few. In this talk we present the instruments that will be hosted at LAHN once the reactor is operative, focusing on those more widely used for the study of biological systems: the Small-Angle Neutron Scattering (SANS) and Neutron Reflectometry (NR) instruments. A brief review of the advantages and challenges posed by neutrons as a probe for structural studies will be given, as well as the instruments' main features and potential applications with impact on the biophysics community.
Keywords: Neutron Reflectometry, Small-Angle Neutron Scattering, X-rays

SM.04.03 - The mechano-sensitive potassium channel and the K/Ca exchanger of the human erythrocyte: A new senescence hypothesis

Jesus G. Romero Muñoz [1]
[1]School of Biology and Institute of Experimental Biology, Central University of Venezuela (Caracas, Venezuela)

The human red blood cell (hRBC), being an enucleated cell, cannot go through a classical apoptosis, although its half-life is 120 days. We know that [Ca]i of hRBC increases over time and its relationship with senescence is widely accepted, however, what are the molecular mechanisms capable of counting time and producing aging remains unknown. In our laboratory we have described for the first time two new ion transport mechanisms in hRBC, a K+ channel whose activation depends on mechanical stress in the membrane and is modulated by the internal concentration of Calcium and intracellular pH: the Mechano-Activated K+ channel (HEMKCA). And an obligatory potassium-calcium exchange mechanism, with a stoichiometry of at least 3 potassium ions for each calcium ion, whose activation depends on the membrane potential and is regulated by oxidative stress and intracellular pH: The voltage-dependent K +/Ca2+ exchanger. With these mechanisms, we have proposed a new hypothesis for hRBC senescence. In brief: the mechanical stress, once the erythrocyte enters the capillaries, will produce a HEMKCA transient activation, and a concomitant loss of water, and will depolarize the membrane and consequently will activate the exchanger, producing a Ca2+ entry, which in turn, will activate proteases, producing a decrease in the capacity of Ca2+ extrusion, among other effects. This will keep repeating, each passage through capillaries, accumulating the effects of elevated Ca2+, leading the cell to an increase in density and rigidity, which, along with other effects, will produce its removal from the bloodstream.
Keywords: hRBC, HEMKCA, Ca2+

SM.04.04 - To be or not to be: what is the function? The exquisite biophysics of the Golgi Reassembly and Stacking Proteins

Antonio José da Costa Filho [1]
[1]Departamento de Física, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo (Ribeirão Preto, SP, Brazil)

Golgi Reassembly and Stacking Proteins (GRASPs) are involved in cell processes that seem paradoxical: they are responsible for shaping the Golgi structure and participate in unconventional secretion pathways that bypass the Golgi. The exact molecular mechanisms underlying each process remain elusive. Their structures are constituted by the C-terminal SPR domain and the N-terminal GRASP domain, formed by two PDZ subdomains. Structural data have been limited to the GRASP domain. This talk will show results obtained with full-length GRASPs, which unravel unexpected structural features: the presence of intrinsically disordered regions (IDRs) and their capacity to form fibril-like structures and undergo liquid-liquid phase separation (LLPS). Our data offers a robust molecular rationale for the long-recognized asymmetry of the PDZs regarding the number and diversity of interacting partners. A comparison of our in vitro biophysical data of GRASPs from fungus, yeast, and humans shows that only one of the human GRASPs (GRASP65) is similar to the lower eukaryotes. We also show that GRASPs can transition to different higher-order oligomers, such as amyloid-like fibrils, and undergo LLPS under conditions that mimic those found during cellular stress. We propose a model of how the cell could use the GRASP sensitivity to changes in its local environment to trigger those transitions, thus impacting its role during different cell-cycle periods.
Keywords: Golgi Reassembly, Stacking Proteins, GRASPs

Index

A - Arthropods 68
B - Bioenergetics and Metabolism 86
C - Cellular and Systems Biophysics 115
D - Computational Biophysics and Biochemistry 123
E – Structural Biology 156
F - Biotechnology and Biomaterials 203
G - Drug Discovery and Delivery 247
H - Science Education 266
I - Photobiology, Optogenetics and Neural Systems 271
J - Glycobiology 277
K - Molecular Mechanisms of Diseases 281
L - Mechanobiology 317
M - Microorganisms and Pathogens 318
N - Neurochemistry 334
O - Omics and Systems Biology 341
P - Membrane-Active Peptides 355
Q - Membrane Permeation: Channels and Transporters 364
R - Plants and Synthetic Biology 368
S - Redox Processes 375
T - Natural Products 392
U - Gene Regulation and Cell Signaling 404
V - Phase Separation in Biological Systems 418
X - Biomimetic Systems and Membrane Biophysics 421

A - Arthropods

A.01 - Tick embryo cell line (bme26) survival and metabolic strategies under hypoxic conditions

Nathan Diniz Silva[1], Beatriz de Oliveira Santos Marques[1], Maria Luísa Corrêa Sampaio[1], Luís Fernando Parizi[2], Itabajara da Silva Vaz Jr[2,3], Carlos Jorge Logullo de Oliveira[1,3], **Leonardo Araujo de Abreu [1,3]**
[1]Instituto de Biodiversidade e Sustentabilidade - NUPEM, Universidade Federal do Rio de Janeiro (RJ, Brazil),
[2]Centro de Biotecnologia and Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul (RS, Brazil),
[3]INCT-EM, Instituto Nacionais de Ciência e Tecnologia - Entomologia Molecular (Brazil)

INTRODUCTION: The hypoxia induced factor pathway (HIF) is highly conserved. Oxygen-dependent hydroxylases act on specific HIFα residues to inhibit its transcription factor activity. Reduced oxygen availability HIFα stabilizes due to hydroxylation inhibition, which favors HIFα dimerization with HIFβ and this complex regulates hypoxia adaptation. OBJECTIVES: Investigate the potential effects of hypoxia treatments in BME26 cell line metabolism. MATERIALS AND METHODS: Cells were kept at 32ºC in L15B300 medium on 24 well plates (5 x10^5 cells /well). Hypoxia was chemically induced by treating cells with 0-500 µM $CoCl_2$ (Co), 0-1 mM Deferoxamine (DFO) or 0-5mM Dimethyloxalylglycine (DMOG) for 24 hours (24hs). Cell viability was determined using MTT assay. Additionally, hypoxia induction was attempted using a vacuum desiccator jar (150 mm) containing fruit salt powder. Cell plates were placed on ceramic desiccator plate. Sterile water was added before closing and vacuum was briefly connected to provide an oxygen-deprived and CO_2 enriched atmosphere (Dss). After 24hs at 32ºC, treated cells were harvested for lactate content, Lactate Dehydrogenase (LDH) activity determination, and MTT assay. DISCUSSION AND RESULTS: BME26 cell viability upon chemically induced hypoxia exhibited IC_{50} values determined at 950 µM, 670 µM and 1.6 mM for Co, DFO and DMOG, respectively. Cell viability decreased by 30% under Dss treatment when compared with control. LDH activity was determined in cell homogenates and it was significantly increased in both 200 µM Co and Dss treatments. Although, lactate content after Co treatment did not differ from control BME26 cells kept in Dss exhibited a 5-fold increase in lactate content. CONCLUSION: These data strongly suggest that BME26 cells exhibit anaerobic metabolism under low oxygen levels (Dss) or hypoxia mimetic conditions (Co), as observed for LDH activity and Lactate Content. Further studies aim to identify and functionally characterize HIF pathway putative components and targets.
Keywords: hypoxia, metabolism, tick
Supported by: PIBIC-UFRJ, FAPERJ, INCT-EM and CAPES

A.02 - Characterization of High Molecular Weight Components from *Tityus serrulatus* Venom

Nicoly Malachize Alano da Silva [1], Iara Aimê Cardoso[1], Isadora Sousa de Oliveira[1], Eliane Candiani Arantes Braga[1], Karla de Castro Figueiredo Bordon[1]
[1]Department of Biomolecular Sciences , School of pharmaceutical sciences of Ribeirão Preto (SP, Brazil)

INTRODUCTION: *Tityus serrulatus*, popularly known as yellow scorpion, is considered the most venomous scorpion in South America and is responsible for the most serious accidents in Brazil. Its venom is a complex mixture of compounds, mainly low-molecular-weight neurotoxins, in which most high-molecular-weight compounds have not yet been isolated and characterized. OBJECTIVES: The aim of this study is to carry out the structural and functional characterization of high molecular weight components from *T. serrulatus* venom, obtained through chromatography of the venom in a reversed-phase or ion exchange columns, followed by functional assays appropriate for each class of isolated molecule. MATERIALS AND METHODS: *T. serrulatus* venom fractionation was performed on C18 column (250 x 10 mm, 5 µm, 300 Å), using fast protein liquid chromatography (FPLC) system. Fractions of interest were characterized structurally by electrophoresis in polyacrylamide gel containing sodium dodecyl sulfate (SDS-PAGE). Enzymatic assays (Phosphodiesterase (PDE) and Hyaluronidase activities) were performed, as well as recognition of the venom by ELISA assay. DISCUSSION AND RESULTS: The fractionation of the venom on C18 was able to separate 33 fractions. The fractions containing high-molecular weight proteins were identified by (SDS-PAGE). Enzymatic assays revealed the presence of hyaluronidase and no significant activity of PDE. CONCLUSION: Our results support that the *T. serrulatus* venom contains several high-molecular- weight compounds, including enzymes, which are poorly explored. Therefore, the study of its actions in the envenoming process and its structural characterizations are relevant to expand the knowledge about this venom.
Keywords: scorpion venom, Tityus serrulatus, high molecular weight toxins
Supported by: FAPESP and CNPQ

A.03 - RNA Interference of Chitin Synthase Gene On *Rhodnius prolixus* Vector Competence: *Trypanosoma cruzi* And ROS Formation

Evelyn Seam Lima Alvarenga[1], Thiago Silva Nascimento[1,2], Francis Monique de Souza Saraiva[3], Georgia Correa Atella[4,5], Marcia Cristina Paes[3,5], **Mônica Ferreira Moreira Carvalho Cardoso [1,5]**

[1]Depto de Bioquímica, Instituto de Química, Univ. Federal do Rio de Janeiro (RJ, Brazil), [2]IFRJ, Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro (RJ, Brazil), [3]Departamento de Bioquímica, IBRAG , Universidade do Estado do Rio de Janeiro (RJ, Brazil), [4]Instituto de Bioquímica Médica Leopoldo de Meis, Univ. Federal do Rio de Janeiro. (RJ, Brazil), [5]Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular-INCT-EM, Univ. Federal do Rio de Janeiro (RJ, Brazil)

INTRODUCTION: Introduction: Chagas' disease has *Rhodnius prolixus* as one of its main vectors. Chitin, synthesized by the chitin synthase enzyme (CHS) is present in the cuticle, peritrophic matrix (PM), ovary, trachea and salivary glands. The PM is a structure found between the intestinal epithelium and the food bolus, present in the most of insects. In Hemiptera and Thysanoptera the MP is absent, being replaced by a structure called perimicrovilar membrane (PMM). Alvarenga et. al. (2016) identified chitin in R. prolixus midgut. OBJECTIVES: Objectives: Evaluate the CHS gene silencing effects on oxidative stress and on the T. cruzi life cycle. MATERIALS AND METHODS: Material and Methods: Adult females/5th instar nymphs were reared and maintained as described in UFRJ-CAUAP 001/011. The CHS clone was used as a template for the synthesis of dsRNA for CHS silencing (dsRNACHS). The dose 1µg dsRNACHS/female was injected into the metathoracic cavity of insects three days before feeding. The silencing phenotype was evaluated in T. cruzi-infected insects, in dsRNACHS-treated groups and in control groups (unrelated gene dsRNA or water). DISCUSSION AND RESULTS: Results and Discussion: The dsRNACHS-group intestine was very fragile with fewer tracheas, ovaries, fat body and oviposition were also affected. The heme amount found in silenced intestines was lower than in controls, but when the hemolymph was analyzed, it was found a greater heme amount for CHS silenced group compared to controls, a possible antioxidant pathway desviation. The hemoglobin amount present in the silenced groups was altered more in the silenced groups, however, when silenced and parasitized, the hemoglobin amount present was lower than of the controls. In dsRNACHS-treated intestines, there was a reduction of trypomastigote and increased epimastigote forms in relation to controls. CONCLUSION: Conclusion: The CHS gene silencing in R. prolixus affects blood digestion, increase oxidative stress, decrease hemozoin formation and altering the growth and parasitic differentiation of T cruzi in intestine.

Keywords: Chitin syntase, *Rhodnius prolixus*, RNA interference / **Supported by:** Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ)/CAPES

A.04 - What happens in the intestinal epithelial cells of *Callosobruchus maculatus* weevil larvae when fed with common bean (*Phaseolus vulgaris*)

Samira Ibrahim[1], Carlos Silva [1]

[1]Biouqímica, Universidade Federal de Santa Catarina (Santa Catarina, Brasil)

INTRODUCTION: Beans are a legume with high nutritional and economic value. Pests cause serious damage to their cultivation and storage. *Callosobruchus maculatus* is the main pest of black-eyed bean (*Vigna unguiculata*). However, in beans of the common bean (*Phaseolus vulgaris*) these insects do not develop. OBJECTIVES: The present research aimed to investigate what happens, at the cellular and molecular level, in the intestinal epithelial cells of *C. maculatus* weevil larvae when they are fed with *P. vulgaris* beans. MATERIALS AND METHODS: For such investigation, an induced feeding approach was used. Weevil larvae *C. maculatus* were transferred to capsules containing bean flour and kept for different feeding periods. A fluorescent probe indicating reactive species was used, assays were carried out to quantify total glutathione, amylase activity and the presence of peritrophic membrane in the larvae of *C. maculatus* weevils were verified. DISCUSSION AND RESULTS: Among the results, it was confirmed the inhibition of intestinal α-amylases and the possible presence of peritrophic membrane in the larvae, which may characterize important factors in the damage to digestion and consequent antibiosis. CONCLUSION: As main conclusions of this work, we highlight the effect of the overexpression of amylases in response to the ingestion of amyalse inhibitors present in the seeds of *P. vulgaris*. The presence of peritrophic membrane as a target for phaseolin binding may also be a contributing factor to the antobiosis observed in *C. maculatus* larvae.

Keywords: Insect, Digestion, Resistance / **Supported by:** FAPESC

A.05 - Antiviral Activity of Eugenol Derivatives on the Survival Rate of Bombyx mori Silkworm Infected with BmNPV Baculovirus.

Raquel Cosin de Souza[1], Lucinéia de Fátima Chasko Ribeiro[2], Poliana Aparecida Rodrigues Gazolla[3], Róbson Ricardo Teixeira[3], Maria Aparecida Fernandez[1], **Flavio Augusto Vicente Seixas [4]**
[1]Depto de Biotecnologia, Genética e Biologia Celular, Universidade Estadual de Maringá (Paraná, Brasil), [2]Centro de Ciências Biológicas e da Saúde, Univ. Estadual do Oeste do Paraná (Paraná, Brasil), [3]Depto de Química, Universidade Federal de Viçosa (Minas Gerais, Brasil), [4]Departamento de Tecnologia, Universidade Estadual de Maringá (Paraná, Brasil)

INTRODUCTION: Sericulture is an agro-industrial activity of great economic importance that revolves around *Bombyx mori*, the silk-producing insect. Many pathogens attack the silkworm causing huge losses in production, with *Bombyx mori* nuclear polyhedrosis virus (BmNPV) being the most lethal, leading to losses between 70 to 100% at the production level. No treatment is available and infection is controlled by handling. OBJECTIVES: To evaluate the antiviral activity of three synthetic compounds derived from eugenol on silkworm caterpillars infected with BmNPV; to identify the compound that promotes the highest survival rate, and evaluate its biodisponibility in hemolymph over time. MATERIALS AND METHODS: Nine groups of 40 caterpillars were identified as Controls (Diluent, Water, Virus); Toxicity (Bm6, Bm7, Bm8); and Infection plus treatments (Virus+Bm6, Virus+Bm7, Virus+Bm8). Groups received 3x10 ^{8} COPs/mL (virus) and/or 10 µL of the respective compound at concentrations of 10 mg/mL. Other 50 caterpillars received 10 µL Bm7 (10 mg/mL) and their hemolymph was collected at a time interval for 7 days. Bm7 was extracted by adding acetonitrile to hemolymph plus centrifugation. A calibration curve was created by adding Bm7 to clean hemolymph and extracted as cited. The compound was quantified by HPLC-ESI-MS/MS. DISCUSSION AND RESULTS: The mortality rates in the treatments Virus, Virus+Bm6, Virus+Bm7 and Virus+Bm8 were 37.5%, 25.0%, 7.5% and 22.5% respectively. The diluent had no antiviral effect and the compounds alone were not toxic (0% mortality). Bm7 was the compound with the best antiviral activity and was present in hemolymph at concentrations above 300 ng/mL for 48h, reaching peak concentration (500 ng/mL) after 12h, which suggests 3 applications of 1 dose every 2 days. CONCLUSION: Bm7 was shown to be a promising antiviral candidate in the treatment of BmNPV infection since it was nontoxic and increased the survival rate of caterpillars by 48% with a single dose. **Keywords:** Bombyx mori, grasserie, BmNPV / **Supported by:** European Union

A.06 - The expression of autophagy genes in the midgut epithelial cells of the Aedes aegypti mosquito.

Jéssica da Costa Ferro [1], Gabriella Nascimento Machado[1], Mauricio de Oliveira Nunes[1], Milena Felix Thompson [1], Helena Dantas de Lima[1], Ana Beatriz Walter-Nuno[1], Isabela Barbosa Ramos[2], Gabriela de Oliveira Paiva e Silva[1]
[1]Laboratório de Bioquímica de Artrópodes Hematófagos, [2]Laboratório de Bioquímica de Insetos, IBqM., Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: Arboviruses are debilitating diseases that affect thousands of people in different regions of the world. The vectors of these diseases are insects with a hematophagous habit, such as mosquitoes. Midgut epithelial cells are the first barriers to these pathogens, which requires the presence of cellular and molecular mechanisms that guarantee the maintenance of tissue integrity, as cell proliferation and the elimination of cells damaged by the infection. Our group has already shown that in Aedes aegypti strains with different types of susceptibility, the dynamics of cell proliferation is related to their resistance to viral infection. OBJECTIVES: The aim of our project is to evaluate whether other cellular mechanisms involved in the defense of this midgut, such as the autophagic pathway, will follow the proliferative profile in these strains. MATERIALS AND METHODS: We initially identified, using bioinformatics tools, the presence of several genes that encode proteins that are components of the autophagy process, in the genome of A. aegypti, based in the orthologous genes in the fly Drosophila melanogaster. Among these genes are two important components of the machinery of this pathway, the proteins ATG1, a kinase that regulates the process and ATG8, which participates in the formation of the autophagic vacuole. DISCUSSION AND RESULTS: Our results also showed that the expression of these genes is higher in the midguts of females fed with blood than in the group fed with sugar, which may show an inverse correlation with cell proliferation in this tissue according to previous data from our group that show that it is in the sugar diet that there is greater induction of proliferation when compared to the blood diet. CONCLUSION: To understand the role of autophagy in mosquito vector competence, we need to mainly silence by Rnai mechanism the ATG1 and ATG8 genes and observe the impacts on mosquito physiology after Zika virus infection.
Keywords: Autophagy, Aedes aegypti, Midgut / **Supported by:** Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) & A Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

A.07 - Characterization of the transglycosydase activity of a membrane-anchored alpha-glucosidase from Lutzomyia longipalpis

Nathália Xavier da Silva [1], Samara Graciane da Costa Latgé[1], Fernando Ariel Genta[1]
[1]Instituto Oswaldo Cruz , Fundação Oswaldo Cruz (Rio de Janeiro, Brasil)

INTRODUCTION: Sugar-rich foods are essential for sandflies development in the adult stage and α-Glucosidases are enzymes involved in sugar digestion in insects. They catalyze the hydrolysis of the α-1,4 glycosidic bonds in disaccharides and in the presence of high substrate concentration, they can perform a transglycosylation reaction. OBJECTIVES: We characterized the transglycosydase activity of the midgut membrane-anchored α-glucosidase from *L. longipalpis* and identified in the genome genes encoding α-glucosidases. MATERIALS AND METHODS: Samples obtained from females sandflies were enriched for obtaining midgut membrane α-glucosidases. The α-glucosidase activity was determined using sucrose as a substrate at different concentrations (10-1400 mM). The assays performed using 1400 mM of sucrose were analyzed by HPLC and collected for total sugars quantification using the sulfuric phenol method. Annotation of alpha-glucosidase genes was performed using the PFAM domain (PF00128) in the platform Vector Base. Multiple alignments with homologous sequences were performed using ClustalW for catalytic site identification. DISCUSSION AND RESULTS: The α-glucosidase from *L. longipalpis* does not follow the classic Michaelis-Menten kinetics, being inhibited at high substrate concentrations. The transglycosylation reaction can explain this inhibition. After analyzing samples by HPLC, we observed products with molecular masses corresponding to tri- and tetra-saccharides. We identified in the genome of *L. longipalpis* three genes coding for membrane-anchored alpha-glucosidases with molecular masses between 59 kDa and 70 kDa and isoelectric points of 5.6 and 4.6. The three proteins indicate N- and O-glycosylation sites and catalytic sites were correctly identified, indicating that these are active enzymes in the insect. CONCLUSION: Understanding the mechanism of transglycosylation that leads to an apparent inhibition of the digestive α-glucosidase in high substrate concentration in *L. longipalpis* allows us to obtain important biochemical and physiological information about this vector. This information is a good resource for developing new insect control strategies tools.

Keywords: α-glucosidase, Lutzomyia longipalpis, Transglycosylation / **Supported by:** CNPQ - Conselho Nacional de Desenvolvimento Científico e Tecnológico

A.08 - The Role of E75 on the Intestinal Cells Proliferation in Aedes aegypti.

Mauricio de Oliveira Nunes [1,2], Roberto Lucas Barbosa e Silva[1,2], Vanessa Bottino Rojas[3], Pedro Lagerblad de Oliveira[1,2], Gabriela de Oliveira Paiva e Silva[1,2]
[1]IBqM, Instituto de Bioquímica Médica Leopoldo de Meis – Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil. (Rio de Janeiro, Brasil), [2]Entomologia Molecular, Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular. (, Brasil), [3]Department of Microbiology & Molecular Genetics, University of California, Irvine (, USA)

INTRODUCTION: The mosquito Aedes aegypti is an important arbovirus vector due to their hematophagic habit, through which they ingest and transmit pathogens that affect millions of people around the world. Dengue, Zika and Chikungunya viruses infect the gastrointestinal tract, reach the salivary glands, and then can be transmitted by the mosquitoe's bite. The mainting of intestinal integrity impacts the vector competence, Thus the study of the blood feeding effects on the mosquito's intestinal homeostasis becomes important. A blood feeding causes a series of specific events that alter the physiology of adult mosquitoes, among which the production of Ecdysone in the ovary stands out. It is known that this hormone is directly related the formation of the eggs. Ecdysone is able to transcriptionally regulate the cells of target tissues, which express a set of Ecdysone initial response genes, among these, the E75 transcription factor. OBJECTIVES: The aim of this project is to investigate the regulation of intestinal stem cell proliferation by Ecdysone through the expression of E75 in the mosquito Aedes aegypti. MATERIALS AND METHODS: Our methodology consists of quantify the expression of E75 by quantitative PCR in different biological conditions and silence it using RNAi machinery. DISCUSSION AND RESULTS: Our results demonstrated the increased expression of E75 24 hours after blood meal in the mosquito midgut, mainly of the isoform B of this gene. Conversely, the exposure to Zika vírus and Pseudomonas entomophila bacteria, do not alter the E75 expression. In addition, we noticed that silencing of E75 increases the rate of mitotic cells in the midgut, suggesting that this transcription fator is required by the molecular apparatus involved in maintaining the intestinal integrity and homeostasis CONCLUSION: Therefore, the future analysis of this gene silencing in response to important challenges for the intestinal epithelium is essencial to elucidate E75 function and to achieve a better comprehension of the mosquito physiology and its vectorial competence.

Keywords: Aedes aegypti, midgut, stem cells proliferation
Supported by: Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES)

A.09 - Proteomic Data of Aedes aegypti Entire Eggs

Brenda Martins Vasconcellos [1], Thiago Nascimento[1,2], Julia Bragança Soares[1,2], Gilberto Barbosa Domont[1], Fabio César Sousa Nogueira[1], Magno Rodrigues Junqueira[1], Mônica Ferreira Moreira[1,3]
[1]Departamento de Bioquímica, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brazil), [2]Graduação, Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro (Rio de Janeiro, Brazil), [3]Entomologia Molecular, Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular (Rio de Janeiro, Brazil)

INTRODUCTION: *Aedes aegypti* is the major vector of the Dengue, Zika and Chikungunya arbovirus. This insect suffered evolutionary adaptations, such as resistance to desiccation of eggs, making them viable for long periods. It is known that possibly *A. aegypti* eggs can host viruses, which are contaminated during follicular development or during oviposition. The knowledge about proteins involved with composition of eggs or exogenous proteins in eggs can help to identify control and vector surveillance targets. OBJECTIVES: The objective of this work was to amplify the protein panel that constitutes the eggs of *A. aegypti* mosquitoes. MATERIALS AND METHODS: Mosquito eggs were lysed with urea:thiurea buffer (8:2M) containing protease inhibitor, followed by sonication on ice and agitation. The soluble proteins were quantified, followed by steps of reduction, alkylation, dilution and trypsinization. The peptides obtained were desalted, dried and later eluted in 0.1% formic acid. Analyzes were performed on a high resolution mass spectrometer, with a 90 min gradient in biological triplicate. The data obtained was processed using Proteome Discoverer software workflow against Uniprot database. DISCUSSION AND RESULTS: The processed dataset resulted in the identification of 3,109 peptides and 779 proteins which were functionally categorized based on their subcellular localization; biological processes and molecular function using Gene Ontology (GO) based annotation available for *A. aegypti* in the Uniprot database. With this approach, it was possible to expand the panel of egg proteins previously described in the literature (130), such as those associated with embryogenesis, lipid transport, energy metabolism, proteins structurally associated with cuticle formation, odor-binding proteins (OBPs), proteins related with iron metabolism, proteins with chitin-binding domains and specific proteins involved in the chitin biosynthesis pathway. CONCLUSION: Protein extraction performed from whole eggs and trypsinization in solution was more efficient than the use of whole ovaries and eggshells precipitated from ovaries separated by SDS-PAGE.
Keywords: Proteomic, Aedes aegypti, Vector diseases / **Supported by:** Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ)

A.10 - Effects of hypercaloric diets on the physiology of the beetle Tribolium castaneum

Matheus Paiva Santos Tavares [1], Julia Moura Silva[1], Fernanda Almeida de Oliveira[1], David Majerowicz[1]
[1]Depto de Biotecnologia Farmacêutica, Faculdade de Farmácia, Univ. Federal do Rio de Janeiro (RJ, RJ), [2]Instituto de Biologia, Univ. Federal do Rio de Janeiro (RJ, Brasil), [3]Instituto de Bioquímica Médica, Univ. Federal do Rio de Janeiro (RJ, Brasil), [4]Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular, Universidade Federal do Rio de Janeiro (RJ, Brasil)

INTRODUCTION: Obesity causes severe consequences to people's health, bringing on diseases like diabetes type II, hypertension and other. In brazil, some forecasts indicate that, by 2050, the major part of its population will get fat and the expenses of treatments can reach billions of dollars. The absence of effective and safe drugs to treat obesity reducts the options to combat this disease. The similarities between the metabolic and signaling pathways in insects and mammals elucidate the use of these arthropods as models to investigate this area. Futhermore, genomic and transcriptomic informations and RNAi tool efficiency confirm the use of the beetle *Tribolium castaneum* as an attractive model for this matter. OBJECTIVES: This project aims to investigate the effects of hypercaloric diets on the lipid metabolism of *T. castaneum*. MATERIALS AND METHODS: Larvae were maintained in wheat flour supplemented with olive oil (high-fat diet), tryptone (high-protein diet), sucrose or glucose (high-carbohydrate diets). The amount of triacylglycerol was measured by a colorimetric enzyme assay. Gene expression was determined by quantitative PCR. DISCUSSION AND RESULTS: An increase in triacylglycerol and a delay in the larvae development in the high-fat diet was observed. The high-protein and high-carbohydrate diets also were tested in various concentrations, but it was only possible to observe a significant increase in triacylglycerol at a 10% w/w tryptone diet. The high-protein diet also altered the gene expression profile of the larve, reducing the expression of glucose-6-phosphate dehydrogenase, the first enzyme of the pentose phosphate pathway, and increasing the expression of an insulin-like peptide. CONCLUSION: These results indicate that hypercaloric diets seem to induce phenotypes similar to obesity and diabetes in *T. castaneum*, making this insect an exciting model for studying metabolic diseases. Supported by FAPERJ, CNPq and INTC-EM.
Keywords: lipid metabolism, triacylglycerol, Tribolium castaneum

A.11 - Structural Characterization of Tick Evasins Proteins from Rhipicephalus microplus

Renato Bernabé [1], Helga Gomes[1,1], Glória Braz[1,2], Itabajara Vaz Jr[1,3], Carlos Logullo[1,4], Eldo Campos[1], Jorge Moraes[1,2]

[1]Laboratório Integrado de Bioquímica Hatisaburo Masuda, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil), [2]Departamento de Bioquímica, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil), [3]Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul (Rio Grande do Sul, Brasil), [4]Laboratório de Bioquímica de Artrópodes Hematófagos, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: Ticks are blood-sucking parasites that transmit diseases to humans and animals. They are responsible for billions of dollars of economic damage to the world's livestock systems. Ticks secrete in their saliva a group of proteins belonging to a family called Evasins. These Evasins modulate the host's immune system allowing the tick to parasitize long enough to feed. This modulation is due to its binding to immune components of the host called chemokines. OBJECTIVES: Of this work were identification, classification, structural models constructions and analysis of the Evasins in transcriptome from tick organs and tissues. MATERIALS AND METHODS: Are basically sequences from tick *Rhipicephalus microplus*' transcriptome, Basic local alignment search tool software like http://www.clustal.org/ and https://blast.ncbi.nlm.nih.gov/Blast.cgi, protein data bank https://www.rcsb.org/ and protein structure homology-modelling server https://swissmodel.expasy.org/. DISCUSSION AND RESULTS: In this work we identified 13 Evasins that were classified 12 as Evasins A and 1 as Evasin B. The majority expressed protein was called EVA-Bm-1A. These encoded protein sequences were found in salivary gland, fat body, ovary, embryo, engorged and partially engorged females. The conformation of the Evasins structural analysis showed chemokine binding sites. CONCLUSION: Therefore, our **CONCLUSION** is that tick evasins can be targets to combat ticks through their inhibition at a specific molecular structural site or by using their biotechnological potential to inhibit chemokines of the signaling cascade that recruit cells involved in the inflammatory process of specific clinical situations.

Keywords: ticks, Rhipicephalus, microplus / **Supported by:** UFRJ, CNPq, FAPERJ

A.12 - Characterization of a New Odorant Binding Protein, RPRC017720, from *Rhodnius prolixus*

Pamela Winnie Da Conceição Ramos[1], Gabrielle Carvalho Miguens Cabo[1], Vitor Nobrega Galvão[1], Nathalia Faro de Brito[1], Ana Claudia do Amaral Melo[1]

[1]Bioquímica, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: Chagas disease is a zoonose caused by *Trypanosoma cruzi*, and transmited by insects from Reduviidae family. In endemic areas, vector transmission is predominant, and *Rhodnius prolixus* is one of most important vector in Latin America. Insects find vertebrate host through semiochemicals exhalated by them, using odorant binding proteins (OBPs) as odor transporters. OBPs deliver the odorants to specific odorant receptors, starting the olfactory signal transduction that evokes insect behavior. Interfering in this process could be the key to reduce Chagas disease transmission in the endemic area. OBJECTIVES: Then, the main goal of this study is characterizing RPRC017720 and suggesting its role in *R. prolixus* behavior. MATERIALS AND METHODS: The *R. prolixus* genome has predicted 27 transcripts for OBPs (Mesquita et al., 2015). However, reanalyzing genome data, we found RPRC017720, and it now needs to be studied. Different platforms, including VectorBase, SIB, NCBI, and software, such as, Mega X, Pymol, Phyre2, Dockthor were used. Then, physico-chemistry characteristics, the protein 3D model and the specific ligands were obtained. DISCUSSION AND RESULTS: Primary sequence analysis revealed it to be a classical OBP, with 6 cysteine in conserved positions forming 3 disulphide bonds. Signal peptide prediction indicated that the cleavage site is between aminoacid 19 and 20 (AQS-ES). The mature protein has 157 aa, MW 19.19 kDa, and pI 5,71, with 22 aa charged positively (5 ARG + 17 LIS), and 24 aa charged negatively (7 ASP + 17 GLU). A tridimensional model was constructed based on odorant binding protein 4 from *Chrysopa pallens*. This model showed 100% confidence, with 6 α-helices regions. Based on the model, the 3 S-S bonds were localized between CYS:47-CYS:78; CYS:74-CYS:134, and CYS:121-CYS:143. Phylogenetic analysis grouped RPRC017720 in hemipteran clade, suggesting that protein is transporting semiochemicals involved in specific hemipteran behavior. CONCLUSION: Docking analysis of selected ligands, and gene expression are being processed.

Keywords: *Rhodnius prolixus*, odorant binding protein, ligands / **Supported by:** FAPERJ, CAPES, INCT-EM, CNPq

A.13 - Characterization of the expression of Heme Responsive Genes (HRGs) in hematophagous insect *Rhodnius prolixus*

Júlia Rodrigues Saldanha de Menezes[1], Ana Beatriz Walter-Nuno da Silva[1,2], Pedro Lagerblad de Oliveira[1,2], Gabriela de Oliveira Paiva e Silva[1,2]

[1]Instituto de Bioquímica Médica Leopoldo De Meiss, Laboratório de Artrópodes Hematófagos, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil), [2]INCTEM, Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular (Rio de Janeiro, Brasil)

INTRODUCTION: Hematophagous insects, such as *Rhodnius prolixus*, are known vectors of numerous diseases that until today affect millions of people around the world. The digestion of host hemoglobin, releases the prooxidant free heme in the intestinal lumen of these insects, representing an oxidative challenge. Previous works from our group described mechanisms that contribute to counteract the toxic effects of heme in *R. prolixus* such as heme aggregation, heme binding-proteins and enzymatic degradation. However, the mechanisms by which molecules are transported from the lumen into epithelial cells remains unknown. OBJECTIVES: The aim of this project is to characterize the expression of two heme transports homologs: HRG1 and HRG2 and how these genes affects heme metabolism in *R.prolixus*. MATERIALS AND METHODS: Using bioinformatics techniques, HRG1 and HRG2 gene were identified in the genome of *R. prolixus*. Primarily, anterior and posterior midguts, ovaries and fat bodies from adult females fed on rabbit blood were dissected at different time points to evaluate the time course expression of these genes. qRT-PCR assays were used to determine mRNA levels. Futhermore, in order to knockdown HRGs' expression and analyze their importance in the metabolism of *Rhodnius prolixus*, HRG1 and HRG2 dsRNA were synthesized and injected in females. DISCUSSION AND RESULTS: Both genes demonstrated an increase in mRNA levels in the midgut after blood feeding. Insects injected with dsRNA for the selected genes showed a reduction of mRNA levels, between the 2nd and 10th day after feeding, revealing an effective silencing of both genes expression. CONCLUSION: HRG1 and HRG2 demonstrated to be heme-responsive genes. With HRG1 and HRG2 knockdown assays we hope to identify in more detail the impact on *R.prolixus* physiological events such as: oviposition, egg viability and survival of adult insects.
Keywords: hematophagy, heme transport, *Rhodnius prolixus* / **Supported by:** CNPq, FAPERJ, CAPES

A.14 - Characterization of the Bithorax complex in the lower Diptera Bradysia hygida

João Vitor Cardoso Uliana [1], Vitor Trinca[1], Nadia Monesi[2]

[1]Programa de Biologia Celular e Molecular, Programa de Biologia Celular e Molecular, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo (São Paulo, Brazil), [2]Análises Clínicas, Toxicológicas e Bromatológicas, Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Univ. de São Paulo (SP, Brazil)

INTRODUCTION: Hox genes encode highly conserved transcription factors in metazoans. In insects, the *Hox* genes act at the top of the genetic regulatory hierarchy and specify the identity of body segments. *Hox* genes were first characterized in *Drosophila melanogaster* and in this organism these genes are organized in the Antennapedia and Bithorax complex, which are separated by 9.7 Mb, in chromosome III. Our laboratory studies the non-conventional model organism *Bradysia hygida*, a sciarid that belongs to the Bibionomorpha infraorder. OBJECTIVES: To characterize the genomic organization of the *Hox* genes in *B. hygida* and the pattern of the expression of the Bithorax complex genes during embryonic development. MATERIALS AND METHODS: The Hox genes were automatically annotated in the *B. hygida* genome assembly. *Hox* transcripts were identified in embryonic transcriptomes. and final manual curation of gene models employed EMBOSS. The Bithorax complex genes were cloned in pGEM-T and recombinants were used as a template for the synthesis of RNA probes. Immunolocalization experiments employed an anti-Ubx/AbdA antibody raised against *D. melanogaster*. DISCUSSION AND RESULTS: The Hox genes reside in the X chromosome, corresponding to about 2 % of the *B. hygida* genome. *Hox* gene sizes range from from 20936 bp (*Abd-B*) to 304013 bp (*Antp)*, and transcriptional isoforms are found for the majority of genes. Analysis of the protein deduced sequences confirm the presence of a homeodomain in all isoforms. Immunolocalization experiments revealed that Ubx and Abd-A are expressed in five segments of the caudal region, in 5-8 days old embryos CONCLUSION: In contrast to what is found in Diptera, in Sciarids the *Hox* genes reside in the sexual chromosome (X). The organization of the Hox cluster in *B. hygida* indicates that the Bithorax complex underwent an inversion, which does not seem to affect the pattern of Hox gene expression along the anterior-posterior embryonic axis.
Keywords: Bradysia hygida, embryonic development, Hox genes / **Supported by:** CNPq and FAPESP

A.15 - An analysis of the Aedes aegypti mosquito's nutritional status and aging and its relationship with the vector capacity and vector competence.

Clara Luiza Rulff da Costa [1], Ana Beatriz Walter Nuno da Silva[1,4], Mauricio Nunes de Oliveira[1], Igor Ferreira da Costa de Almeida[1], Leonan Azevedo dos Reis[1], Tainan Carla Guedes da Silva[1], Gabriela da Silva[1], Marcus Fernandes de Oliveira[1,4], Gabriela Oliveira Paiva e Silva[1,4], Ana Cristina Bahia Nascimento[3,4], Rafael Dias Mesquita[2,4], Pedro Lagerblad de Oliveira[1,4]

[1]Instituto de Bioquímica Médica Leopoldo de Meis , [2]Instituto de Química - Departamento de Bioquímica, [3]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil), [4]Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular, Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular (RJ, Brasil)

INTRODUCTION: The *Aedes aegypti* mosquito is the vector of major arboviruses and represents a menace for global public health. The host-pathogen interaction is important to understand the vector capacity and competence, and the factors in the life history of the mosquito that influence disease transmission. OBJECTIVES: Therefore our goal is to correlate the interference of nutritional and aging factors with the life history traits of the mosquito in order to better understand its relationship with vector capacity and competence. MATERIALS AND METHODS: Here we established a rearing protocol with reduced food availability during larval development that impacts adult mosquito physiology and then we performed survival assay, virus infection, plaque assay and, finally, an Illumina Sequencing correlating nutritional, aging and infection parameters. DISCUSSION AND RESULTS: The suboptimal larval rearing diet (SLRD), which decreases adult mosquito weight, affect vector capacity due to reduced egg laying. However, SLRD produced no significant effect on lifespan, suggesting that the physiological response under nutritional restriction privileged maintenance of homeostasis, prolonging lifespan, even at the cost of producing a reduced offspring. In order to investigate the impact of life history traits on vector competence we evaluated intestinal infection of different oral doses of Zika virus (ZKV) in newly emerged (6 days) and 20 days old mosquitoes fed ad libitum (CT) or reared under SRLD, observing a significant increase in ZKV prevalence in midgut of SRLD insects, but little effect of aging on ZKV infection. These data was used to perform a transcriptomic analysis of midguts of 6 and 20 days old (CT and SRLD) mosquitoes fed on sugar, blood and infected or not with ZKV. PCA analysis confirmed consistent distinct expression profiles were obtained for each condition. CONCLUSION: Quantitative and functional analysis of the differentially expressed transcripts (underway) will help to comprehend how nutrition and aging impacts on mosquito physiology and vector competence.

Keywords: Aedes aegypti, Nutrition, Aging / **Supported by:** FAPERJ, CNPq, CAPES, FINEP

A.16 - Lectin from Myracrodruon urundeuva Leaves Causes Structural Damage in Aedes aegypti Eggs

Robson Raion de Vasconcelos Alves[1], Gabryella Borges Prazeres[1], Abdênego Rodrigues Silva[1], Amanda Luiza Tomaz Soares Silva[1], Jessica da Silva Nascimento[2], Roberto Araújo Sá[3], Gabriel Gazzoni Araújo Gonçalves[4,5], Luiz Carlos Alves[4,5], Fábio André Brayner[4,5], Daniela Maria do Amaral Ferraz Navarro[2], Paulo Euzébio Cabral Filho[6], Adriana Fontes[6], Thiago Henrique Napoleão[1], **Patrícia Maria Guedes Paiva** [1]

[1]Dep. Bioquímica, Univ. Federal de Pernambuco (PE, Brasil), [2]Dep. Química Fundamental, Univ. Federal de Pernambuco (PE, Brasil), [3]Centro Acadêmico do Agreste, Univ. Federal de Pernambuco (Pernambuco, Brasil), [4]Laboratório de Imunopatologia Keizo Asami, Univ. Federal de Pernambuco (Pernambuco, Brasil), [5]Dep. Parasitologia, Insituto Aggeu Magalhães, Fundação Oswaldo Cruz (PE, Brasil), [6]Dep. Biofísica e Radiobiologia, Univ. Federal de Pernambuco (PE, Brasil)

INTRODUCTION: *Myracrodruon urundeuva* leaf lectin (MuLL) showed larvicidal activity against *Aedes aegypti*. This lectin is a chitin-binding protein, resistant to proteolysis by *A. aegypti* larval digestive enzymes and able to modulate protease and amylase activities from larvae gut. OBJECTIVES: Since chitin is an important component of *A. aegypti* eggs and embryos, this work evaluated the ovicidal activity of MuLL. Also, the effect of MuLL on the ultrastructure of the egg surface and its ability to penetrate the egg were investigated. MATERIALS AND METHODS: *A. aegypti* eggs were incubated with MuLL for 72 h to determine the concentration at which the hatching rate is reduced by 50% (EC50). Control eggs (100% hatching) were treated with distilled water. The effect of MuLL on egg surface structure was evaluated using scanning electron microscopy (SEM). The interactions of MuLL with the internal structures of eggs and embryos were investigated using MuLL conjugated to fluorescein isothiocyanate (FITC) and fluorescence microscopy. DISCUSSION AND RESULTS: MuLL was an ovicidal agent with EC50 of 0.88 mg/mL and SEM revealed that eggs treated with MuLL for 24 and 48 h had no tubercles and showed a disrupted exochorionic network. Deformation and degeneration of the egg surface were detected after 72 h. Fluorescence microscopy showed that MuLL penetrated the eggs and bound to serous cuticle and to digestive tract of the embryos. CONCLUSION: MuLL is ovicidal agent against *A. aegypti* and the action mechanism involves damage to the egg surface structure, interaction with serous cuticle of egg and penetration in the embryos body.

Keywords: Chitin, Microscopy, Fluorescence / **Supported by:** CNPq, CAPES and FACEPE

A.17 - Characterization of a Kazal-type Serine Protease Inhibitor Modulated in Aedes aegypti Mosquitoes Infected with Dengue Virus 2

Giulia Scomparim [1], Verônica de Moraes Manzato[1], Camila de Morais Coelho[2], Gloria Alejandra Gallo Enamorado[2], Martin Rodrigo Alejandro Würtele Alfonso[2], Ricardo José Soares Torquato[1], Aparecida Sadae Tanaka[1]
[1]Bioquímica, Univ. Federal de São Paulo (SP, Brasil), [2]Ciência e tecnologia, Universidade Federal de São Paulo (SJC, Brasil)

INTRODUCTION: Arboviruses are epidemic diseases caused by virus such as Dengue, Yellow Fever, Chikungunya and Zika transmitted by the adult female *Aedes aegypti*. The main control method for these arboviruses is still the vector control. Therefore, any knowledge about the physiology of the mosquito can help in the development of alternative methods for the vector control. OBJECTIVES: The aim of this study is to characterize a putative serine protease inhibitor of the Kazal-type family (AaKz211) identified in the *Ae. aegypti* transcriptome. MATERIALS AND METHODS: AaKz211 nucleotide sequence was cloned into pPIC9 vector and expressed using *P. pastoris* GS115. Recombinant AaKz211 was purified by ion exchange and size exclusion chromatographies, respectively. Purified rAaKz211 was used in kinetic assays. The expression of the AAEL000211 gene was determined in different life stages and tissues (ovary, midgut, and salivary glands) of *Ae. aegypti* by qPCR. DISCUSSION AND RESULTS: AAEL000211 gene expression was higher in ovary, followed by the salivary gland and less in midgut. Its expression did not present statistically significant differences between the mosquito life cycle phases. The rAaKz211 strongly inhibited proteinase K and subtilisin A with Ki 0.25 and 2.74 nM, respectively. Interestingly, the rAaKz211 also inhibited the NS2b-NS3 protease of the Zika virus with Ki 42.77 nM but did not inhibit NS2b-NS3 protease of DENV2. rAaKz211 inhibitory activity for subtilisin A and proteinase K suggests a possible role in the control of these enzymes in bacteria. The NS2b-NS3 protease of Zika inhibition suggests a possible role of AaKz211 in the control of this enzyme in the vector. CONCLUSION: AaKz211 is a strong inhibitor for bacteria serine proteases of the S8 family and the NS2b-NS3 protease of Zika but not for NS2b-NS3 protease of Dengue 2. As far as we know, this is the first Kazal-type inhibitor described for proteases for flavivirus.

Keywords: Aedes aegypti, Kazal-type inhibitor, NS2b-NS3 protease of Zika / **Supported by:** FAPESP, CNPq, CAPES, INCT-EM

A.18 - Preliminary characterization of feeding habits and insecticide susceptibility of Bradysia hygida (Diptera: Sciaridae)

Guilherme Magre Fernandes [1], Amanda Gotardi Marques[1], João Vitor Cardoso Uliana[2], Nadia Monesi[1,2]
[1]Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto - Universidade de São Paulo (São Paulo, Brasil), [2]Programa de Biologia Celular e Molecular, Faculdade de Medicina de Ribeirão Preto - Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: Sciarids feed on decomposing biomass and participate in the soil carbon cycle. Some species constitute agricultural pests, mainly in China where widespread insecticide resistance developed. *Bradysia hygida* was isolated in the campus of USP in Ribeirão Preto, in 1965, and has been maintained in the laboratory since then. Over the years, it has been used as a model organism mainly in molecular biology studies. OBJECTIVES: To investigate the feeding habits of *B. hygida* and to determine its susceptibility to the field insecticide chlorfenapyr. MATERIALS AND METHODS: Twelve-days old larvae were fed with standard diet (decomposing *Ilex paraguariensis* leaves), artificial diet, slices of *Lentinula edodes* or *Agaricus bisporus* or no food at all. Diet performance was evaluated by the number of larvae that pupated. In the insecticide assays, three-days old pupae were brushed with different concentrations of chlorfenapyr and rates of emergence were daily assessed. DISCUSSION AND RESULTS: Larvae fed with *Lentinula edodes* did not pupate, and died after nine days, at the same time as the unfed larvae. In contrast, about 73% of larvae fed with *Agaricus bisporus* pupated, at the same time as the standard diet group. Our results also show that *B. hygida* is susceptible to chlorfenapyr (LD50=315 $\mu g.L^{-1}$), and that more than 60% of the pupae did not emerge when treated with a 800 $\mu g.L^{-1}$ solution. CONCLUSION: Preliminary results indicate that *B. hygida* does not feed on *Lentinula edodes* but completes larval development when fed on *Agaricus bisporus*. This may be related to the different compositions of the cell wall of the two tested fungi. Preliminary results also suggest that *B. hygida* is more susceptible to chlorfenapyr than other sciarids. Together our results expand the knowledge about the feeding habits and insecticide resistance of a sciarid laboratory species.

Keywords: Bradysia hygida, feeding habits, insecticide resistance

A.19 - Metabolic remodeling in embryonic tick cells during nutricional restriction as a strategy to survival

Cintia Lopes Nogueira [1], Angélica Fernandes Arcanjo Sampaio[1], Maria Elisa Gosmes Lima[1], Renato Martins da Silva[1], Carlos Jorge Logullo de Oliveira[1]
[1]IBqM, Universidade Federal do Rio de Janeiro (RJ, Brasil)

INTRODUCTION: Ticks are obligatory hematophagous ectoparasites and have very peculiar characteristics in their life cycle. To guarantee their survival, there is a metabolic remodeling in which energy reserves are mobilized. Although much research has been done on the metabolism of the *Rhipicephalus microplus* tick, the mechanisms by which the regulation of energy metabolism occurs for a long period in the complete absence of nutrients remain unknown. OBJECTIVES: In this work, mechanisms are being investigated by which embryonic cells of the *Rhipicephalus microplus* tick, conditioned to a prolonged fasting, can survive. MATERIALS AND METHODS: To assess cell viability, the MTT assay was used. The main genes involved in energy metabolism were evaluated using real-time PCR, the content of glycogen, protein were performed using colorimetric kits. DISCUSSION AND RESULTS: We observed that BME26 cells survive even in the total absence of nutrients, with a drop in viability only after 24 hours. Initially, a transcriptional analysis of the main genes involved in glucose metabolism was performed. It was observed that the transcription of PEPCK, one of the main gluconeogenic enzymes, increases significantly after 6h, 24h and 48h of fasting. In addition, it is possible to show by microscopy a conformational alteration of these cells after fasting, presenting a cytoplasmic retraction. The glycogen content present in these cells showed a significant difference in 24 hours in response to fasting. CONCLUSION: The present set of results indicates that these cells can withstand prolonged periods of fasting through a fine network of metabolic adaptations. These findings will contribute to a better understanding of the metabolism regulation in the Rhipicephalus microplus tick, as well as in other arthropods, since these pathways tend to be highly conserved among disease vectors, giving us greater knowledge for the development of strategies in the blocking the transmission of pathogens.

Keywords: Rhipicephalus microplus, metabolism, starvation / **Supported by:** Capes

A.20 - Silencing of The Heme-Exporter FLVCR Promotes Mitochondrial Biogenesis and Intracellular Redox Imbalance in the Hematophagous Insect *Rhodnius prolixus*

Ana Beatriz Walter Nuno Da Silva [1,2], Marcus Fernandes Oliveira[3,4], Pedro Lagerblad De Oliveira[1,2], Gabriela De Oliveira Paiva E Silva[1,2]
[1]Instituto de Bioquímica Médica Leopoldo de Meiss, Laboratório de Artrópodes Hematófagos, Universidade Federal do Rio de Janeiro (RJ, Brazil), [2]INCTEM, Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular (RJ, BRAZIL), [3]Instituto de Bioquímica Médica Leopoldo de Meiss, Laboratório de Bioquímica Redox (Rio de Janeiro, Brazil), [4]Instituto Nacional de Ciência e Tecnologia de Biologia Estrutural e Bioimagem (I, Laboratório de Inflamação e Metabolismo, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brazil)

INTRODUCTION: The *Rhodnius prolixus* insect, vector of Chagas Disease, have to deal with large amounts of heme released during the blood digestion. When free, heme molecules are cytotoxic. Thus, a tight control of intracellular heme levels must coordinates heme synthesis, degradation, and trafficking between intracellular compartments to avoid cellular damage. Contrary to the well-characterized heme synthesis and degradation pathways, the mechanisms of heme transport in *R. prolixus* remains unclear. We identified the heme-exporter Feline Leukemia Virus C Receptor (FLVCR) orthologue in *Rhodnius* genome OBJECTIVES: The aim of this work is to characterize its function and impact on heme metabolism. MATERIALS AND METHODS: the levels of hemolymphatic heme, biliverdin, a product of heme degradation and citrate synthase activity were monitored in the females midgut by spectrophotometry. ROS production and mitochondrial content were determined in a fluorescence microscopy using the redox-sensitive DHE and mitotracker probes, respectively.The expression of antioxidante genes were measured by real-time PCR. The silence of FLVCR gene was by the RNAi technique. DISCUSSION AND RESULTS: FLVCR KD reduced hemolymphatic levels of heme and increase billiverdin in the midgut. FLVCR-silenced females produced higher levels of ROS then control females. Accordingly, dsFLVCR-injected females had an increase in lipid peroxidation, configuring a redox imbalance in this tissue. Also, FLVCR-silenced insects showed an increase antioxidant enzymes gene expression when compared to controls. Mitochondrial content were higher in FLVCR silenced females, although cytochrome c oxidase activity were reduced, indicating that FLVCR activity interferes in the mitochondria content and activity. CONCLUSION: FLVCR acts as heme exporter in R. prolixus and heme efflux from the lumen to the hemolymph is critical for the control of intracellular heme levels and for the maintenance of intestinal homeostasis/redox balance. FLVCR silencing also promotes the biogenesis of a dysfunctinal mitochondria, indicating that this transporter has a paramount role in mitochondrial physiology.

Keywords: *Rhodnius prolixus*, FLVCR , heme- exporter / **Supported by:** FUNDAÇÃO DE AMPARO À PESQUISA DO RIO DE JANEIRO (FAPERJ)

A.21 - Characterization of a Novel Sphingomyelinase-Like from the Rhipicephalus microplus Tick

Fernando Allan Abreu Silva [1], Gabriel Cerqueira Alves Costa[1], Ricardo José Soares Torquato[1], Itabajara da Silva Vaz Junior[2], Aparecida Sadae Tanaka[1]
[1]Bioquímica, Universidade Federal de São Paulo (São Paulo , Brasil), [2]Patologia Clínica Veterinária, Universidade Federal do Rio Grande do Sul (Rio Grande do SUL, Brasil)

INTRODUCTION: Rhipicephalus microplus is a tick responsible for economic losses in cattle production. To nowadays, there is no effective vaccine available to control this parasite. Therefore, many research groups have been investigating tick proteins with potential to act as novel vaccines. The sphingomyelinase is an enzyme, which catalyzes sphingomyelin hydrolysis. Recently, it was described a sphingomyelinase-like from *Ixodes scapularis* saliva able to modulate the host immune response and it is important to flavivirus transmission via exosomes. Our group identified a coding sequence for a sphingomyelinase-like in *R. microplus* transcriptome. OBJECTIVES: Heterologous expression and characterization of a novel sphingomyelinase-like (Smase) from R. microplus salivary gland. MATERIALS AND METHODS: The amplicon containing the Smase coding sequence was obtained by PCR using cDNA of partially engorged female tick and cloned into pET14b expression vector. The recombinant protein was expressed in *E. coli* BL21 pLysS and purified by His-affinity and ion exchange (Q-sepharose) chromatographs. Purified Smase was used in western blotting assays to detect the presence of anti-Smase antibodies in serum from naturally infested cattle. Smase-gene relative transcription levels in different tissues were analyzed by qPCR. DISCUSSION AND RESULTS: The Smase gene transcripts were higher in salivary glands when compared to ovary and midgut. The recombinant Smase was expressed as inclusion bodies and the active form of the Smase has not obtained yet, although it was used various refolding protocols. The results showed that serum from bovine highly infested by tick can recognize the Smase. CONCLUSION: The sphingomyelinase-like is more transcript in salivary gland and the serum from tick-infested cattle recognized the Smase, suggesting this protein is inoculated during the tick infestation, it can have a role in host-parasite relationship and could be a useful target to vaccine development. The perspective will be to obtain the active Smase using the yeast expression system and perform a functional characterization.
Keywords: tick, vaccine, antigen / **Supported by:** CAPES, CNPq, FAPESP e INCT-EM

A.22 - Activation of Macrophages by Parachartergus fraternus Venom

Jóyce dos Santos Lencina [1], Mônica Cristina Toffoli Kadri[1], Jessica Araujo Isaias Muller[1], Márcia Renata Mortari [2]
[1]FACFAN, Universidade Federal de Mato Grosso do Sul (Mato Grosso do Sul, Brasil), [2]Neurofarmacologia, Universidade de Brasília (Mato Grosso do Sul, Brasil)

INTRODUCTION: Parachartergus fraternus is a wasp species endemic in Brazilian Cerrado and induces inflammation with predominance of mononuclear cells that are activated in this process. Among the functions of these cells, there are adhesion and spreading to phagocytize injurious agents, and to date there is no description that Pfv induces these effects. OBJECTIVES: Evaluate the adhesion, spreading, and phagocytosis activity of macrophages stimulated with P. fraternus venom (Pfv). MATERIALS AND METHODS: For the assays were used three Pfv concentrations (2.5, 5, and 10 µg/mL). For adhesion assay, macrophages suspensions were dispensed into 96-well plates and incubated with Pfv at 37ºC, 5% CO2 for 2h. After incubation, the wells were washed, and the remaining cells were stained with violet crystal and the absorbance read at 540 nm in spectrophotometer. For cell spreading assay, Pfv were used and incubated under the same conditions in 24-well plates. The spread index was determined based on morphological parameters, in phase contrast microscopy, by counting the number of spread cells in a total of 100 cells. For phagocytic activity assay, macrophages and zymosan were incubated in the presence of Pfv for 1h in an incubator. Were counted 100 cells per field and macrophages that phagocytized one or more zymosan particles were considered in phagocytosis. All the results were expressed in %. As a negative control, cells were maintained without stimuli. Results expressed as mean ± SEM; ANOVA; Bonferroni; $p < 0.05$. DISCUSSION AND RESULTS: The increase of adhesion was 23.98% (2.5 µg/mL), 20.50% (5 µg/mL) and 24.30% (10 µg/mL). The spreading increased in 23.86%, 32.22%, and 35.71% at the concentrations 2.5, 5 and 10 µg/mL, respectively. Phagocytosis was potentiated in 62.67%, 59.33% and 68,00% at the same concentrations, respectively. CONCLUSION: Pfv activated macrophage *in vitro*, with increased adhesion, spreading, and phagocytosis activity, indicating that this venom modulates mononuclear cells functions.
Keywords: inflammation, mononuclear cells, wasp / **Supported by:** CAPES and UFMS

A.23 - Role of the E75 Nuclear Receptor in the Lipid Metabolism of the Beetle Tribolium castaneum

Lívia Coutinho da Cruz [1,2], Alessa Macedo[1,3], David Majerowicz[1,4]
[1]Departamento de Biotecnologia Farmacêutica, Faculdade de Farmácia. , Universidade Federal do Rio de Janeiro (Rio de Janeiro , Brazil), [2]Instituto de Biologia, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brazil), [3]Programa de Pós-Graduação em Biociências, Universidade do Estado do Rio de Janeiro (Rio de Janeiro, Brazil), [4]Rio de Janeiro, Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular (Rio de janeiro, Brazil)

INTRODUCTION: E75 is a nuclear receptor with several roles described in insect physiology, such as development, metamorphosis, reproduction, and oogenesis. However, there is little information about the functions of E75 in lipid metabolism. Mutant flies for this gene show an increase in the amount of triacylglycerol stored; e75 knockdown by RNAi has the same effect. Moreover, the inhibition of nitric oxide production, which regulates the activity of E75, leads to an accumulation of fat in the fat body of larvae. These results indicate that E75 must have essential functions in insect lipid metabolism, but the mechanisms by which this regulation takes place are still unknown. OBJECTIVES: To study the importance of the TcE75 nuclear receptor in regulating lipid metabolism in the insect Tribolium castaneum. MATERIALS AND METHODS: We used the beetle T. castaneum as a model for lipid metabolism. e75 gene expression was measured by quantitative PCR. e75 gene expression was inhibited by RNAi. The amount of triacylglycerol was measured by a colorimetric enzymatic assay. DISCUSSION AND RESULTS: The expression of the e75 gene is stable during the development of the immature insect, from the different stages of larvae to pupa. On the other hand, treating larvae with a diet supplemented with powdered egg yolk, with high fat and protein content, seems to increase e75 expression. Furthermore, e75 knockdown by RNAi appears to induce an increase in the amount of triglyceride in adult beetles. CONCLUSION: These results indicate that E75 plays a role in lipid metabolism also in T. castaneum.
Keywords: E75, Lipid metabolism, Tribolium castaneum

A.24 - Intermediate and Final Digestion of Proteins along the Midgut of Musca domestica Larvae. A Transcriptomic Approach

Ignacio Barroso[1], Clélia Ferreira[1], Walter Terra[1]
[1]bioquímica, Instituto De Quimica, Universidade De São Paulo (São Paulo, Brazil)

INTRODUCTION; Protein digestion comprises three stages, primary, intermediate and final. The last two stages are probably performed in the insect midgut by carboxypeptidases (CPs), aminopeptidases (APs) and dipeptidases. OBJECTIVES: The present work aims to disclose the molecular bases of the intermediate and final protein digestion in the midgut of *Musca domestica*. MATERIALS AND METHODS: To this end, gene expression of CPs (A, B, S10C and MC), APs (A and N) and dipeptidases were analysed by RNAseq from seven midgut sections of *M. domestica* larvae, and the local of action was suggested by bioinformatics and proteomics of microvillar membrane-enriched samples. DISCUSSION AND RESULTS: CPA and dipeptidases were expressed along the posterior midgut, CPB and CPS10C between the middle and the posterior midgut, and CPMC and APs in the second half of the posterior midgut. Moreover, the prediction of transmembrane domains, glycosylphosphatidylinositol anchor and signal peptide suggest luminal and microvillar locations for CPs and APs, respectively. In agreement with that, most APs were found in microvillar membrane-enriched samples. After digestion, amino acids and peptides are absorbed along the midgut, mainly by symporters expressed there. Indeed, the expression profile of enzymes and amino acid transporters (AT) suggests a relationship between the enzymatic products and the transporter substrates. Thus, symporters of neutral amino acids such as NAT and PAT (Na^+-driven and Proton-coupled AT) were highly expressed in the same regions as APN and CPA, which release mainly Ala and Phe. In addition, similar expression profiles of the Proton-coupled peptide transporter and the dipeptidases reinforce this relationship. The same connection appears between the CAT family (cationic AT), which transport charged amino acids, and APA, which releases Glu. CONCLUSION: To conclude, intermediate and final digestion occurs along the posterior midgut region involving CPs, APs and dipeptidases and their enzymatic products are absorbed mainly at the end of the midgut by NATs, PATs and CATs.
Keywords: insect, protein digestion, amino acid absorption
Supported by: Fundação de Amparo à Pesquisa do Estado de São Paulo

A.25 - Identification of the enzyme(s) responsible for prostaglandin synthesis in Aedes aegypti

Dayane dos Santos Silva[1], Vivian Garbocci[1], Ana Beatriz Barletta[1], Marcos Henrique Sorgine[1]
[1]Instituto de Bioquímica Clínica Leopoldo de Meis, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: In previous works, our group has demonstrated the importance of prostaglandin as mediator of humoral immune response in mosquitoes. According to the literature, insects do not have the cyclooxygenase family, enzymes responsible for prostanoids biosynthesis from arachidonic acid in vertebrates. In Drosophila melanogaster, a peroxinectin (Pxt) has been described as responsible for the synthesis of these eicosanoids and perform the cyclooxygenase-like function. OBJECTIVES: Identify mosquito peroxinectin responsible for prostaglandin biosynthesis in Aedes aegypti MATERIALS AND METHODS: An in sílico analysis revealed four putative peroxinectins in Aedes genome. We fed the mosquitoes with blood containing or not vírus or bacteria in the presence or absence of prostaglandin inhibitors and analyzed prostaglandin content, microbiota and vírus levels. DISCUSSION AND RESULTS: The expression of Pxt027 is increased after blood or bacterial meal, and depletion of intestinal microbiota decreases its expression. Bacteria containing meals increase total prostaglandin biosynthesis in the mosquito´s midgut. We knocked down the expression of these genes through RNAi. Pxt027 knock down increases intestinal microbiota, and mosquitoes' mortality after bacterial meal. Pxt030 knock down increases Zika susceptibility in mosquito carcass. CONCLUSION: We found evidence that pxt027 is one of the most important enzymes involved in prostaglandin synthesis in Aedes aegypti.

Keywords: Aedes aegypti, peroxinectin, prostaglandin

A.26 - Chitin in the Perimicrovillar Membrane Fraction of *Rhodnius prolixus*, an Insect Vector of Chagas Disease

Thiago Silva do Nascimento [1], Brenda Martins Vasconcellos[2], Evelyn Seam Lima de Alvarenga[2], Jessica Pereira[3], Georgia Atella[3,4], Isabela Ramos[3,4], Mônica Ferreira Moreira Carvalho Cardoso[2,4]
[1]Graduação, Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro (Rio de Janeiro, Brasil), [2]Departamento de Bioquímica, Laboratório de Bioquímica e Biologia Molecular de Vetores, Instituto de Química, [3]Laboratório de Bioquímica de Insetos, Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro. (Rio de Janeiro, Brasil), [4]INCT-EM, Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular (Rio de Janeiro, Brasil)

INTRODUCTION: Chagas disease is a neglected disease and one of its main vectors is *Rhodnius prolixus*. This insect has inside its midgut the perimicrovillar membrane (PMM), a structure that covers the intestinal microvilli and is related to the processes of digestion, mechanical protection, against pathogens, parasite differentiation and possibly other functions not yet discovered. Alvarenga et al. (2016) confirmed the chitin presence in the vector intestine, but not specifically in the PMM. OBJECTIVES: The aim of this work was to investigate and characterize chitin in the PMM fraction of *Rhodnius prolixus* found in the intestinal lumen. MATERIALS AND METHODS: A white precipitate was extracted from the luminal content of *R. prolixus* after lipid extraction, papain and hot KOH treatments. To confirm the chitin nature, the extracted precipitate was analyzed by infrared spectroscopy, NMR, fluorescence microscopy by labeled of wheat germ agglutinin conjugated with fluorescein isothiocyanate (FITC-WGA), scanning microscopy and photography always in comparison with Sigma's commercial chitin. For PMM separation was performed a sucrose gradient (20 to 60%) in ultracentrifugation process and the addition of FITC-WGA to locate the chitin. DISCUSSION AND RESULTS: The IR and NMR spectra of the lumen purified material were very similar to commercial crab chitin. After ultracentrifugation, the sucrose gradient profile was drawn with the aid of a refractometer and the chitin fraction revealed with the FITC-WGA, the fluorescence labeled was found in the 30-40% sucrose gradient fractions. The fluorescence gradient peaks were also evaluated by fluorescence microscopy technique and chitin was co-located with PMM. CONCLUSION: According to the results obtained in this work, chitin of midgut and PMM of *R. prolixus* were found in the same portion of the sucrose gradient. It can be a strong indication of biopolysaccharide presence in the insect PMM.

Keywords: Chitin, Perimicrovilar membrane, *Rhodnius prolixus*
Supported by: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

A.27 - The role of small heat-shock proteins in the physiology and vector competence of *Rhodnius prolixus*, an insect vector of chagas disease

Tainan Carla Guedes da Silva [1], Pedro Lagerblad de Oliveira[1,3], Rafael Dias Mesquita[2], Hugo Diego Perdomo Contreras[1], Felipe Almeida Dias[1]
[1]Laboratório de Bioquímica de Insetos Hematófagos , Instituto de Bioquímica Médica Lepoldo de Meis (Rio de Janeiro, Brasil), [2]Departamento de Bioquímica, Instituto de Química (Rio de Janeiro, Brasil), [3]Entomologia Molecular, Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular (Rio de Janeiro, Brasil)

INTRODUCTION: *Rhodnius prolixus*, a vector of Chagas disease, takes several times its own weight in a single blood-meal. The midgut homeostasis is achieved by preventing tissue damage upon potential stressors generated during blood digestion, microbiota increasing or pathogen's infections. The transformation of non-infecting forms into infective forms of the *Trypanosoma cruzi* protozoan that occurs in the digestive system of its vector, is an important step in the life cycle of the parasite and consequently for the transmission of Chagas disease. Transcriptomic analysis of midgut from first stage nymphs showed differential gene expression of members of the Small heat-shock proteins (sHSP) family upon infection. The sHSP are proteins involved in response to heat shock and other stress responses, and function primarily as chaperones avoiding protein misfold. OBJECTIVES: The present study aims to investigate the role of sHSP in the physiology of *R. prolixus* and in its vector competence. MATERIALS AND METHODS: Analysis of a midgut transcriptome of first stage nymphs indicated candidate genes. sHSP gene function and parasite infection were analyzed by qPCR, gene silencing and exposing insects to heat-shock treatment. DISCUSSION AND RESULTS: Five sHSP genes were upregulated (50-200x) by blood feeding and downregulated by infection (3-20x). These sHSP transcripts were also overexpressed by heat-shock (60-1000x), confirming their *in silico* identification as sHSP. Silencing of the sHSPs genes affected digestion and microbiota abundance. *In silico* gene promoter analysis revealed putative binding sites for the transcription factor Ecdysone-induced protein 74 (E74), suggesting a hormonal signaling regulation. While simultaneous knockdown of the five modulated sHSP caused an increase in the amount of parasites in the insect gut, putative E74 knockdown caused a decrease, confirming our hypothesis that E74 works as an inhibitor of sHSP genes during infection. CONCLUSION: We identified here a group of sHSPs that appears to be involved in homeostatic response to blood ingestion and *T. cruzi* infection.
Keywords: Chagas Disease, Small Heat-Shock Protein, Ecdysone / **Supported by:** CNPq, FAPERJ, INCT-EM

A.28 - Detection of Dengue, Zika and Chikungunya Viruses in Mosquito Eggs - A Tool for Arboviruses Disease Surveillance

Maria Luiza dos Santos M. Conceição [1], Brenda Martins Vasconcellos [1], George V. Alves Neri[2], Ana Carolina Pereira e Silva[5,1], Tiago Souza Salles[1], Maulori Curié Cabral[1], Adriana Sotero-Martins[4], Monica Ferreira Moreira[1]
[1]Depto De Bioquimica - Instituto De Quimica, Univ. Federal Do Rio De Janeiro (RJ, Brasil), [2]Depto De Ensino, Univ. Federal Do Maranhão (Maranhão, Brasil), [3]Depto De Virologia, Univ. Federal Do Rio De Janeiro (RJ, Brasil), [4]Depto De Saneamento E Saúde Ambiental, Fundação Oswaldo Cruz (RJ, Brasil), [5]Graduação, Instituto Federal De Ciências E Tecnologia Do Rio De Janeiro (Rio De Janeiro, Brasil), [6]INCT/Entomologia Molecular, Universidade Federal Do Rio De Janeiro (RJ, Brasil)

INTRODUCTION: Zika (ZIKV), Chikungunya (CHKV) and Dengue (DENV) viruses are spreading by the Aedes mosquitoes in worldwide. In Brazil, the epidemiological bulletin (MS) informs from 1st January to the 3rd week of May 2022 occurred 9,318 severe dengue cases with higher lethality rate, 382 deaths. As for chikungunya, from 02/01/2022 to 05/14/2022 were registered 14 deaths. While for Zika, from 1/2/2022 to 4/30/2022 there were no records of deaths. OBJECTIVES: The aim of this work was to detect the ZIKV, DENV1-4 and CHKV through molecular methods in georeferenced *A. aegypti* eggs from Timon city – MA - Brazil. MATERIALS AND METHODS: Nine pools of 50 eggs from georeferenced mosquitrump were used to detect ZIKV, DENV1-4 and CHKV genomic material by RT-PCR and quantitative- PCR techniques. The positive viral egg samples in qPCR assays were submitted to Sanger sequencing reactions. The RNA sequences from Sanger procedures were used as a template for NCBI BLASTn searches. All sequences obtained with more than 90% of identity were considered homologous, being used as Datasets in the ClustalW online tool analysis, the phylogenetic tree was performed in the MEGA11 software. DISCUSSION AND RESULTS: From 9 pools of egg samples tested, 6 showed positive results for DENV-2. On the other hand, there were no positive results for the other DENV serotypes, as well as for ZIKV and CHIKV. The sequences with the highest similarity for the 6 positive samples were: FJ461309.1, KY849760.1, MW579053.1, all of Asian origin. The phylogenetic tree showed the Asian origin for the DENV2 strains circulating in Timon city, possibly being these strains responsible for the DENV outbreaks that are occurring in Maranhão state. CONCLUSION: The qPCR and Sanger sequencing results together can validate the qPCR technique as a good tool for vector surveillance for DENV2 in georeferenced mosquito eggs, as seen in Timon.
Keywords: Aedes aegypti, Arboviruses, vector surveillance / **Supported by:** Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro – FAPERJ

A.29 - The first step of a relationship: Mechanisms involved in microbiota acquisition of *Rhodnius prolixus*, a Chagas disease vector

Leonan Azevedo dos Reis [1], Pedro Oliveira[1,2]
[1]Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil),
[2]Entomologia Molecular, Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular (, Brasil)

INTRODUCTION: Microorganisms are known to colonize several environments, including living tissues and cells. Its interaction with metazoans shapes their development dynamics on many levels. The classical literature highlights the importance of a mutualist symbiont, the bacteria *Rhodococcus rhodnii*, on the blood-sucking *Rhodnius prolixus* physiology. OBJECTIVES: This study aimed to investigate the acquisition and settlement of the symbiont by axenic first instar nymphs of *R. prolixus*. MATERIALS AND METHODS: Axenic and non-axenic nymphs were reared, and the colonization dynamics was assessed by reintroducing the symbiont on axenic nymphs through contaminated feces. DISCUSSION AND RESULTS: The symbiont growth showed a plateau around day eight post-feeding. Remarkably, feeding nymphs with different amounts of the symbiont resulted in a similar common population size attained by day four post-feeding. These observations indicate a fine tunned control mechanism that limits the symbiont growth within the host. Transcriptomic analysis of gastrointestinal tract of naturally colonized nymphs unveiled the upregulation of over 130 immune-related genes after a blood meal. Expression of antimicrobial peptides such as Defensins and Lysozymes reaches up to 1500-fold change after a blood meal. Therefore, we compared the modulation of several genes related to different immune pathways, in both axenic and non-axenic nymph populations. Surprisingly, the upregulation of antimicrobial peptides such as Defensin C and Lysozyme A seems to occur independently of the colonization by the symbiont. CONCLUSION: These observations indicate the activation of innate immune pathways in the midgut of the newly hatched insect does not occur in response to the proliferation of the microbiota, but rather is part of the developmental program of the insect.

Keywords: Microbiota, Symbiont, Interaction / **Supported by:** CAPES

A.30 - The Role of Kynurenine Pathway on Intestinal Microbiota-control in Aedes aegypti Mosquitoes

Igor Ferreira da Costa de Almeida [1], Pedro L. de Oliveira[1], Vanessa Bottino Rojas [2], Rodrigo Dutra Nunes[3]
[1]Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil),
[2]Department of Microbiology & Molecular Genetics, University of California (Califórnia , Estados Unidos),
[3]Biochemistry and Molecular Biology, Johns Hopkins University (Baltimore , Estados Unidos)

INTRODUCTION: Blood digestion in mosquitoes leads to large amounts of free amino acids in the midgut, reaching concentrations potentially toxic despite its nutritional role. We recently showed that tryptophan metabolites from the Kynurenine Pathway (KP) are involved in fitness maintenance and modulation of intestinal microbiota post blood-feeding (PBF). OBJECTIVES: The goal of this work is to find which mechanisms are behind the KP influence on the control of PBF microbiota expansion, and check the susceptibility of *White-eye* (Wh) mutant strain ($KMO^{-/-}$; lack for the third step of KP) to virus infection. MATERIALS AND METHODS: We knocked down (RNAi) some KP genes and genes of the canonical *Aryl hydrocarbon Receptor* (AhR) transcription factor pathway (which is activated by tryptophan metabolites) and quantified the microbiota via 16s (qPCR). To verify if tryptophan intermediates directly act on microbiota expansion, we performed a comparative growth rate assays with five different isolated bacterial colonies in the presence of three KP metabolites. DISCUSSION AND RESULTS: Silenced Wh females for *Kynurenine Amino-Transferase* (KAT) and AhR gene showed a significant reduction in microbiota expansion PBF. These results indicate that both pathways are involved in establishing intestinal microbiota with tolerance mechanisms. We observed that xanthurenic acid inhibited growth in one of five colonies. In contrast, kynurenine and kynurenic acid just decrease the growth of four other bacteria species from the indigenous microbiota. This result indicates a direct control made by KP metabolites on specific members of the microbiota, and the presence of some intermediates drives the growth pattern. The role of the KP in the regulation of arbovirus infection is being investigated. CONCLUSION: These findings highlights a major role of the KP/AhR axis in the regulation of intestinal ecology in *Aedes aegypti* mosquitoes.

Keywords: Kynurenine-Pathway, AhR-Pathway, Microbiota

A.31 - Characterization of esterase in *Rhodnius prolixus* antennae

Victoria Brigido Lamim [1,2], Larissa Santos de Oliveira[1], Gabrielle Carvalho Miguens Cabo[1], Nathália Faro de Brito[1], Ana Claudia do Amaral Melo[1]
[1]Bioquímica, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil), [2]Programa de Pós Graduacao em Bioquímica, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: American trypanosomiasis is a neglected tropical disease, which affects 7 million people around the world. In the endemic area, vector transmission is the main form of contamination. Insects from the Reduviidae family are responsible for maintaining this zoonosis. The ability to transmit the *Trypanosoma cruzi*, the etiological agent of Chagas disease, by *R. prolixus* depends on the ability to find the vertebrate. Odors and pheromones are transported by specific proteins through antenna until odorant receptors (OR), an olfactory process that culminates in a specific behavior. Odorant degrading enzymes (ODEs) have an important role in semiochemical inactivation. It is well known that some members of the carboxylesterase (CXE) have the ability to degrade odorants. OBJECTIVES: The main goal of this study is the characterization of esterases expressed in antenna. MATERIALS AND METHODS: The methodology consists of bioinformatics analysis. DISCUSSION AND RESULTS: Proteome showed 78 esterases candidates in the antenna, among which 1 enzyme were classified as CXEs. Phylogenetic analysis comparing *R. prolixus* CXE candidates with 10 ODEs from different insects revealed that 6 proteins were grouped with CXEs, suggesting those proteins could be ODEs. Antennae were homogenized and esterases were separated on 10% native polyacrylamide gel stained with α-β-naphthyl acetate, confirming esterase activity. Initially, PRPC001592 from antenna proteome was selected for the present study. Analysis of primary sequence showed that protein is an α-β hydrolase (CL0028), with 1107aa, theoretical pI of 6.29, MW 113.78 kDa. The COesterase domains were localized between 5-500, and 44-502 aa. A tridimensional model was constructed based on α-esterase-7-carboxylesterase from *Lucilia cuprina*, showed 100% confidence. Preliminary docking suggest affinity between PRPC001592 and three esters produced by Brindley's glands: isobutyl propanoate, amyl isobutyrate, and hexyl butyrate. Gene expression profile is being processed. CONCLUSION: Those results suggests that *R. prolixus* antenna present esterase activity, suggesting odorants are degraded by this enzyme, in accordance to what is observed in other insects.
Keywords: *Rhodnius prolixus*, Odorant degrading enzyme, esterase / **Supported by:** FAPERJ, CAPES, CNPq, INCT-EM

A.32 - A Bovine Tick Protein Disulfide Isomerase (Rm-PDI) Promotes Mechano-Redox Control During Sepsis Thrombopathies in Mice

Josias Alves Machado[1], Fernanda Antunes[1], Teresa M. Monteiro[1], Leonarda de Souza Silva[1], Maria Eduarda de Souza Fernandes[1], Itabajara Vaz[2], Carlos Logullo[3]
[1]Clinica e Cirurgia, Univ. Estadual do Norte Fluminense (RJ, Brasil), [2]Depto de Biologia Molecular e Biotecnologia, Univ. Federal do Rio Grande do Sul (Rio Grande do Sul, Brasil), [3]Bioquímica Médica , Univ. Federal do Rio de Janeiro (RJ, Brasil)

INTRODUCTION: Sepsis, one of the deadliest diseases worldwide, results in multiple organ failure, usually caused by hemostasis disorders and uncontrolled inflammatory responses. Protein disulfide isomerase (PDI) promotes thrombus formation but does not affect physiological blood hemostasis. It was recently shown that ERp5, a member of the PDI family, was required for thrombus formation *In vivo* by binding directly to αIIbβ3 integrin. Paradoxically, ERp5 is able to mediate the de-adhesion of fibrinogen from activated platelet αIIbβ3 integrin. Protein chemical studies show that ligand binding to the extended αIIbβ3 integrin makes the Cys177-Cys184 disulfide bond of the βI domain cleavable by ERp5. OBJECTIVES: To investigate the mechano-redox control that governs the blood coagulation cascade, using bovine tick Rm-PDI as a molecular target in mouse models of thrombopathies. MATERIALS AND METHODS: Here, a Cecal ligation and puncture (CLP) mouse model was established to mimic sepsis for *In vivo* testing and hematological, biochemical and histological analyzes of the mice were performed. We also identified and characterized an ERp-5 ortholog Rm-PDI that shows high homology with mouse ERP-5 and is highly expressed in tick salivary glands. DISCUSSION AND RESULTS: Amino acid sequence analyzes indicate that Rm-PDI-1 has a 16 amino acid cleavable N-terminal signal peptide, suggesting that it is similar to a typical PDI molecule. Rm-PDI contains two typical CXXC PDI active sites and encodes 435 amino acids. putative. Comparative amino acid alignment for RmPDI-1 showed high identity with Hemaphysalis longicornis genes (HlPDI) and with Ixodes scapularis genes (IsPDI). Molecular Docking and Molecular Dynamics showed conformational change in the presence of DTNB, which may impair the interaction with αIIbβ3 integrins. The animals submitted to Sepsis showed a decrease in the number of platelets, an increase in renal markers (urea and creatinine) and alteration in the hemostatic tests. CONCLUSION: Future studies will show even better the mechano-redox regulation between RmPDI-1 and αIIbβ3 Integrins. **Keywords:** Protein Disulfide Isomerase, RmPDI-1, Sepse / **Supported by:** Faperj

A.33 - Cytotoxicity and Production of Reactive Oxygen and Nitrogen Species in Murine Macrophages Stimulated with Parachartergus fraternus Venom

Iluska Senna Bonfá[1], Jéssica Muller[1], Joyce Lencina[1], Márcia Mortari[2], Monica Kadri[1]
[1]Faculdade de Ciências Farmacêuticas, Alimentos e Nutrição, FACFAN, Federal University of Mato Grosso do Sul (Mato Grosso do Sul, Brasil), [2]Laboratory of Neuropharmacology, ICB, University of Brasilia (Brasília, Brasil)

INTRODUCTION: *Parachartergus fraternus* is a wasp that inhabits Cerrado areas in Brazil. Its venom triggers inflammatory process in victims with important participation of mononuclear cell, like macrophages. However, the knowledge about this venom on macrophage activation is scarce. OBJECTIVES: Evaluate the cytotoxicity and production of reactive oxygen and nitrogen species in macrophages stimulated with *P. fraternus* venom (Pfv). MATERIALS AND METHODS: Murine macrophages were used at 2×105 cells/well (n=3, triplicate) and incubated at 37ºC, 5% CO2. The concentrations of Pfv were 2.5, 5, and 10 µg/mL, 100 µL. In cytotoxicity (MTT) and production of nitric oxide (NO) assays, Pfv was added and after 48h the supernatant was transferred to a plate with Griess Reagent for NO quantification. MTT was added to the remaining cells for cytotoxicity evaluation. In NO assay the cells were also incubated with Pfv+L-NAME. The absorbance was read in an ELISA reader at 540 nm. In hydrogen peroxide (H_2O_2) assay, Pfv was added in macrophage suspensions with phenol red solution. After 1h, the absorbance was read at 620 nm. NO and H_2O_2 concentrations were obtained by comparison with a standard curve and the results were expressed in percentage and µM NO_2^- or H_2O_2, respectively. As a negative control, cells were maintained without stimuli. Results expressed as mean±SEM; ANOVA; Bonferroni; $p < 0.05$. DISCUSSION AND RESULTS: Pfv at all concentrations evaluated were not cytotoxic. The incubation of Pfv with macrophage at concentrations of 5 and 10 µg/mL increased the production of NO_2^- by 60% and 62%. L-NAME reduced this production by 43% and 45%, respectively. The same concentrations of Pfv increased by 58% and 84% the H_2O_2production. CONCLUSION: Pfv was not cytotoxic in the evaluated concentrations and induced the production of NO and H2O2, maintaining the inflammatory process. The production of NO was inhibited by L-NAME, indicating a production via iNOS.
Keywords: Inflammation, *in vitro*, NO, H2O2 / **Supported by:** CAPES and UFMS

A.34 - Effect of Tityus confluens Venom on CD39 and CD73 Enzymes of the Purinergic System in Lymphocytes and Platelets

Telma Benetti[1], **Laís Corrêa de Lima** [1], Henrique Covali Pontes[1], Malson Neilson de Lucena[1]
[1]Instituto de Biociências (INBIO), Universidade Federal do Mato Grosso do Sul (MS, Brasil)

INTRODUCTION: The purinergic system is a signaling system, where the purine nucleotides, ATP (Adenosine 5'-triphosphate), and ADP (Adenosine diphosphate), and the nucleoside, adenosine, act as extracellular messengers. CD39 (ecto-nucleoside triphosphate diphosphohydrolase 1, E-NTPDase1) converts ATP or ADP into AMP, and CD73 (ecto-5'- nucleotidase, Ecto5'NTase) dephosphorylates AMP into adenosine. CD39 and CD73 regulate the function of several immune cell types, including lymphocytes. Furthermore, there is evidence that changes in CD39 expression and activity affect the potential thrombogenic of a tissue. OBJECTIVES: The objective of this study was to assay the enzymatic activity of CD39 and CD73 in the presence of scorpion venom from *Tityus confluens* (Tc) in human peripheral blood lymphocytes and platelet. MATERIALS AND METHODS: In the assay was used Tc venom (0.2 mg/mL), peripheral blood mononuclear cell (PBMC; 0.2 mg/mL) and platelet-rich plasma (PRP;0.4 mg/mL). The reactions were initiated by the addition ATP or ADP (for CD39) or AMP (for CD73). After 70 min (PMBC) or 60 minutes (PRP), the reaction was stopped by adding 10% perchloric acid solution. Malachite Green reagent (1.0 mM MLG, 0.16% PVA, 6.0 mM sulfuric acid) was added, and the absorbance at 630 nm was measured. The enzyme activity was expressed as nmol Pi/minute/mg protein.DISCUSSION AND RESULTS: The results demonstrate that T.c venom has decreased CD39 activity in ATP hydrolysis in peripheral blood lymphocytes compared to control ($P< 0.05$). On the other hand, the results demonstrate no effect on CD39 and CD73 enzymes in peripheral blood platelets. CONCLUSION: This study shows for the first time the effect of Tityus scorpion venom on CD39 and CD73 enzymes in peripheral blood cells. Other studies can elucidate the main action pathway between venoms and the blood.
Keywords: Enzymes, scorpion, Tityus sp.

A.35 - Antitrypanosomal activity of the venom from *Tityus paraguayensis*

Henrique Ranieri Covali Pontes [1], Mila Marluce Lima Fernandes[1], Laís Corrêa de Lima[1], Maria Antônia Bastos de Oliveira[1], Alda Maria Teixeira Ferreira[1], Malson Neilson de Lucena[1]
[1]Instituto de Biociências, Universidade Federal de Mato Grosso do Sul (Mato Grosso do Sul, Brasil)

INTRODUCTION: The scorpion venom apparatus consists of a pair of glands connected to a telson sting. These glands are responsible for the production and storage of venom. Scorpion venoms contain a vast chemical diversity that includes: peptides, enzymes, polysaccharides, lipids, nucleotides, and inorganic salts, among others. Several studies have identified biological activities in venom scorpions such as antimicrobial, antitumor, bradykinin potentiating and antitrypanosomal. OBJECTIVES: The purpose of this study was to evaluate the antitrypanosomal activity of the venom of the endemic scorpion *Tityus paraguayensis*. MATERIALS AND METHODS: *Trypanosoma cruzi* epimastigotes (Dm28c) were grown in liver infusion tryptose medium (LIT) at 28°C until the logarithmic growth phase. The effect of the venom on the viability of the parasites was evaluated by the MTS assay. Parasites were incubated for 72 hours at different concentrations (60/30/15/7.5/3.75 µg/mL) of the venom and kept at 28ºC. After incubation, MTS reagent (5 mg/mL) was added to the wells, and the plates were incubated for 4 hours at 37ºC. Finally, the plates were read in a microplate reader (λ 550 nm). ANOVA test was applied to compare the venom concentrations, followed by the Tukey post-test, with a p-value < 0.05 being considered statistically significant. DISCUSSION AND RESULTS: The parasite viability has decreased ($p < 0.05$) by 9%, 20%, and 18% at 15, 30, and 60 µg/mL venom, respectively. CONCLUSION: That result reveals a potential biotechnological application of the *T. paraguayensis* venom.

Keywords: Biological activity, Scorpion, T. cruzi
Supported by: FUNDECT, UFMS and CAPES

B - Bioenergetics and Metabolism

B.01 - From Nucleus to Mitochondria: a Platform for Allotopic Gene Expression in Yeast

Letícia Veloso Ribeiro Franco [1,2], Mario Henrique Barros[1], Alexander Tzagoloff[2]
[1]Department of Microbiology, Institute of Biomedical Sciences, Universidade de São Paulo (SP, Brazil), [2]Department of Biological Sciences , Columbia University (NY, USA)

INTRODUCTION: Two of the respiratory complexes and the ATP synthase of *S. cerevisiae* mitochondria are genetic hybrids composed of subunit polypeptides encoded both by mitochondrial and nuclear DNA. Radiolabeling of mitochondrial gene products followed by pulse-chase analysis is a powerful tool for studying the biogenesis of these hetero-oligomeric enzyme complexes. Although it is also possible to selectively label the nuclear gene products in the presence of inhibitor that arrest mitochondria protein synthesis, the analysis of this class of proteins is fraught with technical problems. OBJECTIVES: The present work aimed to develop a platform to allotopically express in mitochondria proteins that are normally encoded by nuclear genes. MATERIALS AND METHODS: Acetylornithine aminotransferase, a product of the nuclear *ARG8* gene, can be expressed as a functional enzyme from a recoded *ARG8^m* gene integrated into mitochondrial DNA. Here, we constructed a plasmid that allows *ARG8^m* to be expressed under the control of the mitochondrial *ATP9* gene promoter placed in a region downstream of *VAR1*. DISCUSSION AND RESULTS: We have successfully constructed a strain that contains mitochondrial *ARG8^m* under the control of *ATP9* promoter in a nuclear *arg8* background. This strain can be used to express genes appropriately modified for compatibility with the mitochondrial genetic code by biolistic transformation with a plasmid containing the gene of interest and selection of recombinants auxotrophic for arginine. CONCLUSION: We have developed a tool for the transfer of foreign genes for their expression in mitochondria.

Keywords: Mitochondria, Allotopic gene expression, *Saccharomyces cerevisiae* / **Supported by:** FAPESP 2019/16015-3, FAPESP 2019/02799-2

B.02 - Placental Neuroprostanes and Isoprostanes are Altered in Gestational Diabetes Mellitus and Maternal Obesity

Carolina dos Santos Ferreira[1], Gabriela Dias Alves Pinto[1], Claire Vigor[2], Vanessa de Araujo Goes[1], Deborah Bauer[1], Alexandre G Torres[3], Thierry Durand[2], Graham J Burton[4], **Tatiana El-Bacha** [1]
[1]Instituto de Nutricao Josue de Castro, Federal University of Rio de Janeiro (Rio de Janeiro, Brazil), [2]Institut des Biomolécules Max Mousseron, University of Montpellier (Montpellier, France), [3]Instituto de Quimica, Federal University of Rio de Janeiro (Rio de Janeiro, Brazil), [4]Centre for Trophoblast Research, University of Cambridge (Cambridge, United Kingdom)

INTRODUCTION: The rise in prevalence of obesity in women of reproductive age in developed and developing countries might propagate intergenerational cycles of detrimental effects on metabolic health. Placental lipid metabolism is disrupted by maternal obesity and gestational diabetes mellitus (GDM), which possibly affects the life-long health of the offspring. OBJECTIVES: In this study we investigated Neuroprostanes and Isoprostanes, which are non-enzymatic metabolites of docosahexaenoic (DHA) and arachidonic (AA) acids, respectively, in placentas from lean women and from women with pre-gestational obesity and with gestational diabetes mellitus. MATERIALS AND METHODS: Samples from two randomized controlled trials (NCT03215784 and NCT05174728; ethical approval 34611513.0.0000.5257 and 66949217.0.0000.5275) was used. Trials were conducted at Maternidade Escola, Federal University of Rio de Janeiro (January/2015 to March/2019). Placentas were collected at delivery from lean (n=11), obese (n=8) and gestational diabetes mellitus (7) women. Placental Neuroprostanes and Isoprostanes were quantified by targeted lipidomics using microLC-MS/MS based on standard calibration curves. DISCUSSION AND RESULTS: Placental Neuroprostanes and Isoprostanes are increased in maternal obesity compared to lean controls, but reduced to normal levels in GDM. These results were not related to differences in maternal diet nor gestational weight gain, indicating that obesity and GDM produce different placental responses in terms of DHA and AA metabolism. Our data highlight the importance of investigating the signalling roles of Neuroprostanes and Isoprostanes in the human placenta. CONCLUSION: The decrease in Isoprostanes suggests adaptive placental responses, whereas the decrease in placental Neuroprostanes suggests impairment in DHA metabolism in GDM.

Keywords: maternal obesity, oxidised fatty acids, placenta
Supported by: Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro [E-26/110.111/2014, E-026/203.254/2017); Isaac Newton Trust research 170 grant [18.07(k)]; Global Challenge Research Fund [102642/A19819]

B.03 - Evaluation of the Oxidative Balance in Hippocampus from Female Rats Submitted to Obesogenic Diet During the Critical Period of Development

Maria Daniele T. B. de Lemos [1], Osmar Santos-Jr[1], Leticia Pachêco[1], Claudia Lagranha[1], Mariana Fernandes[1]
[1]Curso de Educação Física, Universidade Federal de Pernambuco (Pernambuco, Brasil)

INTRODUCTION: The consumption of a high-fat diet during pregnancy may be associated with a greater vulnerability to oxidative stress and in the long term induce neurological diseases in the offspring's hippocampus. OBJECTIVES: To evaluate the effects of an obesogenic diet during pregnancy and lactation on the oxidative balance in the hippocampus of female offspring at 30 days of life. MATERIALS AND METHODS: Approved by the ethics committee (protocol n° 0090/2021). Pregnant rats received, during pregnancy and lactation, either a Presence® vivarium diet or an obesogenic, high-fat, high-carbohydrate diet supplemented with an offer of condensed milk. After the lactation period (21 days), female rats were subdivided into two experimental groups: Control (C) and Obesogenic (O). The analysis were performed when the animals completed 30 days of life. Data were expressed as Mean ± SEM and compared using test-t with GraphPad Prism 6.0 software; significance was kept in 5% ($p < 0.05$) for all analysis. DISCUSSION AND RESULTS: We did not observe a significant difference in MDA levels between the groups, in the enzymatic antioxidant defense system, there was no significant difference between the experimental groups in the enzymatic activity of SOD and CAT, already in the GST activity we observed a significant reduction in the obese group compared to the control (CT:2.609 ± 0.3826 N=6; OB:1.205 ± 0.2223 mM N=7, p=0.0072). Evaluating the non-enzymatic antioxidant defense, Sulphydryls, we also did not observe any difference between groups. CONCLUSION: Our data suggest that in female at 30 days of age, the consumption of an obesogenic diet during pregnancy and lactation did not induce a significant difference in oxidative balance probably due the protective effect induced by estrogens levels.

Keywords: Nutrition, Obesity, Phenotypic Plasticity / S**upported by:** CNPQ

B.04 - An Outline of Energy Metabolism Gene Expression Profile of the Fruit Fly Drosophila melanogaster at Tissue Resolution

Yan Aveiro dos Reis [1], Thaís da Silva Rocha[1], Marcos Tulio de Oliveira[2], Rafael Dias Mesquita[3], Marcus Fernandes de Oliveira[1]
[1]Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brazil),
[2]Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal, Universidade Estadual Paulista (São Paulo, Brazil),
[3]Instituto de Química, Universidade Ferderal do Rio de Janeiro (Rio de Janeiro, Brazil)

INTRODUCTION: Tissues have different gene expression patterns that define their functions and metabolism. Energy metabolism pathways, electron transport system (ETS) and oxidative phosphorylation (OXPHOS) mechanisms are highly conserved between humans and Drosophila melanogaster, making the insect a suitable model to study metabolic diseases. Many publications studied genic expression in different tissues associated to conditions of stress. But there is still a gap of knowledge in describing the tissue-specific energy metabolism differences Drosophila. Our hypothesis is that the metabolic profile of D. melanogaster can be defined by comparative analysis between gene expression profile and metabolic activity at a tissue-specific level. OBJECTIVES: Define tissue-specific differences in D. melanogaster energy metabolism by bioinformatic analysis and biochemical assays. MATERIALS AND METHODS: We gathered raw data from publicly available transcriptomics databases with tissue-specific resolution. Each library was reprocessed and unified in a new comparative platform. In parallel, high resolution respirometry (HRR) assays were conducted to specify the contribution of different substrates for mitochondrial oxygen consumption. DISCUSSION AND RESULTS: the global expression pattern of each tissue differs from the others in different magnitudes. Unsupervised hierarchical clustering of a set of energy metabolism related transcripts, indicated common and distinct expression patterns for each tissue analyzed. Transcripts related to ETS and OXPHOS were highly expressed in all tissues. Thorax had remarkably higher expression of carbohydrates pathways, glycerol phosphate, OXPHOS, ETS. Previous HRR data indicated complex IV specific O2 consumption being the highest in a substrate independent way in young male Drosophila thorax. Testis expression pattern indicated a set paralogous transcripts being exclusively expressed in this tissue, as described in literature, alongside with many mitochondrial transporters. Head had specific set of glycerolipid pathways transcripts described to be associated with retinal maintenance. Ovary showed higher transcript expression of branched chain aminoacids pathway. CONCLUSION: RNA expression profile indicates possible nutritional preferences at a tissue specific level in D. melanogaster.

Keywords: Drosophila melanogaster, Drosophila metabolism, Tissue metabolism
Supported by: CNPq, FAPERJ

B.05 - Characterization of *Saccharomyces cerevisiae* ORF YDL157c gene product

Maria Antônia Kfouri Martins Soares [1], Letícia Veloso Ribeiro Franco[1], Mário Henrique de Barros[1]
[1]Instituto de Ciências Biomédicas Microbiologia, Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: Mitochondrial respiratory complexes and mitochondrial structures are encoded by two different genomes: nuclear and mitochondrial. Fine control of the expression of two genomes is possible due to the spatiality of the processes that link transcription to RNA degradation. From in silico proteomics, metabolomics and lipidomics analysis of proteins, either participating or genetically related to the mitochondrial gene expression system, ORF *YDL157c* was selected to be studied. OBJECTIVES: This work aims to characterize the role of Ydl157p in mitochondrial biogenesis. MATERIALS AND METHODS: Phenotypic analyzes resulting from *YDL157c* removal and overexpression were performed at 30°C and 37°C. The synthesis of mitochondrial polypeptides was accompanied by labeling with 35S-methionine/cysteine; enzymatic activities of respiratory complexes III and IV measured by cytochrome c oxidation/reduction, and mitochondrial supercomplex formation was seen in non-denaturing 4-13% acrylamide gel by BN-PAGE. DISCUSSION AND RESULTS: *Δydl157c* mutant shows profile similarity with respiratory deficient cells and Ydl157cp resembles the C subunit of *S. cerevisiae* vacuolar ATPase. Ydl157cp has as possible orthologs Tam6p from *Schizossacharomyces pombe* and Dmac1p from human, with conserved cysteine residues. Null mutant *Δydl157c* grows slowly in non-fermentable medium at 30°C, intensified phenotype at 37°C, in addition to showing increased resistance to copper. It was not possible to detect variations in the ability to synthesize mitochondrial polypeptides either in the absence or overexpression of *YDL157c*. The decrease in the activity of respiratory complexes III and IV and reduction in the formation of mitochondrial supercomplexes of the null mutant show a probable role in the stabilization of these supercomplexes. CONCLUSION: Ydl157cp is probably involved in mitochondrial supercomplexes stabilization.

Keywords: Mitochondrial biogenesis, respiratory supercomplexes, *Saccharomyces cerevisiae*
Supported by: CNPq

B.06 - Evaluation of Oxidtive Stress Biomakers in an *In vitro* Model of Metabolism-Associated Fatty Liver Disease

Vinícius Marques Arruda [1], Maria Júlia Gonçalves Granato[1], Joyce Ferreira da Costa Guerra[1]
[1]Instituto de Biotecnologia, Universidade Federal de Uberlândia (Minas Gerais, Brazil)

Metabolism-Associated Fatty Liver Disease (MAFLD) is a complex multisystem disorder characterized by lipid accumulation within hepatocytes, known as hepatic steatosis. The spectrum of the disease includes advanced stages, such as steatohepatitis and its progression is strictly associated with oxidative stress. Among the antioxidant mechanisms, glutathione system is the main endogenous response and a potential target for MAFLD, since there is no pharmaceutical treatment approved for the disease. Thereby, we evaluated aminotransferases levels and oxidative stress biomarkers of an *In vitro* model of MAFLD. To induce lipid accumulation, HepG2 cells were maintained in DMEM medium at 37°C, 5%CO 2 for 24hours, and then incubated with solutions of palmitic acid (0.7mM); a mixture of oleic and palmitic acids (1.0 and 2.0mM) in a 2:1 ratio, respectively, conjugated with albumin, at periods of 24 and 48h. Cells were fixed, stained with Oil Red O and lipid content determined pectrophotometrically. Cell viability was determined through MTT assay and hepatic damage through quantification of alanine and aspartate aminotransferases. Moreover, oxidative stress was evaluated through the quantification of lipid peroxidation biomarkers by thiobarbituric acid reactive substances assay (TBARS) and total glutathione levels.All tested concentrations increased intracellular lipid content compared to control, not affecting cell viability. However, higher aminotransferases levels were detected in treatments of 1.0mM at 48h and 2.0mM at both times. Furthermore, only 2.0mM treatment resulted in increased TBARS levels at both times. Surprisingly, all concentrations led to an increase of glutathione levels. These results indicate that the fatty acid overload is efficient in inducing an *In vitro* model of MAFLD, especially at the concentration of 2.0mM, which shows altered levels of tested lipid peroxidation and antioxidant system biomarkers in association with cell damage. Thus, the model here approached represents an important tool for pre-clinical study and possible therapeutic targets for MAFLD.
Keywords: MAFLD, glutathiones, hepatic steatosis / **Supported by:** FAPEMIG, Capes and UFU

B.07 - Understanding the Mechanisms of Photobiostimulation on Mitochondrial Bioenergetics

Natasha Ferreira Mezzacappo [1], Natalia Mayumi Inada[1], Aline Sanches Perez[1], Edilene de Souza Siqueira-Santos[2], Roger Frigério Castilho[2], Anibal Eugênio Vercesi[2], Vanderlei Salvador Bagnato[1]
[1]Instituto de Física de São Carlos, Universidade de São Paulo (SP, Brazil), [2]Departamento de Patologia, Faculdade de Ciências Médicas, Universidade Estadual de Campinas (SP, Brazil)

INTRODUCTION: Light therapeutical effects have been widely employed to treat several health conditions. Photobiomodulation (PBM) is a therapeutic modality based on photons interacting with biological tissues. Light sources are usually LASERs emitting in red and near infrared (NIR). PBM promotes biostimulating effects such as wound healing, pain relief and inflammation reduction, being a useful tool in the treatment of diseases. It is proposed that mitochondria are the main target at the cell level, where the photon absorption would lead to the observable therapeutical results. However, there is still much to explore and understand about the effects of photons on mitochondria. OBJECTIVES: This study investigates photon interactions on mitochondrial bioenergetics measuring mitochondrial respiration through high-resolution respirometry (HRR) techniques, regarding light at 635 nm. We also intend to evaluate biochemical alterations, mitochondrial swelling and membrane potential in the conditions with greater alterations. MATERIALS AND METHODS: Mice liver mitochondria (C57BL/6 females) were isolated and immediately irradiated with a LASER beam emitting at 635 nm and output powers between 200 and 800 mW. Complex I and Complex II substrates were used (glutamate plus malate, and succinate, respectively), non-phosphorylating respiration (state 4) was obtained by adding oligomycin, and CCCP was added to uncouple respiration. DISCUSSION AND RESULTS: No effects were observed in intensities below 200 mW. For Complex I substrates, only values above 600 mW significantly altered both state 4 and uncoupled respirations, inducing changes of around 20% in these parameters. On the other hand, when Complex II substrate were used, we observed slight alterations in state 3 and 4 respirations only when 800 mW was applied. CONCLUSION: Our results suggest that Complex I and Complex II substrates may generate different responses when mitochondria are exposed to light at 635 nm, giving us a direction to proceed with further different analyses.

Keywords: LASER, Mitochondrial Bioenergetics, Photobiomodulation / **Supported by:** FAPESP, CNPq and CAPES

B.08 - Browning-related acetylation modifies PPARγ activation

Marieli Mariano Gonçalves Dias [1], Caique Malospirito[1,2], Giovanna Elias[1], Ana Carolina Figueira[1]
[1]Brazilian Biosciences National Laboratory (LNBio), Brazilian Center for Research in Energy and Materials (SP, Brazil), [2]Institute of Biology, State University of Campinas (SP, Brazil)

INTRODUCTION: The nuclear receptor PPARγ stands out as a therapeutic target for metabolic diseases due to its central role in adipogenesis and glucose metabolism. In this sense, exploring the mechanisms involved in the modulation of PPARγ beyond the canonical pathway, by activation with agonists, is a step forward in optimizing its therapeutic function. OBJECTIVES: In this work, we aim to present details on PPARγ activation, as well as its interaction mechanism between the coactivator PGC-1α in the context of the browning-related deacetylation of PPARγ K268 and K293 by SirT1, and the interaction of this effector with PPARγ itself. MATERIALS AND METHODS: We used acetil-mimic PPARγ mutants to identify differences in PPARγ activation, through reporter gene, mammalian two-hybrid, and *In vitro* assays. DISCUSSION AND RESULTS: Our preliminary results have shown that the acetylation of K268 can increase PPARγ transactivation. Conversely, the deacetylation of the same residue can decrease its activation. Curiously, the K293 mutant had increased activation independent of the acetylation status. CONCLUSION: We aim to relate these PTMs to propose a new avenue in the PPARγ modulation, protecting the occurrence of acetylation, without the known agonist side effects. The elucidation of this mechanism of action may provide a basis for studies in the search of insulin-sensitizing treatments.

Keywords: PPAR gamma, acetylation, PTMs / S**upported by:** FAPESP

B.09 - Investigation of the Potential Targets of Dichloroacetic Acid (DCA) in Toxoplasma gondii

Denis Amilton Dos Santos [1], Mariana Sayuri Ishikawa Fragoso[1], Emanuella de Castro Andreassa[2], Jhenifer Yonara de Lima[2], Sheila Cristina Nardelli[1], Tatiana De Arruda Campos Brasil de Souza[2], Andréa Rodrigues Ávila[1]
[1]Laboratório de Pesquisa em Apicomplexa, Instituto Carlos Chagas (, Brazil), [2]Laboratório de Proteômica Estrutural e Computacional, Instituto Carlos Chagas (, Brazil)

INTRODUCTION: Toxoplasmosis is a worldwide infection caused by the intracellular protozoan *Toxoplasma gondii*. The disease is usually asymptomatic in immunocompetent individuals, but severe clinical cases are reported in the congenital infection or immunosuppressed patients. The treatment is toxic and not efficient in the chronic phase, leading to investigation for new drug targets. Dichloroacetic acid (DCA) is a drug used in cancer treatment and our group has demonstrated that it affects the proliferation and infective processes of parasite without causing toxicity to human cells. However, it is still not clear the precise target of DCA in *T. gondii* metabolism, and the potential targets are the kinases pyruvate dehydrogenase kinase (TgPDK) and branched chain ketoacid dehydrogenase kinase (TgBCKDK). OBJECTIVES: Identify the DCA target through *In vitro* assay to analyze drug's effect on the activity of each kinase using recombinant proteins. In addition, approaches using knockouts parasites will provide an evaluation of phenotypic alterations linked to the activity of those potential targets. MATERIALS AND METHODS: Recombinant production in prokaryotic systems and purification through affinity chromatography. The enzymatic activity of purified kinases will be measure *In vitro* by probing phosphorylation of synthetic peptides and analysis through mass spectrometry. The knockout of genes will be performed by conditional systems. DISCUSSION AND RESULTS: The protocol for recombinant production of TgBCKDK in Nico21 (DE3) strain and its purification were developed. Also, pilot assays of *In vitro* biochemical assay were already performed. The constructs for conditional knockout are already obtained and the mutant parasites will be selected. CONCLUSIONS: Biochemical assays will confirm the specific target of DCA. The identification of the *Toxoplasma* kinases as potential targets will open new alternatives to pharmacological studies. Conditional knockouts will provide further evidence of the precise role of these kinases in the metabolism of *T. gondii*.

Keywords: Kinase activity, carbon metabolism, gene knockout
Supported by: Capes, CNPq, Fundação Araucária

B.10 - Vemurafenib Induces Mitochondrial Fission and Changes in Bioenergetic Parameters in N-RAS Mutated Melanoma Cells

Laura Francisca Leite do Prado de Souza [1], Letícia Silva Ferraz[1], Rayssa de Mello Lopes[1], Denise Costa Arruda[2], Silvya Stuchi Maria Engler[3], Tiago Rodrigues[1]
[1]Centro de Ciências Naturais e Humanas (CCNH), Univ. Federal do ABC (SP, Brasil), [2]Núcleo Integrado de Biotecnologia (NIB), Univ. de Mogi das Cruzes (SP, Brasil), [3]Depto de Análises Clínicas e Toxicológicas, Univ. de São Paulo (SP, Brasil)

INTRODUCTION: Vemurafenib inhibits the MAPK/ERK signaling and proliferation in melanoma cells with the B-RAF ^{V600E} mutation. However, vemurafenib resistance was described in patients due to secondary mutations in N-RAS, leading to the paradoxical activation of MAPK pathway and tumor proliferation. We previously showed that vemurafenib causes mitochondrial fusion in B-RAF mutated melanomas by inhibiting DRP-1 phosphorylation and OXPHOS inhibition enhanced vemurafenib cytotoxicity. OBJECTIVES: To investigate the alterations of mitochondrial morphology induced by vemurafenib in N-RAS mutated melanoma cells to contribute to the comprehension of mechanisms of resistance to vemurafenib. MATERIALS AND METHODS: NRAS ^{Q61R} mutated melanoma cells (SK-MEL-147) were cultured in supplemented DMEM high glucose medium in a CO_2 incubator at 37ºC. Cell viability was assessed by MTT and trypan blue assays. Mitochondrial morphology was visualized using transmission electron microscopy. Protein expression was evaluated by Western blot to investigate MAPK pathway and proteins involved with mitochondrial dynamics. Mitochondrial bioenergetics was estimated by measurement of membrane potential, oxygen consumption, and ATP levels. DISCUSSION AND RESULTS: Vemurafenib increased MAPK/ERK signaling by paradoxical activation in SK-MEL-147 cells, which was associated to DRP-1 phosphorylation and a fragmented mitochondrial pattern (fission). Also, vemurafenib altered bioenergetic parameters, decreasing mitochondrial membrane potential, ATP levels, and basal and ATP-linked mitochondrial respiration after 48 h. Interestingly, the inhibition of mitochondrial fission by Mdivi-1 was able to sensitize SK-MEL-147 cells to vemurafenib action. CONCLUSION: Together, these findings showed that, despite the absence of cytotoxicity in N-RAS mutated melanoma cells, vemurafenib promoted paradoxical activation of MAPK pathway, DRP-1 activation, and mitochondrial fission, highlighting mitochondrial dynamics proteins as potential targets to chemotherapy.

Keywords: cancer, mitochondria, vemurafenib / **Supported by:** FAPESP, CNPq, and CAPES

B.11 - Evaluation of the Oxidative Stress in Lymphomononuclear Cells from Patients With Chikungunya Virus Chronic Infection

Ramon Nascimento Da Silva[1], Wellington Almeida[1,2], Allifer Rosendo Pereira[1], Mariane Cajuba [1,2], Ana Lisa Vale Gomes[1,2], Mariana Pinheiro Fernandes[1,2]
[1]Graduate Program in Nutrition, Federal University of Pernambuco (Pernambuco, Brasil), [2]Graduate Program in Nutrition, Physical activity and Phenotypic Plasticity, Federal University of Pernambuco (Pernambuco, Brasil)

INTRODUCTION: Chikungunya fever is an arbovirus caused by the chikungunya virus (CHIKV), an alphavirus belonging to the Togaviridae family. Febrile illness has a clinical manifestation of high fever, myalgia, skin rashes and moderate to severe arthralgia; polyarthralgia is one of the characteristic symptoms of alphaviruses. Virus infections are usually related to oxidative stress. OBJECTIVES: To evaluate the oxidative balance of lymphomononuclear cells (PBMCs) from patients with chronic infection by the chikungunya virus. MATERIALS AND METHODS: This study was approved by the Human Ethics Committee of the UFPE (2,459,058) C.A.A.E nº72123817.4.0000.5208. Blood samples were collected from women (40-60 years of age) with a positive serological diagnosis for CHIKV for isolation of PBMCs. Control (GC) and Chikungunya (GCHIKV) groups were formed and biochemical analyzes of lipid peroxidation were performed by measuring thiobarbituric acid reactive substances (TBARS), protein oxidation, activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione-s-transferase (GST), in addition to the evaluation of the non-enzymatic antioxidant system by reduced levels of glutathione (GSH), oxidized glutathione (GSSG) and cellular REDOX state (GSH/GSSG). Data were expressed as mean±SEM and compared using the T test of Student considering significant $p < 0.05$. DISCUSSION AND RESULTS: : Our data showed oxidative stress in PBMCs from patients with chronic CHIKV infection evidenced by increase in the lipid peroxidation (three times, $p < 0.002$) and protein oxidation (87%, $p < 0.0005$), increase in CAT (five times, $p < 0.0002$) and GST activities (78%, $p < 0.02$), associated to decrease of the non-enzymatic antioxidante system with GSH decreased levels (24.4%, $p < 0.0004$), GSSG increased (10.17%, $p < 0.0003$) and reduction of cellular REDOX state (GC: 38.58%, $p < 0.0001$). CONCLUSION: Lymphomononuclear cells from patients with chronic CHIKV infection showed REDOX imbalance associated to oxidative stress years after the infection.

Keywords: Chikungunya virus, Imune cells , Oxidative stress

B.12 - Study of Lipase Enzymatic Activity in Human Plasm Samples for a better approach of Covid-19 Pathogenesis Diseases

Adriana Ferreira Lopes Vilela [1], Clara Júlia L. Salmaso[1], Ana Paula Morais Fernandes[2], Carlos Arterio Sorgi[1]
[1]Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto (São Paulo, Brasil), [2]Engermagem Geral e Especializada, Escola de Enfermagem de Ribeirão Preto (São Paulo, Brasil)

INTRODUCTION: Lipase a key enzyme widely studied in diverse biological processes such as; metabolism of dietary triglycerides, cell signaling and inflammation. The lipase family catalyzes the cleavage of the ester bonds of lipids and is critical for the metabolism of lipids. In mammals, this family includes many critical members including pancreatic, hepatic, endothelial, and lipoprotein lipase. Pancreatic enzymes (lipases and amylases) in patients with COVID-19 are increased and this is suggesting that virus may be infectious cause of pancreatic injury. Therefore, the activity enzymes monitoring are crucial for providing the correct care and decreasing the morbidity and mortality of severe diseases. OBJECTIVES: Here we report for the first time the development of an *In vitro* methodology of activity of lipase in human plasm sample infected with Covid-19. MATERIALS AND METHODS: Lipase enzyme from porcine pancreas, 4-nitrophenyl palmitate substrate, human plasm samples, and reagents for samples extraction. The samples were extracted according to the Bligh and Dyer method. Activities of the different lipase and human plasm samples preparations were determined by using 4-nitrophenyl palmitate as substrate. The activities were calculated measuring p-nitrophenol (p-NP) release at 410 nm in buffer PBS at pH 7.4. One unit of activity (U) was defined as production of 1 µmoL of p-NP per minute, under the assay conditions. DISCUSSION AND RESULTS: The lipase-activity method assay developed was reproducible and stable. The parameter KMapp and the conditions for application were optimized. In the human plasm samples the activity lipase was detected and quantified. The comparison of the samples infected with Covid-19 and without infection was carried out and was observed differences between in terms os activity enzyme. CONCLUSION: The *In vitro* of activity lipase assay provided a simple, fast and direct procedure for measuring lipase activity in a human plasm samples. The method assay developed also can be applied for studies inhibition.

Keywords: lipase activity, human plasm samples, Covid-19 / **Supported by:** FAPESP, CAPES e CNPq

B.13 - Effects of Aldose Reductase Overexpression and Sorbitol Accumulation in Renal Cells and their Effects on Diabetic Nephropathy

Luca Lopes Cestari [1], Michelle do Nascimento Sales[1], Juliana Almeida Tamashiro[2], Marcelo Augusto Christoffolete[1]
[1]Centro de Ciências Naturais e Humanas, Federal University of ABC (São Paulo, Brasil), [2]Escola Paulista de Medicina, Federal University of São Paulo (São Paulo, Brasil)

INTRODUCTION: Diabetes presents itself as one of the main diseases in the world today, affecting several organs, being cataract and diabetic nephropathy examples. Both have similarities, as the increase in the activity of the polyol pathway and aldose reductase enzyme, responsible for the transformation of glucose into fructose through the production of sorbitol, causes the accumulation of reactive oxygen species, products of advanced non-enzymatic glycosylation and osmotic stress. However, compared to cataract, diabetic nephropathy has a lack of studies related to the polyol pathway and this pathology. OBJECTIVES: To analyze morphological and molecular changes in renal cells through increased aldose reductase activity and intracellular sorbitol accumulation MATERIALS AND METHODS: For the conditioned overexpression of aldose reductase, the TIGHT promoter was used together with a plasmid containing pMRI-Cherry and a TET-3G system, which conditions the expression of the enzymes of interest based on doxycycline presence. This construct was expressed in HEK-293 cells for further analysis. DISCUSSION AND RESULTS: When aldose reductase was overexpressed, an abnormal size increase of a fluorescent cell in which the promoter was activated was observed. From the experiments carried out, it is observable how the accumulation of intracellular sorbitol causes osmotic stress, a fact expected by the function of this molecule in helping the osmotic balance on renal cells, and that in excess causes several structural and molecular cellular changes. CONCLUSION: There is a need to conduct experiments to obtain new results regarding the quantification of intracellular sorbitol. Furthermore, it is also necessary to analyze intracellular pathways altered by the increase of sorbitol in these cells.
Keywords: Diabetic Nephropaty, Sorbitol, Aldose Redutase
Supported by: FAPESP and CAPES

B.14 - *In vivo* Pravastatin Treatment Reverses Hypercholesterolemia Induced Mitochondria-Associated Membranes Contact Sites, Foam Cell Formation, and Phagocytosis in Macrophages

Leandro Henrique de Paula Assis[1], Gabriel de Gabriel Dorighello[1,2], Thiago Rentz[1], Jane Cristina de Souza[1,2], Anibal Eugenio Vercesi[2], Helena Coutinho Franco de Oliveira [1]
[1]Structural and Functional Biology, Institute of Biology, State University of Campinas (SP, Brazil), [2]Pathology, Faculty of Medical Sciences, State University of Campinas (SP, Brazil)

INTRODUCTION: Statins are successful drugs used to treat hypercholesterolemia, a primary cause of atherosclerosis and cardiovascular mortality. Macrophages are the key cell type involved in all steps of the atherogenesis process. OBJECTIVES: In this work, we investigated how hypercholesterolemia and pravastatin treatment impact macrophage and mitochondria functions, which may impact cardiovascular disease development. MATERIALS AND METHODS: We tested the effects of hypercholesterolemia by comparing bone marrow-derived macrophages (BMDM) of C57BL/6J wild-type and LDL receptor knockout (LDLr−/−) mice. Then, we tested whether pravastatin could restore overall cellular functions by comparing BMDM from three month pravastatin-treated and non-treated (control) LDLr−/− mice. DISCUSSION AND RESULTS: We observed hypercholesterolemia increased the number of contact sites at mitochondria-associated endoplasmic reticulum (ER) membranes (MAM), enhanced mitochondrial hydrogen peroxide release, altered the gene expression of inflammatory markers, and increased oxidized LDL (ox-LDL) uptake and phagocytic activity. Three months of *In vivo* pravastatin treatment of LDLr−/− mice reversed the number of contact sites at the MAM, ox-LDL uptake, and phagocytosis in LDLr−/− BMDM. Additionally, pravastatin increased BMDM mitochondrial network branching and enhanced markedly the expression of the mitochondrial dynamics-related genes Mfn2 and Fis1 in BMDM. CONCLUSION: Our results show that hypercholesterolemia and pravastatin treatment affect macrophage mitochondria network structure as well as their interaction with the endoplasmic reticulum (ER). These effects impact on macrophage conversion rates to foam cell and macrophage phagocytic capacity. These findings associate MAM stability changes with known mechanisms involved in atherosclerosis progression and resolution.
Keywords: Hypercholesterolemia, Mitochondria-associated membrane, Macrophages
Supported by: Fundação de Amparo a Pesquisa do Estado de São Paulo (Fapesp) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

B.15 - Acetyl-CoA biosynthesis in Herbaspirillum seropedicae is regulated by the GlnB protein

Larissa Fonseca Tomazini [1], Eduardo Sabatine Lopes [1], Josielle Abrahão de Souza[1], Marco Aurelio Schüler de Oliveira [1]
[1]Departamento de Bioquímica, Universidade Estadual de Maringa (Paraná, Brasil)

INTRODUCTION: The PII family of proteins regulate the activity of many target enzymes by direct protein-protein interaction. These proteins have long been described as an important regulatory factor of the nitrogen metabolism. However, up to date evidences have shown that the PII proteins can also, beyond the nitrogen control, regulate the carbon metabolism, suggesting a wider regulatory of the PII family of proteins. Recently, our group identified, through a high throughput interactome, the interaction between the PII protein GlnB with the phosphotransacetylase Pta protein of the nitrogen fixing bacteria Herbaspirillum seropedicae. The Pta protein catalyzes the reversible interconversion of acetyl-CoA and acetyl phosphate. The balancing of the direct and reverse reactions is essential for adaptation of the bacteria to different environments. OBJECTIVES: To show the *In vitro* regulation of Pta protein of H. seropedicae by GlnB MATERIALS AND METHODS: We overexpressed and purified the recombinant proteins and tested the acetyl-CoA reaction catalyzed by Pta in the presence of GlnB and its allosteric effectors ATP, ADP and 2-oxoglutarate (2-OG). DISCUSSION AND RESULTS: Our results suggest that GlnB inhibits the acetyl-CoA synthesis by increasing the Km of the enzyme in the presence of either ATP and ADP. The interaction between GlnB and Pta was disrupted by 2-OG in the presence of ATP, releasing the inhibitory effect. The uridylylated form of GlnB, on the other hand, was not capable to inhibit the Pta acetyl-CoA synthesis activity. CONCLUSION: Altogether, the results reported here indicate that the unmodified GlnB protein controls the biosynthesis of Acetyl-CoA in H. seropedicae.

Keywords: Acetyl-CoA, GlnB, Pta / **Supported by:** CAPES

B.16 - The Contribution of AMP-Kinase on Energy Metabolism Control Induced by Redox Modulation in Embryonic Tick Cells

Marcelle Vianna de Carvalho Uhl [1], Rodrigo da Fonseca[1,2], Carlos Logullo[1,2]
[1]Instituto de Biodiversidade e Sustentabilidade da UFRJ, Universidade Federal do Rio de Janeiro (, Brasil), [2]Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro (, Brasil)

INTRODUCTION: The cattle tick *Rhipicephalus microplus* is a widespread parasite in Brazil causing damage and transmitting pathogens. To establish new control strategies, our group has studied molecular and cellular physiology during embryogenesis in order to identify new molecular targets. The embryonic tick cells BME26 can survive in a highly oxidative environment, modulated by a redox control and energy metabolism. The AMP-kinase enzyme (AMPK) is a key enzyme that regulates energy homeostasis of cells under nutrient and oxidative stress conditions and promotes the restoration of the appropriate metabolic state in other organisms. OBJECTIVES: To evaluate the hypothesis that AMPK is involved in the maintenance of metabolism and redox balance in BME26 tick embryonic cells. MATERIALS AND METHODS: Cell viability upon challenge with modulators, bioinformatic sequence analysis, transcript identification and phylogenetics, as well as cell imaging systems were used. DISCUSSION AND RESULTS: Two conditions were established in BME26 cells, one using L15 medium without glucose (low glucose) and with the addition of glucose (normal glucose). In the low glucose condition, a decrease in viability and an increase in lipid content was observed when the AMPK inhibitor compound C was added to the medium. The addition of the AMPK activator AICAR and challenge with H_2O_2 (oxidative condition) led to a reduction in lipid content. The sequences, protein domains and functional prediction of the alpha, beta and gamma subunits were identified. A phylogenic analysis using representatives of the largest orders of arthropods was performed and was able to identify the most similar ortholog for each subunit. CONCLUSION: The set of results demonstrates that the AMPK enzyme is present and may be involved in modulating oxidative response in BME26 cells. Further studies still need to be carried out and it is believed that they can contribute to the discovery of new candidates for tick control.

Keywords: AMPK, Energy Control, Redox Balance
Supported by: FAPERJ, CNPq, INCT-Molecular Entomology, PROAP-CAPES

B.17 - The Contribution of PEPCK on Aedes aegypti Oogenesis and Embryogenesis

Renato Martins da Silva [1,2], Jorge Moraes da Cunha[1], Helga Gomes Fernandes [1], Christiano Calixto da Conceição[1], Leonardo Araujo de Abreu[1], Itabajara da Silva Vaz Junior[3], Carlos Jorge Logullo de Oliveira[1,2]
[1]Laboratório Integrado de Bioquímica Hatisaburo Masuda and Instituto de Bioquímic, Universidade Federal do Rio de Janeiro (RJ, Brasil), [2]INCT, Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular (RJ, Brasil), [3]Centro de Biotecnologia and Faculdade de Veterinária,, Universidade Federal do Rio Grande do Sul (RS, Brasil)

INTRODUCTION: The mosquito Aedes aegypti undertakes a shift in carbohydrate metabolism during embryogenesis, including an increase in the activity of phosphoenolpyruvate carboxykinase (PEPCK), a key gluconeogenic enzyme, at critical steps of embryo development. All eukaryotes studied to date present two PEPCK isoforms, namely PEPCK-M (mitochondrial) and PEPCK-C (cytosolic). In A. aegypti, however, these proteins are so far uncharacterized. OBJECTIVES: Study the PEPCK isoforms expression and its correlation with Aedes aegypti oogenesis and embryogenesis. MATERIALS AND METHODS: In this work we describe two A. aegypti PEPCK isoforms by sequence alignment, protein modeling, and transcription analysis in different tissues, as well as PEPCK enzymatic activity assays in mitochondrial and cytoplasmic compartments during oogenesis and embryogenesis. DISCUSSION AND RESULTS: First, we characterized the protein sequences compared to other organisms, and identified conserved sites and key amino acids. We also performed structure modeling for AePEPCK(M) and AePEPCK(C), identifying highly conserved structural sites, as well as a signal peptide in AePEPCK(M) localized in a very hydrophobic region. Moreover, after blood meal and during mosquito oogenesis and embryogenesis, both PEPCKs isoforms showed different transcriptional profiles, suggesting that mRNA for the cytosolic form is transmitted maternally, whereas the mitochondrial form is synthesized by the zygote. CONCLUSION: Collectively, these results improve our understanding of mosquito physiology and may yield putative targets for developing new methods for A. aegypti control.
Keywords: PEPCK, metabolism, mosquito / **Supported by:** INCT, CNPq, FAPERJ, CAPES

B.18 - Chronic Exposure to Inorganic Mercury Causes Disturbances in Mitochondrial Bioenergetics and Blood Cells in Hypercholesterolemic Mouse

Maiara Ingrid Cavalcante Queiroz [1], Carolina Martins Lázaro[2], Thiago Rentz[2], João Victor Virgilio da Silva[3], Pedro Manoel Moraes Vieira[3], Anibal Eugenio Vercesi[4], Ana Catarina R. Leite[1], Helena Coutinho Franco de Oliveira[2]
[1]Instituto de Química e Biotecnologia, Universidade Federal de Alagoas (Alagoas, Brasil), [2]Dept Biologia Estrutural e Funcional, Universidade Estadual de Campinas (São Paulo, Brasil), [3]Dept Genética e Evolução, Microbiologia e Imunologia, Universidade Estadual de Campinas (São Paulo, Brasil), [4]Dept Patologia, Universidade Estadual de Campinas (São Paulo, Brasil)

INTRODUCTION: There is a significant association between heavy metal exposure and atherosclerosis. Mercury (Hg) is a known toxic metal that leads to increases of oxidants generation in several biological systems. Local arterial oxidative stress is involved in all phases of atherosclerosis development. OBJECTIVES: Considering the central role of oxidative stress for initiation and progression of atherosclerosis, the aim of this study is to evaluate markers and risk factors for atherosclerosis development in the hypercholesterolemic LDL receptor knockout mouse model chronically exposed to Hg. MATERIALS AND METHODS: Mice treated for 30 days with $HgCl_2$ in the drinking water (10 µg/mouse/day) were compared with controls (plain water). Experimental protocol was approved by the Committee for Ethics in Animal Experimentation, protocol nº 5745-1/2021 (CEUA/UNICAMP). Assays include plasma lipids and glucose levels, liver mitochondrial respiration rates, membrane potential and H_2O_2 production, white blood cells counting, erythrocytes oxygen uptake, and phagocytic activity of peritoneal macrophages. DISCUSSION AND RESULTS: Hg exposed mice present increased plasma cholesterol levels, without alterations in glucose and triglycerides levels, body and tissue weights. Liver mitochondrial from Hg treated mice showed worse respiratory control (33%), lower oxidative phosphorylation efficiency (19%) and increased H_2O_2 release (40%). In addition, Hg treatment induced a faster membrane potential disruption upon calcium challenge (38%). Erythrocytes from Hg treated mice showed a 50% reduction in their ability to take up oxygen. The Hg treatment also induced significant alterations in white blood cells counting: reduction in lymphocytes (22%) and increases in monocytes (52%), eosinophils (47%) and neutrophils (100%). Peritoneal macrophages from Hg treated mice showed increased phagocytic activity. CONCLUSION: *In vivo* chronic exposure to Hg worsens the hypercholesterolemia, impairs mitochondrial bioenergetics and redox function, red and white blood cells profile and macrophage phagocytic activity. These findings suggest Hg induces metabolic and immune system alterations that are likely relevant for atherogenesis and inflammation.
Keywords: mercury toxicity, mitochondria, atherosclerosis / **Supported by:** FAPEAL and FAPESP

B.19 - Symbiometabolic Interactions Between Wolbachia pipientis Bacteria in Aedes aegypti Mosquito Embryonic Cells

Angélica Arcanjo[1], Christiano Calixto[1], Daniel Costa Santos[1], Natthan Vellardo[1], Cintia Lopes[1], Renato Martins[1], Luciano Moreira[2], Carlos Jorge Logullo[1]
[1]Instituto de Bioquímica Médica , Universidade Federal do Rio de Janeiro (, Brazil), [2]Centro de Pesquisas René Rachou, Centro de Pesquisas René Rachou, Fiocruz Minas Gerais (, Brazil)

INTRODUCTION: *Wolbachia sp.* are symbiotic intracellular bacteria that live in more than 60% of insects. Although they are commonly found in many species of mosquitoes, some with importance in the transmission of pathogens to humans, they are absent in *Aedes aegyptii. Aedes aegypti* is the main vector of the viruses that cause dengue, zika and chikungunya. As there are no effective vaccines for these viruses, the prevention of cases in humans depends almost exclusively on the control of the vector mosquito. Actually, the bacterium Wolbachia sp. is being used in several parts of the world as an innovative approach to control the transmission of pathogens by mosquitoes. The wMel strain of *Wolbachia pipientis* has been used in field trials with *Aedes aegypti* mosquitoes, having reached and remained at a high population frequency and these mosquitoes show susceptibility to DENV quite reduced several years later. The mechanisms behind this inhibition phenotype remain uncertain. OBJECTIVES: Based on these data, our group aims to study the parasite-host relationship from a metabolic and immunological point of view. MATERIALS AND METHODS: For this, embryonic cells of *Aedes aegypti*, infected or not with *Wolbachia pipientis*, were established and will be characterized. In addition, their metabolic profiles will be evaluated as well as genes related to the immune system to elucidate what are the consequences that the infection by the bacteria *Wolbachia pipientis* on the *Aedes aegypti* mosquito. DISCUSSION AND RESULTS: CONCLUSION: In this concern, the question is: how much these possible consequences contribute to the low transmission profile of Dengue that affects millions of people worldwide?
Keywords: Aedes aegypti, Wolbachia pipientis, Embryonic cells / **Supported by:** Faperj, CNPq, Capes

B.20 - Physical exercise increased the formation of autophagosomes in an Experimental Model of Alzheimer's Disease

Estela de Oliveira Koopman [1]
[1]Departamento de Ciências Biomoleculares, School of Pharmaceutical Sciences of Riberao Preto (São Paulo, Brasil), [2]Departamento de Psicologia, Faculty of Philosophy, Sciences and Letters at Ribeirao Preto (São Paulo, Brasil)

INTRODUCTION: Alzheimer's is a chronic and progressive neurodegenerative disease characterized by memory deficits. The accumulation of beta-amyloid protein and tangles of hyperphosphorylated Tau protein in the hippocampus region are key biomarkers of the disease. Evidence has shown that physical exercise can modulate the neuroplasticity of neuronal cells. Futhermore, recent research suggests an important role for autophagy in the developmental process of Alzheimer's disease. OBJECTIVES: This study aimed to evaluate the effect of aerobic exercise on the hippocampal region autophagy process in an experimental model of Alzheimer's disease. MATERIALS AND METHODS: Female mice, triple-transgenic (TG) and B6129SF2/J (WT) were used. The animals with 5-6 weeks old were divided into 4 groups, two sedentary groups (SED-WT, n=6-6 and SED-TG, n=9-6) and, two exercised groups (EXE-WT, n=8-6 and EXE-TG, n=9-6) - Ethics Committee process: 20.1.322.60.3. The animals were trained for 15 weeks, 5 times per week with 40 minutes for each session and speed 18-21 m/min. The Morris water maze was used for the spatial memory test, as described by Vorhees and Williams (2006). For immunofluorescence analysis, sections of the brain, comprising the dorsal hippocampus, were marked with primary antibodies β-Amyloid, LAMP1 and LC3B, incubated for 72 hours at 4°C. The secondary antibodies Alexa Fluor 488 and Alexa Fluor 594 were incubated for 1 hour at room temperature. DISCUSSION AND RESULTS: Physical exercise induced the formation of autophagosomes, by increasing the expression of LC3B in the hippocampus, independently of the β-amyloid and lysosome content. Furthermore, the increase in β-amyloid content in transgenic animals was not sufficient to produce changes in spatial memory in six-month-old animals. CONCLUSION: Therefore, is suggestive that physical exercise may play a role in the modulation related to hippocampus clearance in these animals before spatial memory deficits were verified.
Keywords: Alzheimer, Autophagy, Exercise / **Supported by:** CNPq

B.21 - The Effects of Eugenia uniflora Extract on the Longevity and Resistance to Hydrogen Peroxide in D. melanogaster

Antonio Alvenir Comis Neto [1], Matheus Bianchini[1], Caroline Quines[1], Robson Puntel[1], Cristiane Denardin[1], Simone Pinton[1]

[1]grupo de pesquisa em bioquimica e toxicologia em eucariontes, Univ. Federal do Pampa (UNIPAMPA), Uruguaiana, RS, Brasil.

INTRODUCTION: The pitangueira (Eugenia uniflora) is a tree native to Brazil. A great diversity of nutrients and bioactive compounds have been found in the leaves and fruits (pitanga) of E. uniflora. E. uniflora has antioxidants properties and the purple pitanga variety has the highest amount of bioactivies compounds among the fruits varieties. OBJECTIVES: To evaluated the effect of purple pitanga extract on the longevity of Drosophila melanogaster (fruit fly) and resistance to a stressor agent, hydrogen peroxide (H2O2). MATERIALS AND METHODS: To longevity test male and female flies were divided into three groups: control and treated groups with pitanga extract (10 or 1000µg). Every 24 hours the number of dead flies was counted. In the H2O2 resistance test, male and female flies were divided into nine groups: control group, groups 2 and 3 were treated with pitanga (10 or 1000µg), groups 4 and 5 were exposed to H2O2 (2,5% or 5%) and the other groups were co-treated with pitanga and H2O2. Mortality was verified at 24 and 48 hours. DISCUSSION AND RESULTS: Our result showed that the average survival time (50%) of the control males live was about 21 days, while the average survival time of the control females was about 16 days. Exposure to the highest concentration of pitanga increased the longevity of flies in both sexes. In H2O2 resistance test, the exposure (48h) to H2O2 (2,5% and 5%) increased the fly's mortality in control group and the extract at the concentration of 1000 µg was able to protected, these results were similar in both sexes and in the two concentrations. CONCLUSION: The bioactive compounds that are present in the pitanga have antioxidants that allow us to suggest that this is the mechanism by which it increases the longevity of fruit flies and protects of stress induced by H2O2.

Keywords: Aging, Pitanga, Longevity

B.22 - Gluconeogenesis as a Metabolic Strategy in Embryonic Cells of Mosquito Under Nutritional Restriction

Daniel Costa Santos [1], Cíntia Lopes Nogueira[1], Maria Elisa Gomes de Lima[1], Christiano Calixto Da Conceição[1], Angélica Fernandes Arcanjo Sampaio[1], Carlos Jorge Logullo de Oliveira[1]

[1]Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

Embryonic arthropod culture cells are considering a good tool for biological research. One of the most impressive features of arthropod embryonic cells is the high tolerance to hydrogen peroxide. The strains arthropod embryonic cells, such as Aag-2 *(Aedes aegypti)* and BME26 *(Rhipicephalus microplus)*, were resistant to hydrogen peroxide challenge when compared to mammalian strains. Recently, a new study was published in which they showed that there was an increase in relative transcription of PEPCK against the challenge with H_2O_2, suggesting that gluconeogenesis can maintain energy homeostasis control in these cells. In addition to resistance to hydrogen peroxide, the BME26 cells proved to be resistant to nutritional restriction. In an unpublished study by our group of research, BME26 cells support long periods of fasting, and it has been observed that levels transcriptional levels of PEPCK increase in the period of 3- to 48-h of fasting. In addition, BME26 cell viability decreases by about 25% at 24 h and 50% at 48 h of fasting. In contrast, Aag2 cells showed a decrease in cell viability of 50% in the first hours with restriction nutritional value, there is an increase of approximately 20% in viability in 24 hours of fasting, when compared to the 3- and 6-h fasting periods. We suggest that gluconeogenesis may be involved in the maintenance cellular energy, having an essential role in the metabolic adaptation that results in increased of cell viability during the fasting period. Our objective is evidence of metabolic strategies that assist in cell maintenance and survival in arthropod cells under nutritional restriction, with the focus on the contribution of gluconeogenesis. It is believed that this study will contribute significantly to expand the knowledge of arthropod physiology, as well as well as in the development and improvement of ways to control disease vectors.

Keywords: Aedes aegypti cell, fasting, gluconeogenesis

B.23 - Soil enzymes detect environmental changes in water body with treated domestic effluent

Danielle Gonçalves Teixeira Dos Santos [1], Samantha Salomão Caramori[1]
[1]Laboratório de Biotecnologia, Universidade Estadual de Goiás (Goiás, Brasil)

INTRODUCTION: Wastewater treatment plants (WTP) reduce part of domestic or industrial pollutants, promoting their subsequent disposal in water bodies. Soil, acting as a residue receptor, has the ability to self-cleanse, however, the addition of organic debris causes structural and functional changes in its microbiota, reducing their ability to cycle and mineralize nutrients. The Meia Ponte River Watershed, the main source of supply in the State of Goiás, receives several polluting contributions, such as the discharge of wastewater from the Dr. Hélio Seixo de Britto, located in Goiânia and managed by the Basic Sanitation Company of Goiás (SANEAGO S/A). OBJECTIVES: The objective of this study was to evaluate the activity of extracellular soil enzymes present in the soil bordered by the Meia Ponte River, in order to demonstrate variations caused by such discharge. MATERIALS AND METHODS: The collection of material was carried out along the water body at 6 sampling points, distributed between the river head, WTP and downstream, in composite samples with a depth of 0-20 cm, in the rainy season. The activities of β-Glycosidase, Acid Phosphatase and Arylsulfatase were monitored by incubating the samples with specific substrates and later reading the supernatant at 400 nm. DISCUSSION AND RESULTS: The results obtained for all enzymes suggest a high environmental correlation, since higher enzymatic activities were observed in a conserved environment, such as in the spring, before the WTP. This is due to the possible interaction of microorganisms with the available organic biomass, resulting from a high biodiversity expressed in the place. CONCLUSION: In addition, the data from consecutive points, referring to enzymatic activity, proved to be low when compared to the literature, which suggests that these areas need a process of biological soil recovery, mainly close to the WTP, reflecting the state of soil conservation and the effectiveness of enzymes as environmental bioindicators.

Keywords: Enzyme activity, Soil bioindicator, Effluents
Supported by: Fundação de Amparo à Pesquisa de Goiás

B.24 - Pro-Inflammatory Polarization of Macrophages is Associated with Reduced Endoplasmic Reticulum-Mitochondria Interaction

Leandro Henrique de Paula Assis [1], Gabriel de Gabriel Dorighello[1,2], Helena Coutinho Franco de Oliveira[1]
[1]Department of Structural and Functional Biology, Institute of Biology, State University of Campinas (São Paulo, Brazil), [2]Department of Clinical Pathology, Faculty of Medical Sciences, State University of Campinas (São Paulo, Brazil)

INTRODUCTION: Macrophages play a role in host defense, tissue remodeling and inflammation. Different inflammatory stimuli drive macrophage phenotypes and responses. OBJECTIVES: In this study we investigated the relationship between macrophages immune phenotype and mitochondrial bioenergetics, cell redox state and endoplasmic reticulum (ER)-mitochondria interaction. MATERIALS AND METHODS: We compared naïve THP-1 macrophages with macrophages treated with lipopolysaccharide (LPS) from Escherichia coli plus interferon-γ (IFNγ) and macrophages treated with interleukin-4 (IL4) plus interleukin-13 (IL13) cytokines. DISCUSSION AND RESULTS: Bacterial LPS and IFNγ pro-inflammatory stimuli decreased oxidative metabolism (basal, phosphorylating and maximal conditions) and increased baseline glycolysis (117%) and glycolytic capacity (43%) in THP-1 macrophages. In contrast, IL4 and IL13 anti-inflammatory stimuli increased the oxygen consumption rates in baseline conditions (21%) and associated with ATP production (19%). LPS+IFNγ stimuli reduced superoxide anion levels by accelerating its conversion into hydrogen peroxide (H2O2) while IL4+IL13 decreased H2O2 release rates. The source of these oxidants was extra-mitochondrial and associated with increased NOX2 and SOD1 gene expression. LPS+IFNγ stimuli decreased ER-mitochondria contact sites as measured by IP3R1-VDAC1 interaction (34%) and markedly upregulated genes involved in mitochondrial fusion (9-10 fold, MFN1 and 2) and fission (~7 fold, DRP1 and FIS1). Conversely, IL4+IL13 stimuli did not altered ER-mitochondria interactions nor MFN1 and 2 expression. CONCLUSION: Our results unveil ER-mitochondria interaction pattern as a novel feature of macrophage immunological, metabolic and redox profiles.

Keywords: Immunometabolism, Macrophages, Oxidants / **Supported by:** São Paulo Research Foundation (FAPESP) and National Council for Scientific and Technological Development (CNPq)

B.25 - Toximetabolomics of articaine compared to lidocaine in Schwann cells

Gustavo Henrique Rodrigues da Silva [1]
[1]Department of Biochemistry and Tissue Biology, Institute of Biology, University of Campinas (Campinas - SP, Brazil), [2]CICECO—Aveiro Institute of Materials, University of Aveiro (, Portugal), [3]Department of Clinical Pathology, Faculty of Medical Sciences, University of Campinas (, Brazil)

INTRODUCTION: Articaine (ATC) and lidocaine (LDC) are commonly used local anesthetics (LA). In dentistry LDC is the gold standard, while ATC is a relatively new agent. ATC is the only aminoamide LA with a thiophene ring plus an ester group in its structure. Due to its rapid hydrolysis (by plasmatic esterases) and reduced systemic toxicity, ATC can be used in higher doses (4%) than LDC (2%) or other LA. Despite of that, ATC displays superior local toxicity in relation to LDC, with reported cases of paresthesia. However, there are no *in vitro*/*In vivo* evidence so far on the greater neurotoxicity of ATC, justifying this study. OBJECTIVES: Use NMR-toximetabolomics to identify possible metabolic differences induced by ATC and LDC on Schwann cells (SC). MATERIALS AND METHODS: SC cells were exposed (24 h) to ATC or LDC at the IC10 and IC50 (concentrations to decrease cell viability in10 and 50%, respectively, as assessed, by the MTT assay) and changes in their exo- (culture medium) and endo- (aqueous cell extracts) metabolomics were determined by 1H-NMR. Besides, Seahorse tests were conducted to analyze the effect of LA on glycolysis and aerobic respiration. DISCUSSION AND RESULTS: Multivariate analysis identified an evident separation between Schwann cells exposed to ATC in relation to LDC, in the culture media (PLS-DA, Q2=0.854) and cell extracts (PLS-DA, Q2=0.975). ATC stimulated glucose consumption and increased intracellular levels of several amino acids (leucine, isoleucine, valine, phenylalanine, methionine, histidine, tyrosine and glycine). Such amino acids were decreased in LDC-treated cells. Moreover, ATC had a greater impact on aerobic respiration, as seem in the Seahorse experiments CONCLUSION: ATC triggers distinct metabolic answers on Schwann cells than LDC. Despite being systemically less toxic, ATC local toxicity on excitable cells seems to involve the energetic metabolism and should be further studied.

Keywords: Toximetabolomics, articaine, lidocaine / **Supported by:** FAPESP BEPE #18/24814-0

B.26 - CLINICAL CHARACTERISTICS OF CONVALESCENT PATIENTS WITH COVID-19 AND THE EFFECT OF INTERMITTENT HYPOXIA OVER METABOLIC RESPONSES ON PHYSICAL EXERCISE

João Pedro Rodrigues Campos Renon [1], Diana Mota Toro[2,3], Pedro Vieira da Silva Neto[2,3], Adriana Ferreira Lopes Vilela[1], Átila Alexandre Trapé[4], Carlos Arterio Sorgi[1,2]
[1]Departamento de Químca, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto (São Paulo, Brasil), [2]Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto (São Paulo, Brasil), [3]Instituto de Ciências Biológicas, Universidade Federal do Amazonas (Amazonas, Brasil), [4]Escola de Educação Física e Esporte de Ribeirão Preto, Universidade de São Paulo (São Paulo, Brasil), [5]Departamento de Bioquímica e Imunologia, Faculdade de Medicina de Ribeirão Preto (São Paulo, Brasil)

INTRODUCTION: The COVID-19 pandemic caused by SARS-CoV-2 was responsible for millions diagnosed cases worldwide and death. This disease left a lot of convalescent patients with sequels over time and the physical training with hypoxic stimulus appears as an alternative that supports the conventional treatments of the COVID-19 patient's recovery, given several benefits of physical training in healthy people. OBJECTIVES: We aim to characterize the biochemical, inflammatory and hematologic parameters on COVID-19 convalescent patients and the effect of hypoxia training on their energetic metabolism. MATERIALS AND METHODS: To describe the health status post-COVID, patients recovered who had presented moderate to severe symptoms was assessed through hematological and serum biochemical, immunological (cytokines) and metabolic parameters (n=84). The individuals carry out the training program on bicycles, around the normobaric hypoxia tents using an individual system with face mask. DISCUSSION AND RESULTS: The main results showed that the changes on the hematological levels, such as neutrophils, differ in the analyzed groups, including severe and non-severe patients ($p < 0,05$). In addition, the amount of the cytokines (IL-6, IL-8 and IL-10) was different between the groups. The training program improved the energetic metabolism and enhanced blood lactate concentration in hypoxia group. CONCLUSION: Thus, a moderate-intensity training program performed in intermittent hypoxia could be an efficient alternative in rehabilitating convalescents to recover lung function, physical fitness, quality of life, mental health, and immunological homeostasis.

Keywords: Hypoxia, Metabolism, COVID-19 / **Supported by:** FAPESP, CNPq, CAPES

B.27 - Development of an *In vitro* model of hepatocyte de novo lipogenesis

Michelle Do Nascimento Sales[1], Juliana Almeida Tamashiro[2], Luca Lopes Cestari[1], Marcelo Augusto Christoffolete[1]
[1]Centro de Ciências Naturais e Humanas, Federal University of ABC (SP, Brasil), [2]Escola Paulista de Medicina, Federal University of São Paulo (SP, Brasil)

INTRODUCTION: De novo lipogenesis (DNL) is a complex and highly regulated pathway that can lead to adverse metabolic consequences when deregulated. Through the tricarboxylic acid cycle (TCA), pyruvate is converted to acetyl-CoA and exported to cytosol. Acetyl-CoA in the cytosol is used as precursors for lipid synthesis via the DNL pathway. Newly synthesized fatty acids are further esterified and stored in lipid droplets. *In vitro* models of DNL in adipocyte (3T3-L1, 3T3-F442A) and primary preadipocytes (OP9) are well described and used for studies in the literature, but there are no *In vitro* models for liver cells. OBJECTIVES: Silence the immortality factor, Transforming Growth Factor - alpha (TGF-α), in AML-12, a hepatocytes of the murine lineage, using RNAi, so that the cell can perform DNL. MATERIALS AND METHODS: RNAi sequence was generated using Thermo (www.lifetechnologies.com/rnai) and synthesized by Exxtend as 2 single DAN strands, annealed and cloned into pENTR/U6 vector (Invitrogen). DISCUSSION AND RESULTS: We have successfully cloned the RNAi sequence for TGFa. Transfection experiments are currently ongoing.

Keywords: de novo lipogenesis, Nonalcoholic fatty liver disease, hepatocite
Supported by: fapesp, capes

B.28 - Placental Metabolomics from Women with Gestational Diabetes Mellitus Unveils Glycolytic and 1 Carbon Metabolism Impairment

Vanessa De Araujo Goes [1], Gabriela Dias[1], Carolina Ferreira[1], Deborah Bauer[1], Layla Ranquine[1], Carla Lai[3], Tatiana El Bacha[1,2]
[1]Programa de Pós graduação em Ciência de Alimentos, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil), [2]Instituto de Nutrição Josué Castro, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil), [3]Centre for Throphoblast Research, University of Cambridge (Cambridge, United Kingdom)

INTRODUCTION: The prevalence of Gestational Diabetes Mellitus (GDM) has been increasing worldwide and it is associated with adverse pregnancy outcomes as well as adverse effects for both the offspring and mother post-partum. The placenta serves various endocrine and transport functions and alterations in placental metabolism are associated to the pathogenesis of GDM. Metabolomics is a valuable tool to understand the molecular basis of many diseases and a thorough analysis of the altered metabolic pathways in placentas from GDM pregnancies has not yet been done. OBJECTIVES: Here we investigated the placental metabolic profile in pregnancies complicated with GDM using semi-targeted (by mass spectrometry-MS) and open profile (by nuclear magnetic resonance-NMR) approaches. MATERIALS AND METHODS: Placental fragments were collected immediately after delivery from both control (n=7) and GDM (n=6) pregnancies from a cohort conducted at the Maternidade Escola/UFRJ. For MS analysis, a Thermo scientific UHPLC+ series coupled with a TSQ Quantiva mass spectrometer was used with an electrospray ion source. For NMR metabolomics, a Bruker Advance III 500.13 DISCUSSION AND RESULTS: Our results showed a significant increase in placental Glucose-6-phosphate (G6P) and a decrease in lactate in the GDM group. Furthermore, the sum of all glycolytic intermediates as well as the ratio lactate:G6P were significantly reduced in placentas from GDM pregnancies. When analysing 1 Carbon (1C) metabolism, we observed an overall decrease in its intermediates with a significant decrease in serine concentration and the sum of metabolites that directly participate as methyl donors was significantly lower in placentas from women with GDM. CONCLUSION: Our data shows impairment in both glycolytic and 1C metabolism pathways that are directly associated with oxidative stress, suggestive of mitochondria and ER dysfunction in the placenta with consequences to fetal growth and development.

Keywords: Placenta, Metabolomics, Gestational Diabetes Mellitus
Supported by: Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro [E-26/110.111/2014, E-026/203.254/2017); Isaac Newton Trust research 170 grant [18.07(k)]; Global Challenge Research Fund [102642/A19819]

B.29 - Evaluation of Oxidative Stress in the Brainstem of Female Young Rats Submitted to an Obesogenic Diet in the Developmental Period

Vanessa Lima de Souza [1], Thyago De Oliveira Rodrigues[1], Gizele Santiago de Moura Silva[2], Mariana Pinheiro Fernandes[1,2], Cláudia Jacques Lagranha[1]
[1]Laboratory of Biochemistry and Molecular Biology, Federal University of Pernambuco (Pernambuco, Brasil), [2]Graduate Program in Nutrition, Federal University of Pernambuco (Pernambuco, Brasil)

INTRODUCTION: The critical period of development comprehend pregnancy, lactation and early childhood. Studies shown that eating a high-fat diet during pregnancy leads to metabolic disorders in the offspring, which can develop neurodegenerative diseases caused by a REDOX imbalance. OBJECTIVES: Evaluate the effects of the obesogenic diet during the critical period of development in the brainstem of female young rats at 30 days of life. MATERIALS AND METHODS: Approved by the ethics committee (protocol n° 0090/2021). The pregnant rats received, during pregnancy and lactation, a Presence® vivarium diet or an obesogenic diet, rich in fat and carbohydrates, with free access of condensed milk. After lactation (21 days), female rats were used in the experiments, divided into two experimental groups: Control (C) and Obesogenic (O). The analysis were performed on the 30th day of life. Data were expressed as Mean ± SEM and compared using t-test with GraphPad Prism 6.0 software; significance was set in 5% ($p < 0.05$) for all analysis. DISCUSSION AND RESULTS: Our data showed a reduction in the levels of lipid peroxidation in the obese group compared to the control (CT:2.641±0.234; OB:3.732± 0.318 M; N=6; p=0,0198) the SOD enzymatic antioxidant activity showed a reduction in the experimental group (CT:158.1 +/- 28.024; OB:60.28+/-15.90 mM N=8; p=0,0103). On the other hand, CAT activity showed no significant difference. In the activity of GST, we observed a significant difference between the experimental groups (CT:0.06523±0.005; OB:0.05047± 0.003 mM, N=7; p=0,0331), when evaluating the levels of total thiols, there was an increase in the experimental group (CT:0.07578±0.007; OB:0.1008±0.009 M, N=8; p=0,0410). CONCLUSION: Our data suggest that consumption of an obesogenic diet during a critical period of development leads to a homeostatic imbalance in the offspring at 30 days of life, leading to damage to the brainstem, the organ responsible for carrying out vital functions of the individual.

Keywords: Obesogenic Diet, Oxidative Stress, Critical Developmental Period

B.30 - Action of Thyroid Hormones and Endoplasmic Reticulum Stress

Hellen Marianne de Oliveira Costa [1], Anderson Teixeira Santos[1], Wagner Seixas da Silva[1]
[1]Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: Endoplasmic reticulum (ER) stress exacerbates several metabolic conditions closely associated with thyroid hormone (TH) signaling, such as obesity and insulin resistance. The enzyme deiodinase type 1 (*D1*) is highly expressed in the liver and acts a protagonist in the signaling of thyroid hormones in a state of hyperthyroidism, controlling the hormonal balance at a systemic level. In this scenario, instability in thyroid hormone signaling, caused by ER stress, may be one of the factors that result in liver disorders. OBJECTIVES: Thus, the aim of the study was to investigate the relationship between thapsigargin-induced ER stress and the TH metabolism in HepG2 (human hepatocarcinoma) cell line. MATERIALS AND METHODS: HepG2 cells were maintained in DMEM (25 mM glucose, supplemented with 10% fetal bovine serum) in a 5% CO2 atmosphere at 37°C. For the experimental procedures, cells were seeded in 6-well plates (density: $1x10^5$ cells/mL, 3 mL/well) and kept for 48 hours. Then, cultures were incubated in DMEM + 4 µM thapsigargin or vehicle, for 24 hours. After treatment, mRNA was extracted and the expression of the genes *BiP*, *CHOP*, *IRE1a*, *XBP1t*, *SPOT14* and *Dio1* was performed through RT-PCR. For statistical analysis, the means and standard error of three different culture wells (same experiment) were compared by unpaired Student's *t* test (considered significant when $p < 0.05$). DISCUSSION AND RESULTS: Preliminary results suggest that thapsigargin treatment tends to increase the expression of *BiP* (p=0.3), *CHOP* (p=0.7), *IRE1a* (p=0.2) and *XBP1spliced* (p=0.0041). The treatment did not change the expression of *SPOT14*, but reduced the expression of *Dio1* (p=0.01). CONCLUSION: In conclusion, thapsigargin was able to induce ER stress, confirmed by the significant increase in *XBP1* activation and decreased the expression of the *Dio1* enzyme, but not of the TH-responsive protein *SPOT14*, which is an unprecedented result in HepG2 cells.

Keywords: Endoplasmic Reticulum Stress, HepG2 Cells, Thyroid Hormones
Supported by: CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior)

B.31 - High-intensity physical exercise alters mitochondrial metabolism in the hippocampus differently in Alzheimer's disease model

Jonathas Rodrigo dos Santos [1], Gustavo Ferrari[1], Ana Azzolini[1], Luciane Alberici[1]
[1]Dep. of Biomolecular Sciences, Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo (SP, Brasil)

INTRODUCTION: Alzheimer's disease (AD) is the most common neurodegenerative disease worldwide. The accumulation of amyloid-β protein (aβ) and presence of neurofibrillary tangles are hallmarks of the disease. Mitochondria plays a central role in AD pathophysiology, since aβ accumulation and binding into mitochondrial membrane channels impairs mitochondrial autophagy (mitophagy), dynamics, respiratory capacity and induces oxidative stress. Physical exercise is known as a powerful neuroprotector, but whether it acts on brain metabolism needs studies. OBJECTIVES: To verify the effect of two protocols of physical training in treadmill (moderate – MOD: 30 min at 50% maximal intensity velocity test (MIVT), 5 times/week; or high-intensity – HI: 4 bouts of 2 min at 70% MIVT with 1 min at 50% MIVT, 3 times/week), during 8 weeks on brain mitochondrial metabolism of mice model of AD – 3xTG-AD and healthy counterpart (WT). MATERIALS AND METHODS: ELISA assay, citrate synthase activity, respirometry and immunohistochemistry of fluorescence in hippocampus and pre frontal cortex. DISCUSSION AND RESULTS: Both protocols of physical exercise increased brain derived neurotrophic factor (BDNF), an important neuroplasticity factor, but did not alter aβ levels. In hippocampus of WT, especially HI exercise promoted a reduction in mitochondrial content and mitophagy events, but increased oxidative phosphorylation and maximal respiratory capacities per mitochondria and LDH-A RNAm expression, indicating a metabolic shift to oxidative phosphorylation. Instead, in hippocampus of 3xTg-AD, HI exercise reduced oxidative phosphorylation and maximal respiratory capacities per mitochondria, did not altered mitochondrial content or mitophagy, and increased LDH-A and GLUT-3 RNAm expressions, indicating an additional requirement of glucose. In pre frontal cortex, HI exercise only increased oxidative phosphorylation capacity per mitochondria in WT. CONCLUSION: Eight weeks of HI physical exercise alters mitochondrial metabolism in brains of healthy and AD models in a different manner, which may represent different forms of neuroprotection induced by exercise.
Keywords: Alzheimer's Disease, Mitochondrial metabolism, Physical exercise / **Supported by:** CNPq, FAPESP

B.32 - Brain and Liver Mitochondrial Bioenergetics of Wistar Rats are Affected by Acute Exposure to Thimerosal, a Mercury Derivative Preservative

Marcos Vinicius dos Santos Sales [1], Aline Félix[1], Rafael Azevedo[3], Francisco Cunha[2], Josué Santos[2], Ana Leite[1]

[1]UFAL, Laboratório de Bioenergética (Alagoas, Brasil), [2]UFAL, Laboratório de Instrumentação e Desenvolvimento em Química Analítica (Alagoas, Brasil), [3]UPE, Laboratório de Biologia Celular e Molecular (Pernambuco, Brasil)
INTRODUCTION: Thimerosal (TM) is a mercury-derived organometallic compound used in vaccines as a preservative that has toxic properties. Mitochondria is an organelle that demonstrates a high vulnerability to many species, such as metals, for example, mercury (Hg) and its derivatives. OBJECTIVES: Evaluate if TM exposition mimicking the vaccination period an animal model has any effects on mitochondrial bioenergetics from liver and brain. MATERIALS AND METHODS: The animals were divided in two groups treated for 24 h: TM (20 µg/kg/day) and control (saline). The total mercury, in tissues, was quantified by Atomic Fluorescence Spectrometer. The electron transport chain (ETC) in the isolated mitochondria was evaluated using an oxygen electrode (Hansatech). The mitochondrial membrane potential and H2O2 production were analyzed using fluorescence probes also in isolated mitochondria. Besides, the activity of some enzymes (SOD, CAT, GPx, and AChE) and the quantification of total thiol were performed in tissues. This project has an ethics committee-approved process 01/2019. DISCUSSION AND RESULTS: The accumulation of Hg in the brain and liver was higher in treated animals when compared to control. In isolated mitochondria from the liver, TM improved respiratory control by ~ 21%, however, steps 3 and 4 of the ETC presented a decrease of 16 and 37%, respectively. furthermore, isolated mitochondria from the brain presented an improvement of ~ 61% in respiratory control. Brain enzyme activity was mostly affected by TM treatment, with a decrease in SOD (32%), AChE (42%), and an increase in GPx (79%) and CAT (100%). For the liver, the unique enzyme that presented a significant difference was GPx activity, with an increase of 37%. Mitochondrial brain and liver membrane potential, H2O2 production, and total thiol showed no difference. CONCLUSION: Thus, the treatment with TM acutely showed a damage more pronounced in brain, demonstrating the toxicity of TM is drived to nervous system.
Keywords: mitochondrial bioenergetics, thimerosal, animal model / **Supported by:** FAPEAL, CNPq, IQB, PPGQB, and UFAL

B.33 - Immunometabolism in the symbiotic relationship between the Aedes fluviatilis mosquito and bacterium Wolbachia pipientis

Jhenifer Nascimento da Silva [1], Christiano Calixto da Conceição[1], Gisely Brito[1], Carlos Jorge Logullo de Oliveira[1]
[1]Instituto de Bioquímica Medica Leopoldo de Meis, Universidade Federal do Rio de Janeiro (RJ, Brasil)

INTRODUCTION: Wolbachia pipientis is a maternally transmitted bacterium that mostly colonizes arthropods, including insects, arachnids, crustaceans, and nematodes. Was seen that Wolbachia strain (wFLU) promotes a modulation of glycogen metabolism in your natural endosymbiont, the mosquito Aedes fluviatilis. When silencing the enzyme glycogen synthase kinase 3 (GSK3), the enzyme regulating glycogen synthesis, the amount of this bacterium and glycogen in embryos Aedes fluviatilis increases. OBJECTIVES: The aim of this study was described metabolic e immune changes mediated by GSK3. MATERIALS AND METHODS: Furthermore, we comparing naturally Wolbachia-infected (Wolb+) and uninfected (Wolb-) embrionic cells of Ae. fluviatilis, thus increasing the knowledge about Wolbachia parasitic/symbiotic mechanisms. DISCUSSION AND RESULTS: Comparing Wolb+ and Wolb- cells, we observed that Wolb+ have double of glycogen content followed by decreased GSK3 transcription and increased PEPCK (Phosphoenolpyruvate carboxykinase) transcription. In addition, Wolb+ cells have down-regulated gambicin. By inhibiting GSK3 activity, we observed an increase in Glycogen content and Wolbachia load, followed by a reduction in Relish2 (REL2) and Gambicin transcripts. To investigate whether the IMD pathway acts to control the amount of Wolbachia, we reduced the levels of REL2 in Wolb+ cells by RNAi. The knock-down of Rel2 leads to amount of Wolbachia increases followed by increase in Glycogen content and up-regulated PEPCK transcription. CONCLUSION: Therefore, these data set suggest a possible correlation between GSK3 and REL2.

Keywords: Wolbachia pipientis, , Glycogen Synthase Kinase 3 (GSK3), IMD pathway / **Supported by:** Capes, CNPq, FAPERJ

B.34 - ATP: ADP Ratio Regulates the Adenylyl-removing Activity of Herbaspirillum seropedicae GlnE Enzyme *in vitro*

Eduardo Sabatine Lopes [1], Larissa Fonseca Tomazini[1], Josielle Abrahão de Souza[1], Ana Paula Silva Alves [1], Lorena Gomes Polizelli[1], Marco Aurelio Schüler de Oliveira [1]
[1]Bioquímica , Universidade Estadual de Maringá (Paraná, Brazil)

INTRODUCTION: Herbaspirillum seropedicae, an endophytic plant growth-promoting bacteria, colonizes economically interesting crops such as maize, rice, and sugarcane and has the potential to be used as a biofertilizer. However, a precise description of its nitrogen metabolism is necessary to improve the nitrogen fixation and release of ammonium to the host plant. In bacteria, the Glutamine Synthetase (GS) enzyme is responsible for ammonium assimilation under nitrogen-starvation conditions. GS activity is regulated by a post-translational modification, a reversible adenylylation catalyzed by the bifunctional GlnE enzyme. In Escherichia coli, both Adenylyl-transferase (ATase) and Adenylyl-removing (AR) activities of GlnE are regulated by GlnB protein. For H. seropedicae the GS regulation by GlnE and GlnB remains elusive. OBJECTIVES: Here, we investigated the *In vitro* regulation of AR activity of H. seropedicae GlnE by GlnB protein and its allosteric modulators MATERIALS AND METHODS: We purified the isolated AR domain of GlnE by affinity chromatography and perform *In vitro* γ-GT GS activity assays in the presence or absence of GlnB/GlnB-UMP3 proteins and ATP, ADP, and ATP/2-OG. In order to show the interaction of both ATP and ADP to GlnE, we carried out Differential Scanning Fluorimetry assays DISCUSSION AND RESULTS: The results showed that GlnB and GlnB-UMP3 did not influence the AR activity of GlnE for direct interaction with the AR domain. We observed that AR activity was activated by ATP and inhibited by ADP. The DSF results showed that AR domain possesses an ATP: ADP ratio sensitivity site. CONCLUSION: En-bloc our results suggest that the deadenylation of H. seropedicae GS is strongly dependent on the cell energy status and that this regulatory signal is directly integrated by GlnE, differently from other organisms, in which this signal is transduced by GlnB. This regulation mechanism of GS activity is, to our knowledge, unique among those described so far

Keywords: Glutamine Synthetase, PII proteins, GlnE
Supported by: CNPq/CAPES

B.35 - Spectroscopic Characterization and Cytotoxicity Evaluation of a Library of Urea-Based Compounds

Gabrielle Silva Damiano [1], Laura Francisca Leite do Prado de Souza[1], Rayssa de Mello Lopes[1], Antônio Augusto Soares-Paulino[2], Hélio Alexandre Stefani [2], Tiago Rodrigues[1]
[1]Centro de Ciências Naturais e Humanas, Univ.Federal do ABC (SP, Brasil), [2]Faculdade de Ciências Farmacêuticas, Universidade de São Paulo (SP, Brasil)

INTRODUCTION: Protein kinase inhibitors have been successfully developed and used in the cancer chemotherapy and targeted therapy. Among them, the urea moyet-based sorafenib is a well-known antineoplasic agent indicated for the treatment of hepatocellular and renal cell carcinoma. Its mechanism of action includes the inhibition of several kinases, including RAF and VEGFR2. However, the development of drug resistance has already described and it significantly limits sorafenib's therapeutic efficacy. Thus, the development of novel kinase inhibitors based on the urea moyet represents a strategy to reduces toxicity, increases efficacy, and also overcome drug resistance. OBJECTIVES: To sinthesize and characterize a serie of novel urea derivatives and also to screen their cytotoxicity against tumor cells *in vitro.* MATERIALS AND METHODS: Seventeen urea derivatives were sinthezised, purified, and solubilized in dimethyl sulfoxide at 50 mM as stock solutions. The UV-vis spectroscopic characterization was performed in a UV1800 Shimadzu spectrophotometer by scanning the absorbance of a 50 µM aqueous solution of each derivative from 200 to 800 nm. Cell viability was evaluated by the MTT reduction test. Values were normalized in percentage, considering the control (absence of drug) as 100%. DISCUSSION AND RESULTS: All urea derivatives presented light absorption in the UV region and most of them had a structured band in the 250-300 nm region. Based in a concentration-response curve from 2.5 to 200 µM, all derivatives showed no or low cytotoxicity in tumor cells *in vitro*. Despite this, further experiments are necessary to evaluate their effects on the viability of tumor cells from different origins and on tumor cell migration and proliferation. CONCLUSION: All coumpounds presenting the urea moyet evaluated in this study exhibited low cytotoxic potency against tumor cells and new experiments are required to evaluate the antitumor potential of these class of coumpounds.

Keywords: kinase inhibitor, cancer, urea moyet / **Supported by:** FAPESP, CNPQ

B.36 - Measurement of Lysophospholipid Acyltransferase Activities on Macrophages Using Substrate Competition Assay

Clara Julia Lima Salmaso [1], Diana Mota Toro[2,3], Pedro Vieira da Silva Neto[2,3], Adriana Ferreira Lopes Vilela[1], Carlos Arterio Sorgi[1,3,4]
[1]Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto (São Paulo, Brasil), [2]Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto (São Paulo, Brasil), [3]Instituto de Ciências Biológicas, Universidade Federal do Amazonas (Amazonas, Brasil), [4]Departamento de Bioquímica e Imunologia, Faculdade de Medicina de Ribeirão Preto (São Paulo, Brasil)

INTRODUCTION: The Lands Cycle remodels the products of membrane lipids by novel pathway, through the sequential action of phospholipase and lysophospholipid-acyltransferase (LPAT), producing different molecular species of phospholipids. LPATs belong to two families represented by lysophosphatidic acid acyltransferases (LPAATs) and membrane-bound O-acyltransferases (MBOATs). OBJECTIVES: We seek to determine the activities of lysophospholipid-acyltransferases on macrophages by methodology associated with tandem mass spectrometry (LC-MS/MS). MATERIALS AND METHODS: Bone marrow-derived macrophages (MDMO) were incubated or not with IL-4 and/or IL-13 to obtain M2 or incubated with IFN-γ to M1 phenotype. Macrophage microsomes were prepared and incubated with eight acyl-CoA species and six lysophospholipid species. The samples were extracted according to the Bligh and Dyer method and injected into an LC system, coupled to a triple quadrupole mass spectrometer. DISCUSSION AND RESULTS: Compared to the different MDMO phenotypes, M0 (non-stimulated) and M2 have a lot of similarities in the constitution of membranes related to the classes of phospholipids containing anchored arachidonic acid (AA) or docosahexaenoic acid (DHA). However, M1 showed a decrease in these species by increased activation of cPLA2 in membranes or lower activity of acyltransferases for the formation of some phospholipid products. The M1 enzyme activity was compatible with the control, and M2 phospholipids species analyzed was increased, suggesting an up-regulation in their enzymes gene expression. CONCLUSION: Comparing differents MDMO phenotypes, we demonstrated deviations in the membrane phospholipids composition and in the activity of acyltransferases. Also, the substrate competition assay perfomed an important methodology for the lipids enzymology study.

Keywords: acyltransferases, macrophages, phospholipids / **Supported by:** FAPESP, CNPq, CAPES

B.37 - Role of IGF-1 in Type II Muscle Fibers: a Look at Metabolism

Maria Catharina Mendes Fenuchi[1], Gabrielle Cristina Morante[1], Michelle Nascimento Sales[1], Marcelo Augusto Christoffolete[1]
[1]CCNH (Centro de Ciências Naturais e Humanas), Federal University of ABC (São Paulo, Brasil)

INTRODUCTION: Hypertrophy is an adaptive process that results in an increase in the cross-sectional area of the muscle fiber. This process involves several factors such as: growth factors, hormones, immune system, satellite cell, among others. IGF-1 is an important regulator of muscle mass through interaction with a specific receptor (IGF-1R), inducing stimulation of protein synthesis, and/or inhibition of protein degradation, through intracellular signaling pathways (Akt/mTOR; calcineurin/NFAT; MAPKs and myostatin control). OBJECTIVES: The present study aims to mimic the effects of IGF-1 by its overexpression in type II muscle cells (C2C12). In this sense, we intend to carry out the construction of a viral molecular tool controlled by the Tet-ON system and evaluate the effects on metabolism and cell morphology. MATERIALS AND METHODS: The tool will be generated by cloning the TET3G-responsive promoter region of the pmRi-mCherry (pTIGHT) vector (Clontech) in the pENTR 5'-TOPO vector (Invitrogen) and subcloning the coding region of the IGF-1 gene to the pENTR11 vector (Invitrogen), to submit them to the double recombination reaction, forming the lentiviral vector pLENTi-TIGHT-IGF-1-IRES-eGFP. C2C12 cells will be submitted to the transfection protocol with Lipofectamine 2000 (Invitrogen). At the end of the experiment, the cells will be fixed with formalin and morphometric analysis will be performed. Protein extracts from transfected C2C12 samples will be subjected to Western Blotting technique using primary antibodies in rabbit (pAkt, Akt, ERK 1/2 and pERK 1/2) and the secondary antibody conjugated to peroxidase made in goat. After the extraction of total RNA, RT-qPCR will be performed for analysis of gene expression. DISCUSSION AND RESULTS: The subclonings were successful, confirmed by sequencing, and it is expected that there will be significant hypertrophy and modulation of metabolism genes. CONCLUSION: It can be concluded that the viral molecular tool is viable and functional for this type of study.
Keywords: C2C12, Hipertrofia, IGF-1 / **Supported by:** FAPESP, UFABC

B.38 - A New Protocol for Assessment of Mitochondrial Oxygen Consumption on Drosophila melanogaster Larval Fillets with High-Resolution Respirometry

Caio Fábio Baeta Lopes[1], **Giulia Covolo Spegiorim** [1], Luciane Carla Alberici[1]
[1]Depto de Ciências Biomoleculares, Faculdade de Ciências Farmacêuticas de Ribeirão Preto (São Paulo, Brasil)

INTRODUCTION: Mitochondrial bioenergetics is a set of vital processes, harnessing free energy for endergonic biochemical reactions in far-from-equilibrium systems. To understand mitochondria in health/disease, multiple models are available, capturing the physiology of this intricate organelle. Non-murine metazoans are promising, since they are cheaper/easier to handle than mammals, generating robust results. *Drosophila melanogaster* has been applied to mitochondrial science successfully, expanding the reach of this model. OBJECTIVES: To advance on this scope, we use 3rd instar larval fillets to assess mitochondrial function in well-preserved, ex vivo preparations, with high-resolution respirometry. MATERIALS AND METHODS: For fillets, 18 larvae/group were dissected, permeabilized with 62.5 µg/mL saponin and used in an Oroboros® oxygraph. 6 male adult thoraces/group were dissected and permeabilized for respirometry. Oxygen consumption rate (OCR, pmol.s-1.IU-1) in BASAL (fillets) and LEAK (thoraces) states was reached with pyruvate, malate and glutamate. OXPHOS was determined with ADP addition. LEAK on fillets was measured adding oligomycin. ETS was defined with CCCP. Non-mitochondrial OCR was determined after NaCN. For citrate synthase (CS) activity, samples were homogenized and assayed for conversion, by CS, of DTNB to TNB, with 0.125 mM acetyl-CoA and 0.125 mM oxaloacetate. DISCUSSION AND RESULTS: Fillets comprise a muscle wall above epidermal cells covering a network of sensory neurons, all contributing to the OCRs. Transgenic fillets (*ppk::GFP* and *ppk-GAL4*) expressing proteins on peripheral C4da neurons have significantly reduced OCRs compared to wild-type (WT) Canton S, but the same mitochondrial content, inferred by CS. The same was observed for thoraces, both results indicating distinct mitochondrial function among WT and transgenic lines. CONCLUSION: Thoraces and fillets have distinct OCR profiles, the latter being more coupled that the former, indicating that tissues other than muscles are consuming oxygen on larval preparations, probably sensory neurons.
Keywords: Drosophila melanogaster, Mitochondria, High-resolution respirometry

B.39 - Bisphenol-A impairs glucose uptake and hexokinase II activity in differentiated L6 myotubes

Beatriz Monteiro Rocha [1], Reinaldo Sousa dos Santos[2,3], Wagner Seixas da Silva[1]
[1]Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro, (Rio de Janeiro, Brasil), [2]Instituto de Investigación, Desarrollo e Innovación en Biotecnología Sanitaria d, Universidad Miguel Hernández de Elche (, Espanha), [3]CIBER de Diabetes y Enfermedades Metabólicas Asociadas , Instituto de Salud Carlos III (, Espanha)

INTRODUCTION: Endocrine disruptors are substances that can interact with the endocrine system and alter its function. Bisphenol-A (BPA), an endocrine disruptor primarily used in the manufacture of plastics, has been associated with insulin resistance and type 2 diabetes development. Besides its known role on glucose homeostasis, skeletal muscle also acts as an endocrine organ, secreting myokines that can modulate different biological processes, such as thermogenesis and inflammation. OBJECTIVES: As the effects of BPA in skeletal muscle energy metabolism are still not fully understood, the aim of our study was to investigate whether BPA would affect glucose uptake and hexokinase II activity in differentiated L6 myotubes. MATERIALS AND METHODS: L6 myoblasts were differentiated into myotubes for 7 days. Upon differentiation, cells were treated with 0.1, 1 and 10 nM BPA for 24 h. Glucose uptake was determined by quantifying the glucose concentration in the culture media before and after the treatment using a commercial kit. Subcellular fractions were obtained by differential centrifugation and hexokinase II activity was measured spectrophotometrically by a coupled enzyme assay. DISCUSSION AND RESULTS: All three BPA concentrations increased hexokinase II activity both in soluble and particulate subcellular fractions. Remarkably, myotubes treated with 10 nM BPA showed a 50% increase in hexokinase II activity in both subcellular fractions compared to vehicle-treated cells. Conversely, 10 nM BPA-treated cells showed a 30% reduction in glucose uptake as compared to vehicle-treated cells. CONCLUSION: Our results suggest that BPA exposure can impair glucose homeostasis in skeletal muscle by changing glucose uptake and hexokinase II activity. These changes might contribute to BPA diabetogenic activity. More analyses have been conducted to better understand BPA effects on skeletal muscle metabolism.
Keywords: Bisphenol-A, hexokinase, skeletal muscle / **Supported by:** FAPERJ

B.40 - Effects of sodium arsenate (AsV) and sodium meta-arsenite (AsIII) on Metabolic Fluxes Linked to Energy Metabolism in the Isolated Perfused Rat Liver

Nairana Melo[1], Rosane Peralta[1], **Adelar Bracht** [1], Livia Bracht[1]
[1]Departamento de Bioquímica, Universidade Estadual de Maringá (Paraná, Brazil)

INTRODUCTION: Sodium arsenate is always mentioned in Biochemistry text-books as a compound that should be toxic to the glycolytic pathway. By transitorily replacing phosphate at the glyceraldehyde 3-phosphate dehydrogenase step the compound is regarded as a kind of glycolysis uncoupler. This is a very old notion but, surprisingly, quantification of its consequences in intact mammalian aerobic cellular systems is still quite precarious. This is especially true for the liver for which no specific measurements at realistic concentrations have yet been reported. OBJECTIVES: To quantify the actions of sodium arsenate (AsV) and sodium meta-arsenite (AsIII) on metabolic fluxes in the liver and, for comparison purposes, in red blood cells. MATERIALS AND METHODS: The experimental systems were the isolated rat liver under once-through perfusion and isolated red blood cells. Oxygen was assayed polarographically and metabolites enzymatically. DISCUSSION AND RESULTS: AsV up to 1 mM was almost inactive on glycolysis, lactate-driven gluconeogenesis and oxygen uptake in the perfused liver. In erythrocytes it stimulated glycolysis by 48%. AsIII, on the other hand, significantly increased glycolysis in the perfused liver, but was inhibitory in erythrocytes at concentrations up to 100 µM. AsIII was particularly active on gluconeogenesis in the perfused liver where 50% inhibition took place at the concentration of 25 µM and almost 95% at the concentration of 100 µM. This effect was paralleled by an inhibition of oxygen uptake. CONCLUSION: The intrinsic deleterious effects of AsV seem to be frequently grossly overestimated, at least with respect to glycolysis and gluconeogenesis. AsIII, however, is remarkably active on hepatic glycolysis and especially inhibitory against hepatic gluconeogenesis, probably in consequence of its action on the mitochondrial energy metabolism. Impairment of metabolism by AsV can only be expected to occur after its reduction to AsIII, which in the liver has been reported to occur as a secondary reaction via the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase.
Keywords: arsenic, metabolism, toxicity / **Supported by:** CNPq and Fundaçao Araucária

B.41 - 4-Phenylbutiric Acid Increases the Glucose Phophorylation by mitochondrial Hexokinase in L6 Myotubes via PI3K/Akt pathway

Aline Machado de Oliveira [1], Wagner Seixas da Silva[1]
[1]Instituto de Bioquímica Médica Leopoldo De Meis, Universidade Federal do Estado do Rio de Janeiro (Rio de Janeiro, Brazil)

INTRODUCTION: The skeletal muscle is the major tissue responsible for the uptake of glucose stimulated by insulin. The type II hexokinase (HK II) is the prevalent isoform expressed in the skeletal muscle tissue and can be found freely in the cytosol or associated in the outer mitochondrial membrane. 4-Phenylbutyric acid (4-PBA) is known as a chemical chaperone, due its capacity of inhibit endoplasmic reticulum stress. Our group showed that the treatment with 4-PBA for 72 h was able to increase HK II activity in L6 myotubes in the mitochondrial fraction. However, the mechanism involved in this regulation unknown. The PI3K/Akt signaling pathway has been described as regulator of type II hexokinase dynamic association in subcellular compartments. OBJECTIVES: The objective of this work was identify which pathway could be related to increase of HK II activity in L6 myotubes during the treatment with 4-PBA. MATERIALS AND METHODS: Rat L6 myoblasts were differentiated in myotubes (Differentiation was induced by DMEM supplemented with 2% HS). The myotubes were pre-incubated with 50 μM LY294002 (well-known PI3K/Akt inhibitor) during 1 h, then treated with 1 mM 4-PBA during 96 h. After that, cultures were submitted to sub-cellular fractionation and hexokinase activity, glucose consumption and HK expression (through western blot) were evaluated. DISCUSSION AND RESULTS: We observed that L6 myotubes treated with the 4-PBA during 96 h showed an increase in HK activity, in particulate and soluble fractions. We detected an increase in glucose uptake, HK II protein expression and basal oxygen consumption. The inhibition of the PI3K/Akt pathway with LY294002, abrogated the reduction in HK II activity in both soluble and particulate fractions and promotes reduction in HK II protein expression. CONCLUSION: Taken together, our results suggest that the 4-PBA is leading to an increase of the HK II activity through the participation of PI3K/Akt pathway.

Keywords: 4-PBA, hexokinase II, PI3K/Akt
Supported by: FAPERJ, CNPq and CAPES

B.42 - Effects of Heat Shock on Carbohydrate Metabolism of Astianax Iacustris (Lütken, 1875)

Diego Mauro Carneiro Pereira [1], Anna Carolina Resende[1], Ieda Cristina Schleger[1], Ananda Karla Alves Neundorf[1], Silvia Romão[2], Maria Rosa Dmengeon Pedreiro de Souza[1], Tatiana Herrerias[3], Lucélia Donatti[1]
[1]Biologia Molecular, Federal University of Parana (Paraná, Brasil), [2]-, Federal University of Fronteira Sul (Paraná, Brasil), [3]-, Faculty Guairacá (Paraná, Brasil)

INTRODUCTION: Temperature is an important factor that conditions the physiological responses of fish, generating a stressful condition when in non-ideal parameters. In this context, fish represent a good study tool, given the disturbances that alter water dynamics, directly affecting these animals. OBJECTIVES: Thus, the objective was to evaluate metabolic aspects in the muscle of freshwater fish *Astyanax lacustris* submitted to thermal shock. MATERIALS AND METHODS: The specimens were subjected to 2, 6, 12, 24, 48, and 96 hours of exposure to low (15 ± 1°C) and high temperature (31 ± 1°C), with their respective controls (23 ± 1°C). DISCUSSION AND RESULTS: At 15 ºC there was a reduction in glycogen phosphorylase (GP) activity at 2 h and pyruvate and lactate levels at 2 h and at 6 h, respectively, indicating a reduction in glycogenolysis in the initial periods. Subsequently, there was an increase in GP activity at 12 h, pyruvate levels at 48 h and hexokinase activity at 96 h, suggesting an increase in energy demand. At 31 ºC the absence of increased anaerobic or aerobic metabolism may suggest that the treatments tested did not cause an increase in ATP demand for temperature maintenance. Other hypotheses are: a) that fish may be using another ATP delivery mechanism such as the phosphocreatine kinase pathway; (b) the low temperature did not affect the animal's muscle homeostasis. In addition, the integrated biomarker response index proved to be a good ally in the evaluation of a set of biomarkers, corroborating the results observed by the biomarkers individually. CONCLUSION: Thus, it is possible to conclude that the acute thermal shock affects the metabolism of *A. lacustris* muscle, which undergoes rearrangements to deal with the variation in temperature, where 15 ºC is more stressful than 31 ºC.

Keywords: Enzymes, Thermal shock, Metabolism / **Supported by:** CNPq and CAPES

B.43 - Effects of Low-Density Lipoprotein-Cholesterol on Mitochondrial Parameters in HT-22 Cells

Hémelin Resende Farias [1], Jéssica Marques Obelar Ramos[1], Natália Baltazar Nascimento[1], Lilian Correa Costa-Beber[1], Leo Anderson Meira Martins[1,2], Gabriela Joras Baumart[1], Fernanda Silva Ferreira[1], Felipe Schmitz[1], Sabrina Somacal[3], Angela Terezinha de Souza Wyze[1], Fatima Theresinha Costa Rodrigues Guma[1], Jade de Oliveira[1]
[1]Departamento de Bioquimica, Universidade Federal do Rio Grande do Sul (Rio Grande do Sul, Brazil), [2]Departamento de Fisiologia, Universidade Federal do Rio Grande do Sul (Rio Grande do Sul, Brazil), [3]Centro de Ciências Rurais, Universidade Federal de Santa Maria (, Brazil)

INTRODUCTION: Hypercholesterolemia is characterized by high plasmatic concentrations of low-density lipoprotein (LDL)-cholesterol. Importantly, hypercholesterolemia has been associated with cognitive impairment both in humans and animals. The exact mechanisms by which hypercholesterolemia leads to cognitive impairment are still unclear. Mitochondria are essential for cellular maintenance as it is responsible for cellular respiration. However, mitochondrial dysfunction has been associated with neuronal damage since mitochondria are the main producer of reactive species (RS) in oxidative phosphorylation. OBJECTIVES: Therefore, in this study, we aimed to evaluate LDL's effect on mitochondrial parameters in HT-22 cells. MATERIALS AND METHODS: HT-22 (Mouse Hippocampal Neuronal Cell) were exposed to human isolated LDL (50 and 300 µg/ml) for 24 hours. After that, we evaluated the RS formation by measuring DCF fluorescence and mitochondrial superoxide production via MitoSOX. Moreover, mitochondrial mass and membrane potential were determined with Mitotracker Green (MTG) and Red (MTR), respectively. In addition, we measured Complex II activity. Cellular damage was measured by extracellular lactate dehydrogenase activity and Annexin V Staining. DISCUSSION AND RESULTS: We observed that LDL increased RS production in both concentrations. Also, mitochondrial superoxide formation was elevated in HT-22 cells exposed to LDL (50µg/ml). HT-22 cells exposed to LDL had decreased MTR/MTG ratio and Complex II activity, indicating mitochondrial dysfunction. Next, we evaluated if mitochondrial dysfunction is associated with cell death. Cells exposed to LDL had increased apoptosis (50 and 300µg/mL) but no alteration in extracellular lactate dehydrogenase activity. CONCLUSION: LDL caused mitochondrial alterations, suggesting mitochondrial dysfunction, which was related to neuronal damage.
Keywords: Mitochondria , Hypercholesterolemia, Cholesterol / **Supported by:** Capes

B.44 - Plasma Lipid Profile and Analytical Macro and Microextraction Method Validation

Miguel Rocha Patricio [1], Tânia Petta[1], Diana Mota Toro[3,4], Pedro Vieira da Silva Neto[3,4], Adriana Ferreira Lopes Vilela[1], Luiz Alberto Beraldo de Moraes[1], Carlos Arterio Sorgi[1,2,4]
[1]Depto de Química, Faculdade de Filosofia, Ciências E Letras De Ribeirão Preto. (SP, Brasil), [2]Depto de Bioquímica e Imunologia, Faculdade de Medicina de Ribeirão Preto (SP, Brasil), [3]Depto de Análises Clínicas, Toxicolocogicas e Bromatológicas, Fac. de Ciências Farmacêuticas de Ribeirão Preto (SP, Brasil), [4]Instituto de Ciências Biológicas. Univ Federal do Amazonas (Amazonas, Manaus)

INTRODUCTION: Metabolomics is the new scientific tool for studying biochemistry components in the cells, tissues and organisms. The complexity of the metabolomics work involved matrices demands from the analytical chemist, the development of new protocols and the modification of existing methods to obtain a full biochemical profile of the sample. Therefore, becoming a great method to identify biomarkers for potential diseases. The ideal sample preparation should be as simple as possible, to minimize the manipulation of the matrix, including its modification and losses. Indeed, it must secure efficient sample cleanup, metabolism quenching and coverage of a wide range of analytes in order to provide a true representation of the studied system. OBJECTIVES: The aim of this study was to establish a solid-phase microextraction methodology for metabolomics using human plasma to comprehensive characterization of their lipid profiles, and to compare them with those macro and liquid-liquid extraction. MATERIALS AND METHODS: The liquid-liquid extractions were carried out as follow Blight & Dyer methodology, and the solid-phase extractions (macro and micro) were conducted using different absorptive coatings. The quantitative analyses of lipids were performed by LC-MS/MS. DISCUSSION AND RESULTS: The results have shown that it is possible to extract mostly of lipid metabolites with a single coated film or fibre with sensitivity and precision comparable to that of traditional methods, such as ultrafiltration, protein precipitation or liquid-liquid extraction. CONCLUSION: In summary, the difference between the analytical methods is explicit. It was possible to categorize them in order to define groups in which each extraction could benefit from stationary phases of the macro or microextraction methods, in addition to designing analytical parameters to improve the metabolomics lipid profile.
Keywords: metabolomics, microextraction, lipids / **Supported by:** FAPESP

B.45 - Initial Characterization of the Lipid Metabolism of the Beetle Tribolium castaneum

Marcelle Pessoa do Nascimento [1,2], Pamela Verdan Pimenta do Nascimento[1], Thayná dos Santos Assunpção Regis[1], David Majerowicz[1,3]
[1]Departamento de Biotecnologia Farmacêutica, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil), [2]Instituto de Biologia, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil), [3]INCT-EM, Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular (Rio de Janeiro, Brasil)

INTRODUCTION: The beetle Tribolium castaneum, also known as the red flour beetle, is a cosmopolitan pest of stored grains, flour, and other cereal products and a model organism for developmental biology. Its genomic data, transcriptomics, and the efficiency of RNA interference tools make this insect an attractive model for the proposed approach. However, information on the lipid metabolism of this beetle is still scarce. OBJECTIVES: Therefore, our objective is to carry out the initial characterization of the lipid metabolism of T. castaneum. MATERIALS AND METHODS: The amount of triacylglycerol in insects was measured by a colorimetric enzyme assay. Gene expression was measured by quantitative PCR. DISCUSSION AND RESULTS: During development, the larvae store triacylglycerol, reaching the maximum amount of fat in the last larval stage and the pupae. After the emergence of adults, the amount of stored triacylglycerol drops suddenly, reaching a minimum level in seven-day-old adults. Several genes of lipid metabolism had their expression analyzed. At the earlier stages of development, the larvae presented higher gene expression for carnitine acyltransferase 1, the limiting step of beta-oxidation. On the other hand, the Brummer lipase and fatty acid synthase genes showed lower expression in pupae. Preliminary results in adult beetles suggest that the acetyl-CoA carboxylase and fatty acid synthase genes increase their expression after the emergence. CONCLUSION: These results indicate that T. castaneum has a dynamic lipid metabolism, as seen by variations in the amount of stored lipids and the expression profile of genes involved in lipid synthesis and degradation.
Keywords: Beetle, Metabolism, Genes

B.46 - Repercussions of Different Intensities of Resistance Training During Pregnancy on Maternal Fasting Glucose and Biochemical Profile

Débora Priscila Lima de Oliveira [1], Thaynan Raquel Dos Prazeres Oliveira[1], Maira Beatriz Matos de Paula[3], Rafael Victor Lira Brito[3], Jose Antonio Dos Santos[2], Raquel da Silva Aragão[1,2]
[1]Programa de Pós-Graduação em Nutrição, Universidade Federal de Pernambuco (PE, Brasil), [2]Núcleo de Educação Física e Ciências do Esporte, Universidade Federal de Pernambuco (P, Brasil), [3]Departamento de Nutrição, Universidade Federal de Pernambuco (PE, Brasil)

INTRODUCTION: Pregnancy is a critical period of development and can be influenced by environmental factors such as physical training. OBJECTIVES: To investigate the effects of different intensities of resistance training during pregnancy on maternal fasting glucose and biochemical profile. MATERIALS AND METHODS: Female Wistar rats were used. Resistance training (RT) was performed in ladder. Maximum load (ML) test occurred every week to provide training intensity. Before pregnancy, all trained rats performed 10 climbs/section, 5 sessions/week; 80% of MLC. During pregnancy, the trained group performed the same number of climbs and section but were divided according to load in first/second/third week of gestation: Constant-Intensity Training (CIT, n=8, 80%/80%,80%), Variable-Intensity Training (VIT, n=7, 50%/80%/50%), Decreasing-Intensity Training (DIT, n=7, 80%/80%/50%). There were also a Control (no training, C, n=7) and Maximum load (only MLT, ML, n=6) groups. Fasting glucose was measured weekly during gestation. Serum was collected at 20th gestational day. DISCUSSION AND RESULTS: CIT group had higher fasting glucose than VIT group at 2nd gestational week ($p < 0.05$). At 20th gestational day, serum glucose was reduced in CIT group compared to C and DIT groups ($p < 0.05$); triglycerides were higher in VIT group compared to ML and CIT ($p < 0.05$) group, albumin was lower in the ML group compared to the other groups ($p < 0.05$), and there was no difference in total cholesterol levels. RT may have induced an increase in GLUT-4 which resulted in reduced glucose concentrations in the higher intensity group, as demonstrated in a study with mice and RT (BRONCZEK et al., 2021). Commonly, at the end of pregnancy, there is a high degradation of fat deposits resulting in an increase in triglyceride levels (ZENG, Liu and Li, 2017), which may justify the increase in the VIT group. CONCLUSION: Distinct intensities of resistance training during pregnancy differently modulate maternal biochemical profile and fasting glucose.
Keywords: Gestation, Glycaemia, Physical Training

B.47 - Excess of Mrx9p Impairs COX1 Expression in RNA Processing, Translation, and Respiratory Metabolism Adaptation

Jhulia Almeida Clarck Chagas [1], Maria Kfouri Soares[1], Leticia Veloso Franco[1], Mario Henrique de Barros[1]
[1]Instituto de Ciências Biomédicas - Dep. de Microbiologia, Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: Mitochondria are responsible for cellular respiration in eukaryotic organisms harboring a genetic system that provides a small set of subunits to the respiratory complexes. *Saccharomyces cerevisiae* is a facultative anaerobic organism, and a model organism to study mitochondrial biogenesis. Recently, aspects related to the optimization of mitochondrial gene expression have been described, such as the spatial organization of mitochondrial DNA and RNA with the mitoribosomes, and named complexes MIOREX. OBJECTIVES: The present work aimed to identify the function of uncharacterized Mrx proteins of the MIOREX complexes in yeast. MATERIALS AND METHODS: The overexpression of MRX genes was tested and the phenotypes derived from Mrx components excess accompanied by *In vivo* labeling of newly synthesized mitochondrial products, mitoribosome and associated proteins sedimentation properties on sucrose gradients, and Northern blots assays for mitochondrial transcripts. DISCUSSION AND RESULTS: The Mrx9p excess lead to a diminishment of COX1 translation and specifically the accumulation of an aberrant translation product that depends on the presence of mitochondrial introns. Overexpression also impairs the adaptation to the respiratory metabolism of cells and interferes in COX1 RNA processing and COB RNA steady-state level. Moreover, Mrx9p removal or overexpression altered the Mss51p sedimentation pattern. CONCLUSION: The results showed that Mrx9p is part of the regulatory system that the mitochondrial introns exert on gene expression based on the toxicity of COX1 translation in the Mrx9p excess conditions.
Keywords: Miorex-complex, Mitochondrial translation, *Saccharomyces cerevisiae*
Supported by: CAPES e FAPESP

B.48 - Study of gene regulation network related to energy metabolism in BME26 embryonic cells

Yolanda Porto Muniz Martins [1], Satoru Konnai[2], Kazuhiko Ohashi[2], Francisco José Alves Lemos[1], Carlos Jorge Logullo de Oliveira[3], Renato Martins da Silva[3]
[1]Laboratório de Biotecnologia, Universidade Estadual do Norte Fluminense (RJ, Brasil), [2]Laboratory of Infectious Diseases, Hokkaido University (Hokkaido, Japão), [3]Lab. Integr. de Bioq. Hatisaburo Masuda e Inst. de Bioq. Méd. Leopoldo de Meis, Universidade Federal do Rio de Janeiro (RJ, Brasil)

Introduction: Infectious diseases are responsible for the death of millions of animals around the world. Among these arthropods, hematophages gain particular importance, as they transmit infectious agents between humans or between animals and humans during the blood meal performed by these vectors. The study of molecules and enzymes involved in metabolic pathways such as glycolysis, pentose phosphate pathway, gluconeogenesis, glycogen synthesis and degradation, and citric acid cycle in ticks under stress conditions could help in understanding the metabolism regulation of this important vector. **Objectives:** Therefore, this work aimed to investigate the gene response of glucose metabolism regulators in embryonic BME26 cells. **Material and Methods:** Double-stranded RNAi were synthesized for G6PDH, IDH and AKT from R. microplus, and gene silencing was performed in BME26 cells. Subsequently, the transcription analysis of the main genes of energy metabolism in the silenced tick cells and their respective controls (dsGFP) was performed. **Results and Discussion:** With the silencing of G6PDH (approximately 90%) the transcriptional profile of IDH-NADP+ increased significantly. On the other hand, IDH silencing increases the transcriptional level of G6PDH. This last silencing significantly suppressed the glycolytic genes, HK and PK, as well as the genes involved in the regulation of glycogen synthesis and degradation, GSK3, PGM and DE. The results suggest that the IDH-NADP+ enzyme is not only essential to the redox balance in the cell, but that it could also generate a compensatory effect on NADPH production when G6PDH was previously silenced. The results also suggest that AKT silencing may affect the insulin signaling pathway and glucose uptake, and consequently glycolysis. **Conclusions:** These results together describe a metabolic panorama that leads to a better understanding of the modulation of glucose metabolism in embryos of this important vector of diseases, in addition to contributing to the identification of new targets for vaccines that integrate strategies for the control of the species.
Keywords: glucose metabolism, embryonic cells, arthropods / **Supported by:** FAPERJ, CNPq, UENF.

B.49 - Evaluation of Oxidative Parameters in Prefrontal Cortex of Overfed Neonates

Severina Cassia de Andrade Silva [1], Maria Daniele Teixeira Beltrao de Lemos[2], Osmar Henrique dos Santos Junior[3], Mariana Pinheiro Fernandes[1,3], Claudia Jaques Lagranha[1,2,3]
[1]Graduate Program in Neuropsychiatry and Behavior Science, Federal University of Pernambuco (PE, Brazil), [2]Graduate Program in Biochemistry and Physiology, Federal University of Pernambuco (, Brazil), [3]3Laboratory of Biochemistry and Molecular Biology, Federal University of Pernambuco (, Brazil)

INTRODUCTION: Introduction. Experimental evidence points to an association between overnutrition during childhood, and chronic degenerative diseases in adulthood. However, there is a gap in the literature relating overnutrition and oxidative stress in the CNS of neonates. OBJECTIVES: Objectives. Investigate at 22 days of life in the Prefrontal Cortex (PFC) of male overfed rats during lactation, the parameters associated with oxidative stress. MATERIALS AND METHODS: Material and Methods. The present study followed the recommendations of the Animal Use Ethics Committee-CEUA of Federal University of Pernambuco (protocol nº: 0024/2018). The selected rats Wistar females were housed in animal facility with light-dark cycle (12/12 hours) under standard conditions of temperature, lighting and humidity, with free access to water and food (commercial diet– Presence). After birth, male neonates were randomly divided into two experimental groups. Normofed (N) (n=9 per litter) and Overfed (O) (n=3 per litter). At 22 days of life the animals were euthanized, the PFC was collected to analyze: Malondialdehyde (MDA), Carbonyl content, enzymes activity Superoxide dismutase (SOD), Catalase (CAT) and Glutathione-S-transferase (GST). DISCUSSION AND RESULTS: Results and Discussion. Our data showed an increase of body weight (BW) on overfed groups at 22° days of life (22° days-N= 46.25±1.25 and O= 55.5±1.8g, p=0,0001). In addition to the increase in BW, we observed an increase in oxidative stress biomarkers: (MDA N= 7.602±0.057 and O= 11.14±0.817 Mol/mg of protein p= 0.005, Carbonyls N= 6.84.01±0.62 and O= 9.92±0.72 mM/mg of protein, p= 0.01). In addition to decrease in antioxidant enzymes (SOD-N= 0.052±0.005 and O= 0.03±0.004 p=0.0039, and CAT-N= 0.073±0.005 and O= 0.055±0.005 U/mg of protein p=0.04. For GST we did not observed statistical difference. CONCLUSION: Conclusions. Our results suggest that neonatal overnutrition induces oxidative stress in PFC, which may lead to lifelong neurodegenerative diseases.
Keywords: Children, Overfed, Oxidative stress

B.50 - Energy metabolism in mosquito cell lines is differently triggered by Wolbachia association/presence

Christiano Calixto da Conceicao [1], Jhenifer Nascimento da Silva[1], Angélica Arcanjo[1], Leonardo Araujo de Abreu[1,2], Carlos Logullo[1,2]
[1]Bioquímica, Univ. Federal do Rio de Janeiro (Rio de Janeiro, Brazil), [2]Bioquímica, INCT - Entomologia Molecular (, Brazil)

INTRODUCTION: Mosquito cell line can be powerful tools to unveil the mechanisms associated with either its defenses against pathogenic organisms, or adaptation to establish persistent and harmless associations. Such studies aim bacteria/virus-mosquito interactions, how they occur, and may contribute to the study and control of this arthropod vector. In this context, Wolbachia bacterium is an endosymbiont that lacks a number of essential enzymes, including part of glycolytic production, which indicates dependence on host cell machinery for energy production and other metabolic functions. On the other hand, little is known about which resources this bacterium may provide to its host, which makes it successful in nature. The metabolic changes that Wolbachia can promote depend on the host and the different type of bacterial strains, and can be quite different when comparing a natural symbiosis with recent infections. OBJECTIVES: Establish different mosquito cell lines under the presence or absence of Wolbachia. Energy metabolism on cell lines was analyzed whether Wolbachia was naturally present of artificially transfected in mosquitos. MATERIALS AND METHODS: In the present work, we established four novel embryonic cell lines from *Aedes fluviatilis* and *A. aegypti* mosquitos either with or without Wolbachia bacteria and compared main energy resources mobilization, mitochondrial mass by mitotraker green stain and resprirometry on Oroboros O2k-FluoRespirometer. DISCUSSION AND RESULTS: The presence of Wolbachia in a natural symbiosis improves energy performance in *A. fluviatilis* cells; it affects the regulation of key energy sources, decreasing lipids about 20 %, and increase glycogen about 70 % and protein 15 %. In addition, Wolbachia increase mitochondrial mass and reduce oxygen consumption rates. Interestingly, determination of duplication time was shown to be increased only in *A. aegypti* cells artificially transfected with Wolbachia while glycogen and protein did not change. CONCLUSION: The results presented here provide relevant information and widens the current knowledge of Wolbachia-mosquito interactions.
Keywords: Wolbachia, Symbiosis, Aedes aegypti / **Supported by:** CNPq, CAPES, FAPERJ

B.51 - UCP1-Independent Mechanisms Involved in the Increased Energy Expenditure and Protection Against Obesity Induced by Fish Oil in Mice.

Tiago Eugênio Oliveira da Silva[1], Érique de Castro[1], Albert Souza Peixoto[1], Thayna dos Santos Vieira[1], Bianca Franco Leonardi[1], Caroline Aparecida Tomazelli[1], Adriano de Brito Chaves Filho[2], Marcos Yukio Yoshinaga[2], Maria Nathália Moraes[1], Sayuri Miyamoto[2], William Tadeu Lara Festuccia[1]
[1]Biofísica e Fisiologia, Universidade de São Paulo (São Paulo, Brasil), [2]Bioquímica, Univ. de São Paulo (São Paulo, Brasil)

INTRODUCTION: The obesity, a disease defined as an excessive increase in white adipose tissue (WAT) mass, has increased dramatically in recent decades. Intake of a high fat diet rich in fish oil and omega 3 fatty acids protects mice from body weight gain and obesity by increasing energy expenditure in an UCP1-independent manner. The oxidation process of n-3 fatty acids necessarily involves their oxidation in the peroxisomes, generates hydrogen peroxide and medium and short chain fatty acids, which are oxidized in mitochondria. The importance of the metabolic connection between peroxisomes and mitochondria for energy balance is unclear. OBJECTIVES: In the present study, we investigated the effects of a hyperlipidic diet enriched with lard and fish oil on body weight of wild type (WT) and uncoupling protein 1 knockout (UCP1 KO) mice and the incorporation of n-3 fatty acids in the liver of wild type (WT) and peroxin 5 knockout (PEX5 KO) mice. MATERIALS AND METHODS: Protocol 1: WT and UCP1 KO mice will be fed for 10 weeks at 30°C with high fat diet produced using either lard (HFDLD) or fish oil (HFDFO) as fat source and evaluated for body weight. Protocol 2: WT and PEX5 KO mice will be fed for 11 weeks at 25°C with the same diets were evaluated for incorporation of of n-3 fatty acids in the liver. DISCUSSION AND RESULTS: Our results indicate that in UCP1 KO mice, the HFDLD induced higher body weight gain when compared to UCP1 WT. Mice fed with HFDFO, however, displayed reduced body weight gain, independently of UCP1. Finally, PEX5 KO mice fed with HFDFO accumulated DHA in liver higher than in PEX WT. CONCLUSION: Under thermoneutrality conditions, the UCP1 is important for the control of body weight in mice fed with HFDLD. The absence of PEX5 specifically in hepatocytes, compromises the oxidation of DHA in mice fed with HFDLD.

Keywords: ácidos graxos ômega 3, obesidade, gasto energético / **Supported by:** Fundação De Amparo Á Pesquiso Do Estado De São Paulo

B.52 - The Use of Metformin as Adjuvant to Restore the Reduction of Cisplatin Cytotoxicity in the Presence of Allantoin

Grazielle Silva Paz [1], Gisele Cardoso de Amorim[1], Janaina Fernandes[1]
[1]Numpex-Bio, Instituto de Biofísica Carlos Chagas Filho, UFRJ, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: The tumor lysis syndrome (TLS) is a metabolic disorder frequently associated with hyperuricemia. To treat TLS, rasburicase has been used, producing allantoin. Recently, our group showed that high levels of allantoin causes impairment of cisplatin cytotoxicity in K562 cell line. Due the importance of the maintenance of the concomitant use of cisplatin and rasburicase, our group evaluated and showed in a recent study the capacity of metformin in recover cisplatin cytotoxicity in the presence of allantoin in K562 cells. OBJECTIVES: This study aims to understand how metformin restores cisplatin cytotoxicity. MATERIALS AND METHODS: For this study, the K562 cell line was maintained in RPMI 1640 medium supplemented with 10% of fetal bovine serum and 0.5% of penicilin-streptomicin at 37ºC and 5% of CO2. During the experiments, the cells were incubated in RPMI 1640 low glucose medium (0.5mM) for 2h and then treated with metformin (0.1 to 3mM), cisplatin (16.5 to 33µM), allantoin (100 and 200µg/ml), rotenone (0.125 to 2µM) and their combination for 48h. Cell viability, cell cycle, morphology analysis and NMR assays were performed. DISCUSSION AND RESULTS: Our results showed: (1) the synergism between metformin and cisplatin; (2) the recovery of cisplatin cytotoxicity by metformin at morphology level; (3) the alterations of cisplatin effect in cell cycle by allantoin; (4) the absence of metformin-cisplatin interaction; (5) the interaction between metformin-allantoin; (6) the synergism between rotenone and cisplatin; (8) the capacity of rotenone to restore cisplatin cytotoxicity in the presence of allantoin at mitochondrial and cell cycle level, such as done by metformin. CONCLUSION: This study showed that metformin recovers cisplatin cytotoxicity in the presence of allantoin through its action on mitochondria and in an independent way.

Keywords: Metformin, Cisplatin, Allantoin / **Supported by:** FAPERJ

B.53 - PROJECT Capsaicin-Induced Metabolic Alterations in Tumor Breast Lines

Sara Eloy de Oliveira [1], Julia Mello Barros[1], Luisa Andrea Ketzer[1]
[1]NUMPEX-BIO, Núcleo Multidisciplinar de Pesquisa UFRJ – Xerém em Biologia (RJ, Brazil)

INTRODUCTION: Breast cancer is a public health problem that affected about 88.492 Brazilian women in 2020. It is characterized by the disordered proliferation of cells of the mammary epithelium. The current oncological treatments compromise the well-being of patients and do not always contribute to increasing life expectancy. In this spectrum, phytochemicals have been described as treatment potential alternatives. Among them, capsaicin (CAP), a selective agonist of the transient receptor potential vanilloid subtype 1 (TRPV1), is responsible for the pungency of red peppers from the genus Capsicum plants. Previous studies have been described for its pro-apoptotic, anti-metastatic, and anti-angiogenic effects of CAP on tumor cells. Tumors have a high energy demand, this need added to the survival in hostile environments corroborated the overexpression of enzymes and receptors of some metabolic pathways, such as the glycolytic one. However, the energy issues and the tumor respiratory mechanisms are not clearly elucidated. Knowing its relevance, it becomes pertinent to investigate the responses induced by capsaicin on glycolytic and oxidative metabolism in breast tumor cells. OBJECTIVES: The aim of this study is to investigate the glycolytic and oxidative metabolism of breast tumor and non-tumor cells after treatment with capsaicin. MATERIALS AND METHODS: The cytotoxicity of the breast cells (MCF-7 non-invasive tumor, MDA-MB-231 invasive tumor and MCF 10 A non-tumor) will be analyzed after a dose- and time-response of capsaicin exposure as follows: cell proliferation through flow cytometry; viability of cells (MTT and trypan blue); cell migration assay; quantification of enzymatic activity of hexokinase, citrate synthase and lactate dehydrogenase; and measurement of oxygen consumption by respirometry. DISCUSSION AND RESULTS: To investigate the cytotoxicity of the compound on the aforementioned cells; to elucidate the possible glycolytic and oxidative alterations post-treatment. CONCLUSION: This project has great relevance for the understanding of glycolytic and oxidative mechanisms in tumors.
Keywords: Capsaicin, Breast Cancer, Bioenergetic Metabolism / **Supported by:** FAPERJ

B.54 - Evaluation of the Role of Allantoin in Drug Resistance in Leukemia

Rafaela Ramos De Oliveira Dos Santos [1], Janaina Fernandes[1]
[1]UFRJ - Campus Duque de Caxias, Núcleo Multidisciplinar de Pesquisa em Biologia – UFRJ Xerém (Rio de Janeiro, Brasil)

INTRODUCTION: Leukemia is a hematological cancer characterized by the exacerbated proliferation of cells of hematopoietic tissue. In the beginning of chemotherapy treatment, there may be the development of Tumor Lysis Syndrome (TLS) due to the large amount of intracellular content from lysis of tumor cells. For this, the treatment of TLS, carried out in parallel to the chemotherapy treatment, uses a recombinant urate oxidase enzyme. The enzyme is responsible for converting high serum uric acid levels into allantoin, which is more easily eliminated in the urine. However, there are no clinical studies that show the action of allantoin during chemotherapy treatment until its complete elimination by urine. OBJECTIVES: Our objective was to investigate if allantoin interferes with the action of cisplatin, *in vitro*, in chronic myeloid leukemia cells. MATERIALS AND METHODS: Sensitive and resistant chronic myeloid leukemia cells were cultured in RPMI 1640 culture medium supplemented with 10% fetal bovine serum, 1% antibiotic, and maintained in a stove at 37°C and 5% CO2. The cells were treated with cisplatin and allantoin at different concentrations. The cell viability assay was performed using the MTT assay, the loss of mitochondrial membrane potential was observed by fluorescence microscopy and flow cytometry was used to analyze the induction of DNA fragmentation and efflux pump activity. DISCUSSION AND RESULTS: Our results show that allantoin does not induce cell death and cisplatin leads to decreased viability, in sensitive and resistant leukemia cells. However, we observed that in the presence of allantoin there is a reduction in death caused by cisplatin, allowing us to deduce that allantoin enhanced the survival of sensitive cells, preventing the efficient action of cisplatin. In the presence of allantoin it was also observed that the activity of efflux pumps changes. CONCLUSION: Allantoin interferes with the action of cisplatin, however more experiments are needed to conclude our study.
Keywords: allantoin, cisplatin, leukemia
Supported by: FAPERJ

B.55 - Modulations of respiratory parameters in oxidative metabolism of C2C12 myoblasts and myotubes induced by capsaicin.

Julia Barros [1,2], Lorena Siqueira[2], Sara de Oliveira[1], Gabrielle da Silva[1], Juliany Rodrigues[1], Luisa Ketzer[1,2]
[1]Núcleo Multidisciplinar de Pesquisa UFRJ – Xerém em Biologia (NUMPEX-Bio), Univ. Federal do Rio de Janeiro, Duque de Caxias/RJ, Brasil (RJ, Brasil), [2]Laboratório de Bioquímica de Vírus, Instituto de Bioquímica Médica Leopoldo de M, Universidade Federal do Rio de Janeiro, Rio de Janeiro/RJ, Brasil (RJ, Brasil)

INTRODUCTION: Capsaicin (CAP) is a selective agonist for the transient receptor potential vanilloid subtype 1 (TRPV1) and gives pungent characteristics in the genus Capsicum plants. Previous studies show that CAP can modulate muscle energy metabolism. However, the molecular mechanisms of action of the CAP are not elucidated. OBJECTIVES: The objective of this study is to investigate the effects of CAP in oxidative metabolism of skeletal muscle. MATERIALS AND METHODS: C2C12 myoblasts and myotubes were grown in DMEM-high glucose supplemented with 10% fetal bovine serum (FBS) and antibiotics. 24 hours after growth, the culture medium was changed for DMEM high (HG, 25 mM), medium (MG, 15 mM), or low glucose (LG, 5.5 mM) and treated with CAP (25 – 400 µM) for 24 or 48 h for the experiments. Cell viability was determined by MTT assay. Oxygen consumption was measured by high resolution respirometry in intact or permeabilized cells. Glucose uptake was quantified by flow cytometry with the fluorescent glucose analogue. Expression of hexokinase I and II was measured by Western Blotting. The cellular ultrastructure was analyzed by transmission electron microscopy. DISCUSSION AND RESULTS: Myoblasts treated with CAP (25 µM) for 24 h kept at HG had a higher basal respiration, maximum respiration, non-mitochondrial respiration, and complex activities compared with the same treatment in LG. In starvation of nutrient for 1 h, it was possible to observe that control and treated myoblasts present a reduction in oxygen consumption after glucose addition, indicating a profile of the Crabtree effect. While the control presented an insulin-resistant profile, treatment with CAP induces a stimulus to insulin-like glucose uptake, and increased levels of HKI expression. CONCLUSION: These data suggest that muscle cells present in supraphysiological glucose concentrations may present mitochondrial damage, and can be reduced, stimulating the oxidative metabolism of cells by action of capsaicin. **Keywords:** capsaicin, skeletal muscle, glucose metabolismo / **Supported by:** FAPERJ

B.56 - Metabolic Changes in Myoblast Cell Lines Through Sodium Selenite

Matheus Alves De Moura [1], LUISA ANDREA KETZER[1]
[1] Campus Duque de Caxias, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: Selenium (Se) is a micronutrient that is associated with several biological functions, being characterized as an antioxidant in physiological concentrations. In muscle tissue, low concentrations of this trace element are associated with sarcopenia. In addition, studies show that dietary supplementation with selenium reduces blood glucose and HbA1c levels, in addition to inducing changes in cellular energy metabolism. However, the mechanism by which selenium acts on such tissue is not yet elucidated. OBJECTIVES: The aim of this work is to evaluate metabolic alterations associated with treatment with sodium selenite (Na2SeO3) in a murine myoblast cell lineage. MATERIALS AND METHODS: C2C12 cells were exposed to 200 nM of Na2SeO3 for 24 hours and then measured: I) cell viability by trypan blue associated with the H2O2 0,25 mM exposure; II) creatine kinase (CK) and citrate synthase (CS) activities by commercial assays; III) high resolution respirometry (OROBOROS) of intact and permeabilized cells. DISCUSSION AND RESULTS: Pretreatment of myoblasts with Na2SeO3 prevented the damage caused by oxidative stress induced by H2O2. In addition, it promoted an increase of 11.6 ± 1.2% in CK activity compared to the control. The evaluation of CS activity showed a non-significative increase of 2 ± 0,7% in the function of this enzyme in the fraction of treated cells. The whole cell respirometry assay showed that the treatment increased p−trifluoromethoxyphenylhydrazone (FCCP)-induced maximal respiratory capacity by 97.5 ± 7.1%. This increase was also observed in permeabilized myoblasts, in the presence of respiratory substrates (pyruvate, malate, succinate and ADP). CONCLUSION: The results showed that Na2SeO3 protects myoblasts from damage induced by H2O2, increases maximal respiratory capacity and CK activity. The mechanism for these effects is unclear, requiring further experimental analysis. However, it may be related to an increase in the cell's antioxidant capacity.
Keywords: muscle, selenium, energetic metabolismo / **Supported by:** FAPERJ

B.57 - I*n vivo* metabolomics of alcoholic fermentation by nuclear magnetic resonance: a study in beer

Werner Florentino Brandão [1], Marcel M. Lyra da Cunha[1], Gisele C. de Amorim[1], Celso B. de Sant'Anna Filho[2]
[1]Núcleo Multidisciplinar de Pesquisa Xerém (NUMPEX-BIO), Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil), [2]Laboratório de Metrologia Aplicada a Ciências da Vida, Instituto Nacional de Metrologia, Qualidade e Tecnologia (RJ, Brasil)

INTRODUCTION: Wort fermentation by yeast is the initial step at beer production and has a high impact on its flavor. Elucidate how the fermentation can be influenced by the source and the sugar concentration is interesting for the academy and industry. A complete map yeasts, synthesis and consumption can help in the fine adjustment of fermentation and be a tool to improve beer production yield. OBJECTIVES: This project aims the detailed analysis of alcoholic fermentation of high sugar content to accurately identify the metabolites and analyze the correlations between synthesis and consumption of compounds with sensory interest in beer. MATERIALS AND METHODS: *Saccharomyces cerevisiae* strains were inoculated in malt extract medium (15% sugar) at 20 ° C. The 1D ^{1}H NOESY and ^{1}H ^{13}C HSQC spectra were obtained on a Bruker Avance 500 MHz spectrometer for 3 days with intervals of approximately 2 hours between the series, and at intervals of 1, 7 and 14 days with variation of 3 initial inoculation conditions. The spectra were processed using the Topspin 3.5 software and the compounds were identified on the Colmar NMR platform and in the literature. The 1H NOESY and 13C 1H HSQC spectra were used using online metabolomic databases, the Chenomx program and previous work. DISCUSSION AND RESULTS: We quantify the consumption of glucose and maltose as a function of time and relate it to the production of ethanol. Other molecules related to the fermentation processes were mapped and, in the case of acetate esters, their production was increased in the condition of greater yeast inoculum. The results indicate that NMR can be a strong allied for the efficient identification of substances in beer. CONCLUSION: Further investigations are underway to expand the identification and the relationship between the compounds produced during fermentation and the common variables of modern beer production, such as sugars, adjuvants and different yeast strains.

Keywords: nuclear magnetic resonance, beer fermentation, *In vivo* metabolomics / **Supported by:** FAPERJ

C - Cellular and Systems Biophysics

C.01 - Proximal Tubule Albumin Endocytosis Is Modulated by ACE2

Helena Ribeiro Peixoto [1], Rodrigo P. da Silva de Aguiar [1], Diogo de Barros de Barros Peruchetti [1], Ana A. de Sá Pinheiro[1,2], Celso Caruso-Neves [1,2,3]
[1]Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro (Rio de Janeiro, Brazil), [2]Rio de Janeiro Innovation Network in Nanosystems for Health-NanoSAÚDE/FAPERJ, Rio de Janeiro Innovation Network in Nanosystems for Health-NanoSAÚDE/FAPERJ (Rio de Janeiro, Brazil), [3]National Institute of Science and Technology for Regenerative Medicine, National Institute of Science and Technology for Regenerative Medicine (Rio de Janeiro, Brazil)

INTRODUCTION: Introduction: Renal proximal tubule cells, PTECs, are the tubular segment responsible for virtually all reabsorption of proteins filtered through the glomerular filtration barrier, including albumin. This function is essential since urinary albumin loss, a condition called albuminuria, is an independent risk factor for all-cause mortality. Albumin reabsorption occurs through receptor-mediated endocytosis. The receptor is formed by a complex between megalin, cubilin and amnionless. A complex endocytic machinery is involved in this process, with multiple steps of maturation and trafficking between endosomes and lysosomal delivery of endocytosed albumin. Importantly, recent evidence suggests that this process is highly dynamic and is regulated by different peptides, including renin-angiotensin system, RAS. ACE2 is a protease expressed at the cell surface of PTECs involved in the breakdown of Angiotensin II, Ang II, in Angiotensin (1-7), Ang(1-7). Ang(1-7), through its receptor MAS, often counteracts AngII/AT1 receptor effects. Our group has shown that AngII/AT1R signaling decreases PTECs albumin endocytosis through downregulation of megalin. The effects of ACE2/Ang(1-7)/MASR is unknown. OBJECTIVES: Objective: Study the role of ACE2/Ang(1-7)/MASR signaling in PTECs albumin endocytosis. MATERIALS AND METHODS: Methods: Proximal tubule cells lines LLC-PK1, HEK-293 and HEK-293 cells overexpressing ACE2, ACE2-HEK-293, were used. Albumin-FITC and Dextran-FITC endocytosis were measured by cell-associated fluorescence. DISCUSSION AND RESULTS: Results: ACE2-HEK-293 cells showed selective increase in albumin endocytosis (2.2 fold) in comparison with HEK-293, with no changes in fluid-phase endocytosis, measured by Dextran-FITC endocytosis. In LLC-PK1 cells, overnight treatment with ACE2 inhibitor MLN-4760 at 100 nM increased extracellular Ang II levels, measured by ELISA kit, and inhibited albumin-FITC endocytosis by 30%.CONCLUSION: Conclusion: These results indicate that ACE2 modulates albumin reabsorption in PTECs, highlighting new mechanisms involved in protein homeostasis in the proximal tubule.
Keywords: ACE2, Proximal Tubule, Albumin endocytosis / **Supported by:** FAPERJ, CAPES and CNPq

C.02 - Oleanolic and Ursolic Acid Interaction with Organelle Model Membranes Under Oxidative Stress

Samuel Utrabo Pizzol [1], Pedro Nunes[1], Rosangela Itri[1]
[1]Física Aplicada, Universidade de São Paulo (SP, Brazil)

INTRODUCTION: Modulation of autophagy is a promising strategy to develop anti-cancer therapies as well as some related to aging as neurodegenerative diseases. Ursolic acid (UA) and oleanolic acid (OA) are pentacyclic triterpenoids, with molecular structure similar to cholesterol. As a consequence, they may interact with biological membranes. Although they have similar physical-chemical properties, it has been shown that their pharmacological properties as well as their mechanisms of cytotoxicity can be quite different. In this work, we explore the binding and mechanism of action of UA and OA on two key organelles: mitochondria and lysosome. Further, it has been also shown that membrane photosensibilization can compromise the autophagic pathway. In this way, in this work we will also explore a possible synergic effect between the action of tripertenoids and lipid oxidation. OBJECTIVES: To comprehend the action mechanism of the isomers OA and UA in mimetic membranes of cell organelles such as mitochondria and lysosome in comparison to plasma membranes, by tuning the composition of lipid membranes. MATERIALS AND METHODS: Model membranes represented by giant unilamellar vesicles (GUVs) are grown by electroformation method with the main lipids that compose the cell organelle (for instance, cardiolipin to mitochondria and BMP to lysosome) The morphological effects of the acids on GUVs were analyzed by optical microscopy in phase contrast and fluorescence modes. Binding assays were carried out through a Langmuir trough. DISCUSSION AND RESULTS: The interaction of OA with POPC GUVs results in significant membrane fluctuations while an increase in membrane permeability is observed for ternary mixtures of DOPC:SM:Cholesterol mixtures that display raft-like phase separation. Evaluation of membrane area and volume increase as well as the features of permeability increase are in progress. CONCLUSION: It's possible to conclude that OA and UA can indeed interact with mimetic membranes of cell organelles. The work is ongoing.
Keywords: Photo-oxidation, Photosensibilisation, Triterpenoic Acids / **Supported by:** CNPq

C.03 - Biochemical and Biophysical Characterization of Extracellular Vesicles obtained from Fetal Bovine Serum.

Julia Lima Oliveira [1], Luiz Henrique da Silva Andrilli[1], Heitor Gobbi Sebinelli[1], Gabriela Guaraldo Totoli[2], Ana Paula Ramos [1], Pietro Ciancaglini[1]
[1]Department of Chemistry, University of São Paulo (SP, Brazil), [2]Bone Research Lab, University of São Paulo (SP, Brazil)

INTRODUCTION: Extracellular vesicles (EVs) are bioactive organelles that can be capable to perform several functions between cells, for example, gene signaling and regulation, transport genetic information, among others. OBJECTIVES: The present project seeks to identify, isolate and evaluate whether the EVs present in commercial Serum Bovine Fetal (SBF) affect the growth of osteoblast cultures. The initial methodology focused in isolating and characterizing the EVs present in the SBF. MATERIALS AND METHODS: Cultilab® SBF (named SBFo) was filtered by using a tangential filtration, with a 100 kDa Vivaflow 200 cassette, resulting in two samples (SBFf correspond to MW < 100kDa and SBFr, corresponds to MW > 100 kDa). EVs were isolated from the SBFr by ultracentrifugation process 2 h at 100.000 x g (4oC). All the samples were characterized by Dynamic Light Scattering (DLS/NTA), protein quantification and SDS-PAGE. DISCUSSION AND RESULTS: A 50 mL SBFo sample, containing 42 mg/mL of total protein was filtered, obtaining a SBFf and SBFr volume of 30 and 20 mL, with 3.2 and 76.4 mg/mL of total protein, corresponding to 4.5 and 76.4 % respectively. DLS analysis of the SBFo revealed the presence of vesicles with diameters of ~10 and 50 nm vesicles that were isolated efficiently by tangential filtration. SBFf revealed principally the presence of vesicles with ~10 nm and SBFr vesicles with 50 nm (with little amount of vesicles with about 6 nm of diameters). The pellet obtained was resuspended in Tris/HCl buffer, pH 7.4. This sample was submitted to DLS analysis results in as homogenous distribution with a ~200 nm of diameters. NTA revealed 2.65x1011/mL containing about 0.34 mg/mL of total protein. CONCLUSION: From the preliminary results obtained, the use of tangential filtration associated with ultracentrifugation resulted in the isolation of homogeneous preparations of EVs to be used in second step in cell culture assays.
Keywords: Extracellular Vesicles , Fetal Bovine Serum, Biochemical and Biophysical Characteriza

C.04 - Characterization Of Nonionic Cubosomes In The Presence Of Model Proteins: A Structural Approach

Heidie Da Silva Torres [1], Leandro Barbosa[2]
[1]Departamento de Tecnologia Bioquímico-Farmacêutico, Faculdade de Ciências Farmacêuticas (São Paulo, Brasil), [2]Laboratório Nacional de Luz Síncrotron, Centro Nacional de Pesquisa em Energia e Materiais (São Paulo, Brasil)

INTRODUCTION: Nanomedicine, with particular attention to drug delivery systems, has gained much attention in recent years; they are widely used to increase the effectiveness of diagnostic drugs, including anticancer, antimicrobial and antiviral drugs. In addition, they reduce the toxicity of existing drugs, minimizing drug interactions and overcoming systemic barriers. Cubosomes are nanostructured particles composed of a specific combination of some types of lipids, such as monolein and phytantriol. Lysozyme is an animal-produced antimicrobial enzyme that is part of the immune system, used as anti-inflammatory, antiviral, antiseptic, antihistamine and antineoplastic. OBJECTIVES: In this project, we will highlight the systems formed by lipids and polymers, as cubosomes, studying the structural influence of a Lysozyme encapsulated by it. These systems will be composed of cubosomes in the absence and presence of the enzyme and will be analyzed using small angle X-ray scattering (SAXS) and dynamic light scattering (DLS), potential zeta besides essays *In vivo* . MATERIALS AND METHODS: Cubossomes' preparation is made so that phytantriol (PHY) solution, anfifilic molecule which forms a cubic structure in aqueous solution, and nonionic polymer are prepared. These solutions stay in 50°C bath and after, phytantriol solution is dripped in the polymer solution, forming cubosomes by agitation. When it's ready, the final solution passes through a machine that evaporates it until the sample final volume, 5 mL. The same procedure is made for multiple concentrations of the Lysozyme (1, 3 and 5 mg/mL).
Keywords: nanostructured systems, cubosomes, lysozyme
Supported by: CAPES

C.05 - Micro reatores enzimáticos formados por separação de fase líquido-líquido em moléculas biológicas.
João Paulo de Mauro Faccin[1]
[1]Departamento de Física, Universidade de São Paulo (SP, Brasil)

INTRODUCTION: The objective of this work was to obtain a chimera protein of the P450 monooxygenase protein complex; this chimera contains two low-complexity domains (LCD), one in N terminus and one in C terminus, formed by a liquid-liquid phase separation (LLPS). Moreover, a study was made using bioinformatics tools, of the secondary structures of the wild version of the protein, as well as of the chimera protein, got experimentally. OBJECTIVES: The main objective of the project is to establish conditions for the production of chimera enzymes, in which sequences known to LCDs enable the formation of LLPS aggregates. Enabling micro-reactors with potential biotechnological applications. MATERIALS AND METHODS: In the first part of the project, cloning was made using the pEt29a plasmid, which already contains the coding sequence for LCD. For expression, E.coli cells were used. During the execution of this project, the P450 in its wild form was expected to be connected to two LCDs. For protein characterization, the circular dichroism (DC) technique was realized in order To verify the secondary structure of the linked chimera protein. To evaluate the thermal denaturation of the chimera protein, differential scanning calorimetry (DSC) and the thermal stability of the chimera protein were compared with that of the wild type protein. DISCUSSION AND RESULTS: Using the AlphaFold 2 artificial intelligence tool, the structure of the chimera protein was got, along with the two protein-bound LCDs, demonstrating that it Is possible to achieve liquid-liquid phase separation. The CD spectra obtained demonstrate that there is a predominance of α-helix in the protein structure. The results obtained by DSC reveal that the maximum temperature at which the protein is folded is 43ºC. CONCLUSION: The results obtained showed that, in fact, it is possible to build an enzymatic micro reactor with the P450 chimera version. The ability to form LLPS generates a range of applications such as biosensors, tissue engineering.
Keywords: Micro reatores, Enzimáticos, Separação de fase / **Supported by:** CNPQ

C.06 - Ultrastructural Analysis of Cancer Cells Treated with Radium Dichloride [223Ra] RaCl2: Understanding the Effect of Alpha-Particle on Cell Structure
Joel Félix Silva Diniz Filho [1], Mariana Baroni[2], Clenilton dos Santos1[1], Luciana Alencar[1], Ralph Santos-Oliveira[3,4]
[1]Department of Physics, Federal University of Maranhão (MA, Brazil), [2]Department of Natural Science and Mathematics, , Federal Institute of Education, Science and Technology (SP, Brazil), [3]Laboratory of Radiopharmacy and Nanoradiopharmaceuticals, Zona Oeste State University (RJ, Brazil), [4]Laboratory of Nanoradiopharmacy and Synthesis of New Radiopharmaceuticals, Brazilian Nuclear Energy Commission, Nuclear Engineering Institute (RJ, Brazil)

INTRODUCTION: The use of alpha-particle (α-particle) radionuclides, especially the [223Ra] RaCl2 (radium dichloride), in targeted alpha therapy is increasing each day. Besides the clinical achievements of this therapy, very little data is available about the effect on the ultrastructures of the cells. OBJECTIVES: The purpose of this study was to evaluate the nanomechanical and ultrastructure effect of radium dichloride {[223Ra] RaCl2} on cancer cells. MATERIALS AND METHODS: To evaluate the effect of [223Ra] RaCl2 on tumor cells, cells of human breast cancer cell lineage (MDA-MB-231) were cultured and treated with the radiopharmaceutical at doses of 2mCi and 0.9mCi. The effect was evaluated using Atomic Force Microscopy (AFM) and Transmission Electron Microscopy (TEM) allied with Raman spectroscopy. DISCUSSION AND RESULTS: The results showed massive destruction of the cell membrane with preservation of the nucleus membrane. No evidence of DNA alteration has been observed. Was observed the formation of lysosomes and phagosomes, probably regulated by the proton pump [vacuolar H(+)-adenosine triphosphatase ATPase (v-ATPase)] in the lysosomal membrane, leading to autophagy. CONCLUSION: The data provided may help elucidate the main mechanism involved in cell death during α-particle therapy. Also, the data corroborate the efficacy of the safety use.
Keywords: cell mechanism, targeted alpha therapy, radiopharmaceutical
Supported by: Capes

C.07 - Effects Of Electromagnetic Radiation on the Bioenergetics of Isolated Mouse Liver Mitochondria

Aline Sanches Perez [1], Natália Mayumi Inada[1], Natasha Ferreira Mezzacappo[1], Vanderlei Salvador Bagnato[1], Roger Frigério Castilho[2], Aníbal Eugênio Vercesi[2]
[1]Institute of Physics of São Carlos, University of São Paulo (São Carlos, Brazil), [2]Department of Biochemistry, University of Campinas (São Paulo, Brazil)

INTRODUCTION: Mitochondria were recently known as the main targets of electromagnetic radiation; they also play an important role in the cell maintenance. Mitochondria are responsible for more than 85% of the cell energy production in ATP molecules. Despite the advances already achieved in the understanding and use of photonic therapies, there are gaps regarding the detailed understanding of the action mechanism at atomic, cellular and bioenergetics levels, especially in the action of radiation outside the visible spectrum. OBJECTIVES: This study proposes to investigate the effects of electromagnetic radiation, outside the visible spectrum using X-ray and ultraviolet, on the bioenergetics of isolated mouse liver mitochondria. The main parameters treated in this study are mitochondrial respiration (RCR) and structural changes. MATERIALS AND METHODS: High resolution respirometer and confocal fluorescence microscope are used. DISCUSSION AND RESULTS: Our study indicates that at 1 and 10 Gy of X-ray RCR are decreasing by a factor of 30 and 10% respectively, but at 5 Gy, the RCR are increaded in 15%. Now, irradieted with UVB (8W) for 3 and 5 seconds the RCR are increseaded in 5 and 15% respectively. CONCLUSION: X-ray and UVB photons are, some how, disturbing the mitochondrial respiratory chain, depending of the dose or radiation time exposure the respiratory pattern decreases or increases which are still been investigated. Confocal measurements (data not shown) can provide the number of isolated mitochondria in the sample, which will be used to estimate the photon energy necessary to disturbe one mitochondrion.
Keywords: isolated mitochondria, radiation effects, respiratory control ratio / **Supported by:** CNPq

C.08 - Ultrastructure and Biophysical Properties of Hippocampus from Alzheimer's Disease Carriers

Maria do Socorro do Nascimento Amorim [1], Bruno Araújo Serra Pinto[1], Luciana Magalhães Rebêlo Alencar[1]
[1]Departament of physical, Universidade Federal do Maranhão (MA, São Luís)

INTRODUCTION: Alzheimer's disease (AD) is one of the neurodegenerative diseases with the highest incidence globally and leads to a progressive loss of mental, behavioral, and functional decline. The hippocampus is one of the main brain structures affected by AD. The relationship between AD, hyperglycemic diets, and diabetes is increasingly evident. OBJECTIVES: Characterize the early ultrastructural, nanomechanical, and vibrational changes in the hippocampus tissue of diabetic subjects with cognitive alterations compatible with AD. MATERIALS AND METHODS: Animal hippocampus tissues were obtained in partnership with the Laboratory of Experimental Physiology (UFMA). The animals were divided into two groups: the control and the obese, which received a sucrose-rich diet for ten weeks. Cognitive tests were performed to confirm AD. The samples were frozen, and two µm cryosections were made. MM8 AFM (Bruker) was used in Peak Force Quantitative Nanomechanics mode. Scans of 30, 10, and 5 μm^2 of 5 regions in 6 samples were performed. Ultrastructural and nanomechanical data such as surface roughness, PSD, Young's Modulus, adhesion, and energy dissipation were evaluated. The hippocampus samples were deposited on glass slides for Raman measurements and analyzed in the T64000 Spectrometer (Horiba), using a λ=632.8 nm laser. Four spectra were obtained in different regions of each sample. DISCUSSION AND RESULTS: The roughness and PSD data showed significant statistical differences, evidencing early changes in the hippocampus. As confirmed by Raman's results, adhesion data evidence changes in tissue molecular composition. MY data indicate changes in the organization of tissue components with AD. The PCA analysis differentiates the groups. The individual spectra show that the most significant changes are in the 1670 cm^{-1} and 1440 cm^{-1} bands, compatible with modes already reported in the literature for AD. CONCLUSION: Even if the tissues did not show evident morphological alterations, the AFM analyses show the alterations at ultrastructural and nanomechanical levels.
Keywords: AFM, Alzheimer's Disease, Biomechanics

C.09 - The Arrhythmogenic Role Of The Pesticide Aldrin

Samuel dos Santos Beserra [1], Diego Santos Souza[1], Michael Ramón de Lima Conceição[1], Leisiane Pereira Marques[1], Jorge Lucas Teixeira Fonseca[1], Danilo Roman-Campos[1]
[1]Biofísica, Universidade Federal de São Paulo (São Paulo, Brasil)

INTRODUCTION: Aldrin is a high toxic pesticide used in agriculture to control prague. However, due to its toxicity it was withdraw from several countries, however, it remains used in few countries and it is still found in soil, due to its high stability. The pesticide can cause severe toxicity, inducing headache, nausea, convulsions, and myotonia. However, few reports described the cardiotoxic effect of aldrin. OBJECTIVES: Thus, we aimed to uncover the potential acute cardiotoxic effect of Aldrin in heart from mice (Mus musculus) lineage. MATERIALS AND METHODS: Male mice (C57BL/6) eight-week-old were used in study. Ex vivo ECG was measured upon exposition of the heart to 0.1, 1 and 10 nM of the pesticide. Whole-cell patch-clamp experiment in the current-clamp mode was also performed to measure the impact of the pesticide on the action potential (AP). DISCUSSION AND RESULTS: The treatment with 10 nM of aldrin increased the QT interval on the ECG (119.10±10.38 ms) compared to the CTR group (92.45±3.38 ms). Considering the same drug concentration the impact in the AP at the same concentration was significant. For example, a significant reduction in AP amplitude (from 109.05±12.06 for 86.91±8.58 mV) and maximum rise slope (171.49±32.78 mV/ms CTR group versus 78.36±30.37 mV/ms aldrin group). CONCLUSION: Thus, aldrin is able to induce changes in the ECG and in the AP which highlight the ability of the pesticide to induce cardiac complications.
Keywords: Aldrin, Cardiotoxicity, NaV1.5
Supported by: Fapesp e Capes

C.10 - Experimental hypothyroidism induces cardiac arrhythmias and ranolazine reverts and prevents the phenotype

Diego Santos de Souza [1], Leisiane Pereira Marques[1], Alexandre Dantas Costa[3], Jader dos Santos Cruz[3], Paula Rhana [3], Artur Santos Miranda [2], Julliane V.J. Santos [1], Aimee Obolari Durco[8], Carla Maria Lins Vasconcelos[8], Danilo Roman Campos [2]
[1]Biofísica, Univ Federal de São Paulo (São Paulo, Brasil), [2]Biofísica, Univ. Federal de São Paulo (SP, Brasil), [3]Fisiologia, Univ. Federal de Minas Gerais (MG, Brasil), [4]Bioquímica e imunologia, Univ. Federal de Minas Gerais (MG, Brasil), [5]Bioquímica e imunologia, Univ. Federal de Minas Gerais (MG, Brasil), [6]Biofísica, Univ. Federal de São Paulo (SP, Brasil), [7]Biofísica, Universidade Federal de São Paulo (SP, Brasil), [8]Fisiologia, Universidade Federal de Sergipe (SE, Brasil), [9]Fisiologia, Universidade Federal de Sergipe (SE, Brasil), [10]Biofísica, Universidade Federal de São Paulo (SP, Brasil)

INTRODUCTION: Hypothyroidism can induce cardiac dysfunction and increase the risk of life-threatening arrhythmias. Sodium current (INa) generated by Nav1.5 is the main ion current responsible for the upstroke phase of action potential (AP) in the working myocardium in the heart. Altered biophysical properties of Nav1.5 can enhance the late component of sodium current (INaL) and increase the susceptibility to arrhythmias. OBJECTIVES: In the present study, we evaluated the potential involvement of INaL in cardiac arrhythmias in an experimental murine model of hypothyroidism and we probed the benefits of blocking INaL using the clinically approved drug Ranolazine, which can block INaL. MATERIALS AND METHODS: Male Swiss mice were treated with methimazole (0,1%, w/vol, 21 days) to induce experimental hypothyroidism. Afterward, ECG, AP, and intracellular Ca2+ dynamics were evaluated. Susceptibility to arrhythmia was measured *In vitro* and *In vivo* . DISCUSSION AND RESULTS: Results revealed that hypothyroidism animals display ECG alteration (e.g. increased QTc) with the presence of spontaneous sustained ventricular tachycardia. These changes were associated with depolarized cell membrane potential and increased AP duration and dispersion at 90% of the repolarization in isolated cardiomyocytes. Aberrant AP waveforms were related to increased Ca2+ sparks and out-of-pace Ca2+ waves compared to control. These changes were observed in a scenario of enhanced INaL. Interestingly, ranolazine corrected the *In vivo* ECG. Also, ranolazine reduced Ca2+ sparks and aberrant waves, and decreased the *In vitro* events and severity of arrhythmias observed in isolated cardiomyocytes from hypothyroidism animals. Using the *In vivo* dobutamine+caffeine protocol, the animals with hypothyroidism had the development of catecholaminergic bidirectional ventricular tachycardia, and pre-treatment of animals with ranolazine prevented them. CONCLUSION: We concluded that animals with hypothyroidism have increased susceptibility to developing arrhythmias due to INaL and ranolazine, a clinically used blocker of INaL, can correct the arrhythmic phenotype.
Keywords: Hypothyroidism, arrhythmia, ranolazine / **Supported by:** Fundação de Amparo a Pesquisa do Estado de São Paulo

C.11 - Expression, Purification and Characterization TMED 1 Human Protein

Danielly Christine Adriani Maia Mota [1], Iara Aimê Cardoso[1,2], Renan Minin de Mori[1,2], Mariana Raquel Bunoro Batista[1], Luís Guilherme M. Basso[3,3], Maria Cristina Nonato[1,2], Luis Felipe S. Mendes[1], Antonio José Costa-Filho[1]
[1]Department of Physics, University of São Paulo (São Paulo, Brasil), [2]Department of Biomolecular Sciences, University of São Paulo (São Paulo, Brasil), [3]Science and Technology Center, Univ. Estadual do Norte Fluminense Darcy Ribeiro (RJ, Brasil)

INTRODUCTION: TMED proteins are eukaryotic transmembrane proteins located in all subcompartments of the initial secretory pathway, i.e. the endoplasmic reticulum (ER), Golgi and the intermediate compartments. TMEDs are essential during bidirectional transport between the endoplasmic reticulum and the Golgi, however, information about their structure, oligomeric state and how they anchor the transport cargo is still lacking. OBJECTIVES: In this work we aim to perform a detailed biophysical characterization of the GOLD domain of human TMED1. MATERIALS AND METHODS: The recombinant TMED1 GOLD protein was purified in two steps and analyzed through: Circular Dichroism, Size Exclusion Chromatography with Multi-Angle Light Scattering, Size Exclusion Chromatography, Differential Scanning Calorimetry, X-ray crystallography, Phylogenetic analyses, Molecular dynamics simulations and MM/PBSA free energy calculation. DISCUSSION AND RESULTS: The high−resolution structure as determined by protein crystallography diffraction to 1.72 Å resolution and showed a structural organization in a β−sandwich composed of two four-strand antiparallel β−sheets and a conserved disulphide bond. This results agreed with the previous data. The protein formed a dimer structure in the crystal organization, confirmed experimentally in solution and showed to be salt-dependent. The proposed dimeric structure was further analysed using molecular dynamics simulation and it is entropically stabilized in approximately −28 kcal.mol $^{-1}$ .CONCLUSION: The residues important for oligomerization were mapped. Finally, the dimer model orientation towards the lipid membrane was proposed based on our data.
Keywords: TMED, Early Secretory Pathway, Oligomerization
Supported by: CNPq

C.12 - The Effects of Enterovirus Infection on Amyloid Protein Aggregation in SH-SY5Y Cells

Gabriela Assis de Lemos [1], Rayanne Moura Nascimento[1], Isabele Mello Vieites[2], Ivanildo Pedro Sousa Junior[2], Tuane Cristine R. G. Vieira[1]
[1]Instituto de Bioquímica Médica Leopoldo De Meis, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil), [2]Instituto Oswaldo Cruz, Fundação Oswaldo Cruz (Rio de Janeiro, Brasil)

INTRODUCTION: The role of viral infection in the pathogenesis of brain amyloidosis has been raised in previous papers and has recently been strengthened as some mechanistical insights have been proposed. Enterovirus is a genus of the Picornaviridae family, whose infection may lead to serious brain diseases such as meningitis and encephalomyelitis. OBJECTIVES: In the present study, we aim to investigate if the infection of the human neural cell line SH-SY5Y with E30 and EV-A71, highly neurotropic variants of Enterovirus, may lead to aggregation of amyloid proteins such as Prion and α-synuclein. MATERIALS AND METHODS: Firstly, we evaluated the cytotoxicity of both viruses, by morphological characterization of the cells, at increasing times of infection in order to establish a time point before cell death. DISCUSSION AND RESULTS: We found that 48 hours post-infection the viruses already have a cytopathic effect, although the cells are still alive, representing a good time point for evaluation of protein aggregation. Preliminary Western Blot analysis suggests that infected cells express higher levels of Prion protein, with a more prominent effect of EV-A71 compared to E30. Interestingly, a Dot Blot assay revealed that total fibers, as well as Amyloid Protein Precursor contents are lower in infected cells. CONCLUSION: These findings suggest that the infection of neural cells with Enterovirus seems to alter cell proteostasis. Further studies must be conducted to better understand the possible relationship between enteroviral infection and the onset of neurodegenerative disorders, which is still unexplored.
Keywords: Amyloidosis, Enterovirus, Protein aggregation

C.13 - IL-1β Sensitizes Mice to Atrial Fibrillation

Oscar Jesus Moreno Loaiza [1], Oscar Jesus Moreno Loaiza[1], Emiliano Horacio Medei[1]
[1]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: The innate immune system recognizes pathological conditions and signals by producing cytokines as IL-1β. OBJECTIVES: To test whether IL-1β creates a substrate that favors atrial fibrillation (AF). MATERIALS AND METHODS: C57BL/6 mice were treated with 4 ng of IL-1β for 15 days. Inflammatory response to IL-1β was studied with Flow cytometry. Atrial action potentials and Ca2+ transients were recorded with Local field fluorescence microscopy. In addition, collagen deposition was measured with Fourier-transform infrared spectroscopy and second-harmonic generation microscopy. Finally, AF susceptibility was studied with transesophageal electrical stimulation. DISCUSSION AND RESULTS: Mice treated with IL-1β presented increased number of neutrophils and macrophages. In addition, IL-1β gene expression was augmented in atrial tissue. Atrial action potential duration (APD) was shortened in mice treated with IL-1β (APD 90: Control = 46.8 ± 4.5 ms vs IL-1β = 34.7 ± 3.8 ms). The rise and fall time of Ca2+ transients were also shortened in IL-1β treated mice. The remaining Ca2+ transient was studied after perfusion of Ryanodine 10 μM (Ry) and Thapsigargin 2 μM (Tg). Ca2+ transients were decreased by 87 ± 3.0% in control group, while it were reduced by 96.1 ± 3.9% in hearts from IL-1β treated mice. IL-1β injection, also increased collagen deposition with a disarranged pattern, while enriching the tissue beta-sheet structures. In addition, IL-1β treated mice showed more cardiac Triggered-activity (TA) events (Control N = 1/11, 9.1% vs IL-1β N = 11/16, 68.8%). Pharmacological prolongation of APD with 4-aminopyrdine (2.5 mM), and atropine (10 μM), prevented TA. Altogether, the electrical and structural remodeling observed increased the susceptibility to AF (Control 16.7% vs IL-1β 75.0%). Finally, IL-1r -/- mice did not depicted the arrhythmic events nor the APD reduction seen in wild-type mice after IL-1β treatment. CONCLUSION: IL-1β treatment sensitizes mice to AF by shortening APD, reducing Ca2+ release, and increasing fibrosis.

Keywords: Atrial Fibrillation, Inflammation, Electrophysiology / **Supported by:** Faperj, Capes, CNPq

C.14 - The ML365 Task-1 Channel Blocker Does Not Exert Chronotropic And Ionotropic Effect On Isolated Right Atrium

Jorge Lucas Teixeira da Fonseca [1], Danilo Roman Campos[1]
[1]Biofísica, Universidade Federal de São Paulo (São Paulo, Brasil)

INTRODUCTION: The TASK-1 channel (TWIK-related acid-sensitive K+ channel-1) belongs to the family of potassium channels two-pore. Among the pharmacological tools available for studying the biophysical properties of the TASK-1 channel are ML365 and ONO-RS-082, able to block and enhance the current, respectively. There is evidence in the literature that TASK-1 is important for the control of right atrial function, although functional evidence is missing. OBJECTIVES: Our objective was to determine the role of TASK-1 in the chronotropical and ionotropical properties of the isolated right atrium (RA). MATERIALS AND METHODS: All animal handling procedures were approved by the Ethics Committee for Animal Use (CEUA) of the Federal University of São Paulo (number 9073161118). The RA was mounted isolated tissue bath containing Tyrode's nutrient solution, stretched to 0.5 gF. DISCUSSION AND RESULTS: The atrial frequency was decreased in more acid extracellular pH and enhanced in more basis pH, when compared to pH 7.4. We probe the involvement of TASK-1 channel using cumulative concentrations of ML365 (0.01-100 μM, pH 7.4). The ML365 was not able to significantly affect RA frequency, maximum amplitude of contraction, maximum rise slope and maximum decay slope. It was previously reported that ONO-RS-082 increases TASK-1 current density. The drug caused only a gradual reduction in RA frequency. To evaluate the involvement of TASK-1 channel on ONO-RS-082 ability to modulate RA function, RA was pre-incubated with ML365 (10 μM) and then the impact of cumulative concentrations of ONO-RS-082 (0.1-100 μM, pH 7.4) on RA function. The pre-incubation with ML365 did not impact the ability of ONO-RS-082 function. CONCLUSION: We show that spontaneous contraction of isolated RA from rats can be modulated by extracellular acidosis and alkalosis and these changes are independent of TASK-1 channel. Thus, acute ex vivo blockage of TASK-1 channel does not impact the control of automaticity of isolated RA from rats.

Keywords: Right atrium, Contractility TASK-1, ML365
Supported by: This work was supported by the State Funding of Sao Paulo [FAPESP] # 2019/21304-4

C.15 - Aerobic Exercise Attenuates Cardiac Remodeling During Chagas Disease in Mice

Pedro Vieira de Moraes dos Santos [1], Alexandre Jayme Lopes Dantas Costa[2], Artur Santos Miranda[2], Julliane Vasconcelos Joviano dos Santos[3], Ivan Lobo de Sousa Marques[1], Mayra Fernanda Ricci[1], Jader dos Santos Cruz[1]
[1]Bioquímica e Imunologia, Universidade Federal Minas Gerais (Minas Gerais, Brasil), [2]Fisiologia e Biofisica, Universidade Federal Minas Gerais (Minas Gerais, Brasil), [3]Biofísica, Universidade Federal de São Paulo (São Paulo, Brasil)

INTRODUCTION: Chagas disease (CD) is a complex disease caused by infection with the protozoan parasite *Trypanosoma cruzi*. There is ≈6–7 million people currently infected with *T. cruzi*, Chronic chagasic heart (CCC) disease represents extensive remodeling of the cardiovascular system, manifested as cardiac denervation and interstitial mononuclear infiltrate. Myocyte degenerative changes, fibrosis, and hypertrophy characterize the main pathologic features of CCC. These morphological alterations coexist and are associated with abnormalities of the electrical and contractile cardiac activities, which are characterized mainly by conduction faults, frequent and complex ventricular arrhythmias, and systolic ventricular dysfunction. Studies in animals and humans show positive results of physical exercise in *T. cruzi* infection. OBJECTIVES: The aim of this study is to investigate whether physical activity would attenuate cardiac remodeling during the development of chagasic cardiomyopathy. MATERIALS AND METHODS: BALB/c mice were infected(i.p) with 100 trypomastigote(Colombiana strain). Animals were randomly distributed in four groups (Control, Trained, Chagasic and Chagasic Trained). Parasitemia and mortality was evaluated starting on the 5 th day after infection. The exhaustion test was used to define the maximum velocity and to calculate the exercise intensity(60% of maximum velocity). Histological analyses were made to investigate nuclei, presence of inflammatory infiltrate and the abundance of other cell types. Cardiomyocytes were isolated after 48h of the final training section using the method described by Shioya (2007). We measured Ca^{2+} transients and contractility using the method described previously by Santos-Miranda (2021). DISCUSSION AND RESULTS: Infected trained animals have lower peak parasitemia and lower mortality rate compared to sedentary chagasic animals. Physical training also reduced inflammation and restored cardiomyocyte occupancy in the chagasic heart. The CD reduced the amplitude of the Ca^{2+} transient and the cell shortening fraction in cardiomyocytes of sedentary chagasic animals and the physical training was efficient to restore these parameters. CONCLUSION: Physical training reduces parasitemia, decreases mortality and attenuates remodeling in Chagas disease.

Keywords: Chagas Disease, Aerobic Exercise, Cardiac Remodeling / **Supported by:** Fapemig e CNPq

C.16 - Project: Effects Investigation of Radiotherapy of High Dose (FLASH) In Cell Model of Tumoral Progression

Deborah Carneiro[1,2], Simone Cardoso[2], Mariana Stelling[1], Cristiano Ponte[1]
[1]Biotecnologia, Instituto Federal do Rio de Janeiro (RJ, Brasil), [2]Instituto de Física, Univ. Federal do Rio de Janeiro (RJ, Brasil)

INTRODUCTION: Flash radiotherapy (RT) is a new RT modality in preclinical studies. Its differential and promising potential is the application of a very high dosage in a short period of time. In conventional RT, a patient receives the full dose in several sessions lasting an average of 5 to 10 minutes. RT Flash proposes to deliver the same total dose, but in a single session lasting in the millisecond range. This protocol leads to the Flash effect, in which the tumor tissue suffers acutely and is eliminated with high efficiency, while the adjacent healthy tissue is protected. As some tumors are radioresistant to conventional RT, it is necessary to study the application of Flash RT and its effects. OBJECTIVES: The biological bases that explain the Flash effect have not yet been discovered and there are still open hypotheses. In addition, the safety of using RT Flash in patients has not yet been established, although there are promising data in the literature. For this reason, this project, in collaboration with the Physics Institute of UFRJ, aims to carry out a systematic analysis of the different radiotherapy conditions using cellular models, in order to establish a protocol for future large-scale tests. MATERIALS AND METHODS: Creation of a cell bank – Lineages of glioblastomas, endothelial cells and astrocytes; establish a radiological treatment protocol for conventional RT; establish a radiological treatment protocol for RT Flash; Cell survival assays. DISCUSSION AND RESULTS: Establish experimental protocols for conventional RT and Flash RT and assess the harm gained from the analyses. CONCLUSION: RT Flash has the potential to introduce a new era of cancer treatment in patients with radioresistance and/or inoperable tumors and projects like this will pave the way for the establishment of this new modality of RT.

Keywords: Cancer, Flash Radiotherapy, Radioresistance

D - Computational Biophysics and Biochemistry

D.01 - TopBuilder: A Tool to Automate the Construction of Topologies and Generation of Atomic Coordinates for Lipopolysaccharide Molecules

Manuela Leal Nader [1], Denys Ewerton da Silva Santos[1], Thereza Amélia Soares da Silva[2]
[1]Depto de Química Fundamental, Univ. Federal de Pernambuco (PE, Brasil), [2]Departamento de Química, Univ. de São Paulo - Ribeirão Preto (SP, Brasil)

INTRODUCTION: The cell wall of Gram-negative bacteria functions as a barrier against antibiotics and is well known to incite an immune response in mammals. This bacterial outer membrane is mainly made of lipopolysaccharide (LPS), which possesses a chemically complex structure. This complexity comes from several aspects, such as its highly negative charged nature, due to the presence of numerous phosphate and carboxyl groups, and the wide diversity of its chemical variants or chemotypes, which can differ whether in the length and quantity of acyl chains or in the variety of its oligosaccharides. In that regard, manually constructing atomistic models for different chemotypes of LPS is proven to be a very time-consuming and error-prone task OBJECTIVES: Therefore, we are developing a software to automate the construction of topologies and generating atomic coordinates compatible with our set of atomic parameters for LPS. MATERIALS AND METHODS: The code is written in Fortran 90. DISCUSSION AND RESULTS: So far, we are able to successfully create a final structure, that is the atomic coordinates, from building blocks - smaller molecules. We are also able to generate a topology for that final structure, which describes all the bonds, angles and dihedrals through parameters previously developed in our group. CONCLUSION: Automatization of the generation of topologies and coordinates is crucial for studying such complex systems through atomistic simulations, especially for not specialized users. We also expect to implement a tool that converts our atomistic models to coarse-grain, as well as back-mapping.

Keywords: Software, Lipopolysaccharide, Bacterial Outer Membrane / **Supported by:** FACEPE

D.02 - Chikungunya nsP2 Virtual Screening Parametrization: Decoy Generation and Molecular Docking

Matheus Fernandes Bittencourt [1], Alessandra Sbano[2], Manuela Leal da Silva[3,2,1]
[1]Biologia Compuacional e Sistemas, Instituto Oswaldo Cruz (Rio de Janeiro, Brasil), [2]Biotecnologia, Instituto Nacional de Metrologia, Qualidade e Tecnologia (Rio de Janeiro, Brasil), [3]Instituto de Biodiversidade e Sustentabilidade , Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: Chikungunya virus (CHIKV) is an arbovirus endemic to Brazil with high associated morbidity, for which there isn't yet an approved specific antiviral. Within its genome, nonstructural protein 2 (nsP2) has an essential role for the virus, being a key component for viral replication, and therefore, a promising potential drug target. As of now, there are no PDB structures of CHIKV nsP2 crystalized with a ligand, making redocking an unviable strategy for validating virtual screening for this target. An alternative strategy is to use the enrichment of annotated ligands versus presumed non-binding molecules, the so-called decoys, as benchmark. OBJECTIVES: Validating the molecular docking protocol for virtual screening through the strategy of enrichment of annotated ligands versus property matched decoys. MATERIALS AND METHODS: The softwares GOLD and AutoDock Vina were used in the molecular docking procedures against Chikungunya nsP2. Ligand preparation was done with Open Babel. The decoys were generated using DUD-E server based on ligands with *In vitro* evidence of nsP2 protease inhibition found in literature. Enrichment factor (EF) and receiver operating characteristics (ROC) curves were used as the metrics for evaluation. DISCUSSION AND RESULTS: Database with 6 active ligands described in literature and 300 decoys generated, all protonated in pH 7.4. CONCLUSION: Using decoys for molecular docking benchmarking is a promising method for parametrizing the system for future virtual screening steps.

Keywords: Chikungunya, nsP2, Antiviral
Supported by: CAPES, FAPERJ, IOC

D.03 - Chiral Enhancement of Aldol Reactions Catalyzed by Lipopeptides Investigated using Molecular Dynamic Simulations

Pedro Tendrih Sodre[1], Mauricio Domingues Coutinho Neto [1]
[1]CCNH, Universidade Federal do ABC (São Paulo, Brasil)

INTRODUCTION: Proline containing lipopeptides can efficiently catalyze aldol reactions with high yield and enantiomeric excess (ee). The proline catalyzed aldol reaction between cyclohexanone and p-nitrobenzaldehyde originates a product with a new carbon-carbon bond formed in a key step that determines the product's stereochemistry, putatively involving the reaction between an enamine intermediate and p-nitrobenzaldehyde. Lipopeptides supramolecular aggregates generate a chemical environment different from isotropic solvent that, when properly controlled, can improve both yield and ee. OBJECTIVES: Self assembly of catalytic lipopeptides offers an interesting platform for greener chemistry by reducing the use of environmentally harmful solvents. In this work, we aim to explain the role of these aggregates in improving the reaction ee. MATERIALS AND METHODS: In this study, we investigate the role of a micellar microstructured environment formed by proline containing lipopeptides in enhancing the aldol reaction's ee. We investigate the influence of the micelle on enhancing pro-chiral encounters using atomistic molecular dynamics simulations employing the AMBER package and force fields. DISCUSSION AND RESULTS: Our results suggest that the micellar aggregate favors pro-chiral encounters that would yield an enhanced ee by favoring the major product with stereochemistry (S,R) in detriment to the (S,S) isomer, in agreement with experimental results. Interestingly, results from simulations in which we used lipopetides with inverted chirality (D instead of L) lead to the enhancement of the same (S,R) isomer. Further investigation of the intermolecular interactions that cause of such enhancement is underway. CONCLUSION: Molecular dynamics offers an atomistic view of the mechanism by which a supramolecular environment can influence the enantiomeric excess of a proline catalyzed aldol reaction.

Keywords: chiral enhancement, molecular dynamics, lipopeptide / **Supported by:** CAPES, CNPq

D.04 - Atomistic md of bis(monoaclyglycero)phosphate-containing lipid bilayer

Pedro Nunes de Oliveira Júnior [1], Antonio Rodrigues[2], Thereza Soares[3], Rosangela Itri[1]
[1]DFAP, University of São Paulo (São Paulo, Brasil), [2]DFGE, University of São Paulo (São Paulo, Brasil), [3]FFCLRP, University of São Paulo (São Paulo, Brasil)

INTRODUCTION: Lysosomes and endosomes are cellular organelles composed of a single heterogeneous membrane. These compartments are crucial to the composition of the endocytic pathway. BMP (bis(monoaclyglycero)phosphate) is one of the main lipids that compose these membranes, with biological functions widely studied, but its structural functions remain elusive. In order to compare previous studies developed by our group, which indicated multivesicular bodies formation depending on the pH of the medium, atomistic simulations of bilayer composed by BMP protonated and deprotonated were performed. OBJECTIVES: To obtain structural properties of BMP that help us understanding the relation between multivesicular formation and curvature depending on BMP protonation degree. MATERIALS AND METHODS: To do this, classical atomistic simulations were performed using GROMACS. Force fields CHARMM-GUI was the one adopted. Bilayers with different lipid compositions were studied, including a structural isomer of BMP, DOPG (1,2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol)), protonated and deprotonated BMP. SuAVE software was also used for some analysis. DISCUSSION AND RESULTS: The following parameters will be discussed: Area and Volume per lipid, electronic density profile, Hydrogen bonds, RDF, Order parameter, membrane thickness and curvature, lateral diffusion coefficient, and angle distribution of hydrophobic chains. CONCLUSION: With adequate validation of Molecular dynamics simulations, this methodology complements experimental data, and it walks alongside experimental data for a better understanding of nature, including at an atomic level.

Keywords: Molecular Dynamics, BMP, Lysosomes
Supported by: conselho nacional de desenvolvimento científico e tecnológico (CNPq)

D.05 - Computational Modeling of ZIKV MTase NS5-SAH Complex

Lilian Mendonça Alves de Oliveira [1], Pedro Henrique Torres[1], Rafael Souza[1], Pedro Geraldo Pascutti[1]
[1]UFRJ, Instituto de Biofísica Carlos Chagas Filho (, Brasil)

INTRODUCTION: The Zika virus (ZIKV) has emerged as an international public health concern due to its serious symptoms, notably the evidence that has accumulated to conclude that infection during pregnancy is a major cause of microcephaly and other severe fetal brain defects. Parallel to the development of an efficacious vaccine, research on chemotherapy focusing on specific proteins are excellent alternatives for the inhibition of viral replication. The NS5 protein of ZIKV is one of the most important and conserved Flaviviridae enzyme, which contains two domains: a N-terminal methyltransferase (MTase) and a C-terminal RNA polymerase. The function of the MTase domain depends on an S-Adenosyl methionine (SAM) cofactor that acts as a methyl group donor, and is thus converted to S-Adenosyl Homocysteine (SAH). The MTase domain methylates the viral RNA and prevents it from being recognized by the host's immune system. Therefore, NS5 MTase can be considered a promising target for drug design. OBJECTIVES: To investigate the NS5 MTase domain in complex with SAH (PDB ID: 5NJU) through Molecular Dynamics (MD) simulations to design an inhibitor for this enzyme. MATERIALS AND METHODS: MD simulations were performed using AMBER18 package in four different conditions: (I) protein without SAH solvated with 70% water and 30% ethanol. (II) protein without SAH in simple aqueous system, (III) protein-SAH complex in water and (IV) protein-SAH complex in mixed solvation. DISCUSSION AND RESULTS: he map of water and ethanol binding microsites on the protein surface showed important regions to explore with pharmacophoric groups for the design of an inhibitor. CONCLUSION: Furthermore, we detected a conformational change around the active site in mixed solvation, which led to the opening of a cavity that might be explored as a target for allosteric inhibitors.
Keywords: Molecular Dynamics, ZikV, NS5

D.06 - In silico design of peptides capable of binding the SARS-CoV-2 spike protein's receptor binding domain

Paula Fernandes da Costa Franklin[1], **João Sartori** [1], Ana Carolina Ramos Guimarães[1], Lucas de Almeida Machado[1,2]
[1]Laboratório de Genômica Funcional e Bioinformática, Instituto Oswaldo Cruz (, Brasil), [2]Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (, Argentina)

INTRODUCTION: COVID-19 is an infectious disease caused by SARS-CoV-2, a highly transmissible and pathogenic coronavirus. The infectious process is highly dependent on the binding of the spike protein to the human ACE-2 receptor. Despite the existence of vaccines, there is still need for advances in therapy and diagnostic. OBJECTIVES: Here, we aim at developing peptides capable of binding to the receptor-binding domain (RBD) of the spike protein with higher binding affinity than ACE2, through an in silico optimization routine MATERIALS AND METHODS: From the cryo-EM structure (PDB code: 7df4) of the RBD-ACE-2 complex, we constructed the structure of RBD in complex with an alpha-helical peptide derived from ACE-2. The protein design procedure was carried out with pyRosetta. It consisted of a repacking routine to add mutations and to optimize rotamers in the vicinity of the mutated site, followed by FastRelax protocol. After the structure optimization, the ΔG values were estimated. All the procedures were carried out using the score function ref2015_cart. The peptides were optimized for 50 generations or until no significant change in ΔΔG was observed. DISCUSSION AND RESULTS: A set of peptides was generated using pyRosetta's design protocol. Their sequences and structures are currently under analysis. We are currently implementing the use of a genetic algorithm to enhance the optimization routine. CONCLUSION: Here, we investigated the possibility of using in silico protocol to optimize the binding of an ACE-2-derived peptide to the RBD. The approach makes it possible not only to generate peptides for therapeutic purposes, but also for diagnostics. The best candidates will be tested experimentally in the future.
Keywords: SARS-Cov-2, ACE-2, peptide design
Supported by: CNPQ

D.07 - Optimization of Allosteric Inhibitors Against SARS-CoV-2 Main Protease (3CLpro)

Ingrid Bernardes S. Martins [1], Jorge Enrique H. González[2], Thamires R. Machado[1], Pedro Geraldo Pascutti[1]
[1]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil), [2]Departamento de Física, Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista (São Paulo, Brasil)

INTRODUCTION: The coronavirus disease 2019 pandemic caused by the severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) is a major health problem. The SARS-CoV-2 main protease (3CL ^{pro}) is considered an attractive target for the development of new drugs to combat this disease due to its key role in the polyprotein processing, together with the absence of human homologs. OBJECTIVES: To characterize, using Molecular Dynamics (MD), the allosteric sites of 3CL ^{pro} enzyme with three inhibitors previously found and combine data from the inhibitors at the respective binding sites with those obtained by FTMAP (site mapping by small molecular probes) and simulations with mixed solvent. MATERIALS AND METHODS: MD simulations of the previously found inhibitors with 3CL ^{pro} were performed using Amber20, as well as the same systems with mixed solvent (70:30 water and ethanol). MD trajectory clustering to select representative conformations of these systems, analysis of hydrophobic contacts and hydrogen bonds between protein and water or the inhibitor were carried out with *cpptraj* module. FTmap server was also used. DISCUSSION AND RESULTS: The simulations were performed for 1 μs each replica and each system (500 ns for mixed solvent simulations). Through analysis of hydrophobic contacts and hydrogen bonds we mapped the interactions between each inhibitor and the 3CL ^{pro} . The more representative conformations obtained were submitted on the FTmap server and we analyzed the probes that anchored on the protein near the ligand position, suggesting a new chemical interaction that can be formed. Through mixed solvent MD simulations we obtain the interactions between the protein and ethanol or water that are formed on the ligand position. CONCLUSION: Combining these analyses, we are capable of proposing modifications on the ligands in order to improve its interaction with 3CL ^{pro} , subsequent inhibition assays and experimental analysis will be performed with new ligands.

Keywords: Allosteric Inhibitors, Sars-Cov-2, Main Protease / **Supported by:** CAPES

D.08 - VISUALIZATION OF THE ACETYLATION ENERGY LANDSCAPE OF H4 HISTONE TAILS

Rafael Giordano Viegas [1,2], Hao Wu[3], Murilo Nogueira Sanches[2], Garegin Papoian[4,5], Vitor Barbanti Pereira Leite[2]
[1]Instituto Federal de Educação, Ciência e Tecnologia de São Paulo (São Paulo, Brazil), [2]Departamento de Física, Universidade Estadual Paulista (São Paulo, Brazil), [3]Department of Physiology and Biophysics, Weill Cornell Medicine (New York, USA), [4]Biophysics Program, Institute for Physical Science and Technology, University of Maryland (Maryland, USA), [5]Department of Chemistry and Biochemistry, University of Maryland (Maryland, USA)

INTRODUCTION: Histones are core structural proteins on which DNA is wrapped around to form the chromatin. They have long intrinsically disordered N-terminal tails that protrude from the nucleosome core. These tails are a target of many posttranslational modifications, like acetylation, that are involved in chromatin regulation. A recent study on H4 histone has shown that the acetylation of the lysine K16 leads to a drastic reduction in its conformational heterogeneity, inducing the formation of specific motifs, and that progressive acetylation has a cumulative effect on the conformational space (JACS, 137(19): 6245–6253, 2015). Describing the energy landscape of this protein is a challenging task due to a lack of reference structures. Nevertheless, such analysis can be carried out by using a method that does not require reaction coordinates, such as the recently developed tool – the Energy Landscape Visualization Method (ELViM, JCTC, 15(11): 6482, 2019). OBJECTIVES: This study aims to explore the effects of acetylation on the energy landscape of H4 tails using the ELViM. MATERIALS AND METHODS: All-atom H4 replica-exchange molecular dynamics trajectories were analyzed through the ELViM. By this method, it is possible to project a single effective energy landscape containing conformations from non-acetylated, mono-, di-, tri-, and tetra-acetylated models. DISCUSSION AND RESULTS: This approach allows us to compare how the individual landscape of each model relates to the overall phase-space. We show how acetylations reshape the individual landscapes, changing the number and distributions of local minima, which are characterized by high-density clusters. We can also highlight regions of the phase space that are populated by a single acetylated H4 form and quantify the heterogeneity of each model by calculating the entropy of each case. CONCLUSION: Our results show that ELViM can provide an intuitive visualization of this complex energy landscape, bringing new insights into the conformational preferences of the H4 in relation to different acetylation modes.

Keywords: Energy Landscape, Histone, Acetylation

D.09 - Magnesium Dependence of Hydrogen Bond and Stacking Potentials in DNA

Maria Izabel Muniz[1], Adrian H. Bustos[2], Sofie Slott[2], Kira Astakhova[2], Gerald Weber[1]
[1]Departamento de Física, Universidade Federal de Minas Gerais (Minas Gerais, Brazil), [2]Department of Chemistry, Technical University of Denmark (Kongens Lyngby, Denmark)

INTRODUCTION: DNA structure is strongly influenced by the presence of cations, such as Na^+ and Mg^{2+}, which stabilize the double helix and influence its structural folding. These cations are present in cells and used in molecular biology techniques. OBJECTIVES: Investigate the influence of buffers with different concentrations of Mg^{2+} and K^+, and positions of base pairs: terminal (last base pair of each end) and internal, in denaturation of DNA sequences, and compare these results with a previous studies of Na^+ MATERIALS AND METHODS: We use the mesoscopic Peyrard-Bishop (PB) model via thermal equivalence approach, which describes the denaturation of double-stranded helix through the use of interaction potentials: Morse and harmonic. Furthermore, we also consider equivalent sodium concentration and ionic strength. To calculate these potentials, we consider 182 DNA sequences and their melting temperature data set for buffers containing different concentrations of Mg^{2+}, with or without K^+. DISCUSSION AND RESULTS: The internal AT and CG Morse potentials were found to be nearly constant for any cation valence. For terminal base pairs, the Morse potentials are well described by sodium equivalent. In contrast, stacking potentials are better described by ionic strength as they show a distinct valence signature and signs of Mg^{2+} and K^+ ion competition in the mixed Mg^{2+} +K^+ buffer. CONCLUSION: We performed a comparative study of DNA in varying concentrations of Mg^{2+} and Mg^{2+} +K^+, and compared them to previous studies in Na^+. We believe that, in view of our results, detailed temperature measurements could be used to evaluate the ionic charge distribution as well as to resolve the ion competition in DNA.

Keywords: DNA, Magnesium, Mesoscopic model / **Supported by:** Fapemig, CNPq e CAPES

D.10 - Quantifying the Bromide vs. Chloride Adsorption at Cationic Surfactant Interfaces

Laura Mortara [1,2,3], Tetiana Mukhina[2], Iolanda Midea Cuccovia[1], Hernan Chaimovich[1], Gerald Brezesinski[2], Nico van der Vegt[3], Emanuel Schneck[2]
[1]Departamento de Bioquímica , Universidade de São Paulo, Instituto de Química (São Paulo, Brazil), [2]Institute for Condensed Matter Physics, Technische Universität Darmstadt, Department of Physics (, Germany), [3]Computational Physical Chemistry, Technische Universität Darmstadt, Department of Chemistry (, Germany)

INTRODUCTION: Specific ion effects determine colloidal stability. The efficiency of Dioctadecyldimethylammonium ($DODA^+$), a gene delivery agent, differs depending on the counterion, Cl (DODAC) or Br (DODAB). We quantified the preferential (Cl/Br) adsorption in cationic monolayers to understand anion selectivity. OBJECTIVES: Quantify the preferential interaction of Br^-/Cl^- with monolayers of $DODA^+$ and of the shorter chain analog Dihexadecyldimethylammonium ($DHDA^+$) at the air/water interfaces. MATERIALS AND METHODS: DHDAC obtained by ion exchange from DHDAB. Pressure/Area isotherms of DHDAC and DODAB measured on aqueous subphases (2 mM) of NaBr, NaCl, or equimolar NaBr/NaCl. Brewster Angle Microscopy images (BAM) were taken. Total-reflection X-ray monolayer fluorescence (TRXF) was performed at the synchrotron beamline P08 at PETRA III of DESY (Hamburg, Germany). Spectra were analyzed by fitting with multiple Gaussian functions. Solvent-explicit molecular dynamics simulations (NAxyPzT)(GROMACS 2021.2) of monolayers at saline solution/vacuum interface were performed. DISCUSSION AND RESULTS: DODAB surfactant monolayer showed distinctive phases depending on the surface pressure, confirmed by BAM images. DHDAC exhibited only single fluid phase under all conditions. For DODAB, the isotherm on the equimolar NaBr/NaCl mixture subphase was similar to that on the NaBr subphase indicating that Br^- plays the dominant role in forming the electrical double layer. The x-ray fluorescence of bare salt solutions and that of the monolayer were determined. The fluorescence intensity of the anions increased in presence of the cationic monolayer, and 80% of the counterions at the interface were found to be Br^-. Several simulation force fields were tested, and only GAFFlipids reproduced the monolayer organization observed experimentally. The simulations qualitatively reproduced the preferential adsorption of Br^- compared to Cl^- but to a lower extent than observed experimentally. CONCLUSION: Experiments and simulations consistently showed that monolayers displaying cationic quaternary ammonium moieties preferentially interact with Br^-. This preference is independent of the monolayer phase state.

Keywords: Cationic Surfactant, Specific Ion Effect, Molecular Dynamics SImulation / **Supported by:** FAPESP

D.11 - Design Peptides as Inhibitor for Molecular Targets involved on the Metabolism of Leishmaniose

Endrew Torres-Bonfim[2,1], Leoonardo Calderon[2], Angélica Nakagawa[1], **Ana Ligia Scott [1]**
[1]CMCC, Centro de Matemática, Computação e Cognição, Universidade Federal do ABC, UFABC, Santo André, SP, Brazil (São Paulo, Brasil), [2]Fcauldade de Medcina, Universidade Federal de Rondônia, UNIR, Porto Velho, RO, Brazil. (, Brazil)

INTRODUCTION: Peptides can have an action in several physiological processes from biological responses as well as modulation of biochemical responses. Its biological activity is determined by functional sequences of specific amino acids and can be mimicked to design short synthetic peptides, which have high specificity, stability and ease of synthesis. The characterization of molecules has developed and made them more and more applicable to the pharmaceutical, and biotechnology industry. In this context, aim to reduce the amount needed to promote the therapeutic effect of suppressing the infection, linking negligible affinity, efficiency and toxicity, we propose the use and development of synthetic peptides with activity against Leishmania infantum, L. panamensis and L. major, these species with information deposited on online platforms. OBJECTIVES: Define molecular targets for Leishmania infantum, L. panamensis and L. major, as well as develop and characterize synthetic peptides with leishmanicidal potential using. MATERIALS AND METHODS: We design a synthetic peptide database with their physical chemical characterization. In a second step, we select molecular targets from Leishmania infantum, L. panamensis and L. major, applying in silico filters, biophysical characterization . A Large-scale virtual screening of the scalar peptide bank crossing in phase I with selected proteins from Leishmania species in phase II, Docking, ranking and selection of the 20 best ranked, Normal Modes Realization, Molecular Dynamics, Refinement (Jury Score) and select candidate peptides with potential Leishmanicidal action DISCUSSION AND RESULTS: We select seven peptides that look like goods candidates to be an inhibitor (lead compound) Now, the Calderon Group (Fiocruz-Rondônia) is conducting the *In vitro* trials CONCLUSION: These results will direct *In vitro* and *In vivo* tests for better responses and application in alternatives for the treatment of leishmaniasis, as well as use of Brazilian biodiversity for the development of innovative products based on new protein and peptide engineering technologies.

Keywords: leishmaniasis, synthetic peptides, virtual screening / **Supported by:** Capes

D.12 - Adaptive collective motions: a flexible method to accelerate molecular dynamics using normal modes

Pedro Túlio de Resende Lara[1,2], Maurício Garcia Souza Costa[3], David Perahia[2]
[1]Centro de Ciências Naturais e Humanas, Universidade Federal do ABC (, Brasil), [2]Laboratoire de Biologie et de Pharmacologie Appliquée, École Normale Supérieure Paris-Saclay (, França), [3]Grupo de Biofísica Computacional e Modelagem Molecular, Fundação Oswaldo Cruz (, Brasil)

INTRODUCTION: Protein function is closely related with its structure and dynamics. Due to its large number of degrees of freedom, proteins adopt a large number of conformations, which describe a highly complex potential energy landscape. Considering the huge ensemble of conformations in dynamic equilibrium in solution, detailed investigation of proteins dynamics is extremely costly. Several methods have emerged to engage this issue, among them the Molecular Dynamics with excited Normal Modes (MDeNM) in which normal modes (NM) are used as collective variables to accelerate molecular dynamics. However, NM vectors do not efficiently describe domain rotational motions or extensive structural displacements due to large anharmonic couplings which penalize conformational sampling. OBJECTIVES: With the adaptive MDeNM (aMDeNM) approach, we adaptively change the excitation vectors during the simulations such that the tensions resulting from the inherent internal non-linearities and from the environment are reduced allowing greater distances to be explored. MATERIALS AND METHODS: We tested the new approach on bacteriophage's T4 lyzozyme, human calmodulin and the Staphylococcus aureus monofunctional transglycosylase. DISCUSSION AND RESULTS: Results showed that aMDeNM outperformed free MD sampling and preserved the structural features comparatively to the original MDeNM approach. We also observed that by adaptively changing the excitation direction during calculations, proteins follow new transition paths preventing structural distortions. We observed that small adaptive modifications in collective movement directions allow a more extensive exploration of the conformational space. This leads to further structural changes which would not have been possible if only the initial unchanged NM vectors were considered. Furthermore, we demonstrate that the direction changes takes place when low and medium frequency motions are intrinsically activated during the trajectories. CONCLUSION: In summary, our data reinforce the power of aMDeNM in plentiful applications, such as protein−protein flexible docking, structural interpretation of experimental data, conformational exploration of large systems in atomistic level and recognition of transition paths.

Keywords: Conformational sampling, Collective Variables, Protein dynamics / **Supported by:** CAPES

D.13 - Lipid Composition and the Structure of Bacterial Outer Membranes

Diane Lima[1], Thereza Soares[1]
[1]Depto de química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto - Universidade de São Paulo (SP, Brasil)

INTRODUCTION: Bacterial resistance is a public health emergency, and in recent years there has been an increase in studies aimed at understanding the mechanisms of resistance. It is known that the outer membrane (OM) of Gram-negative bacteria plays a crucial role in the process of antibiotic resistance due to the presence of lipopolysaccharides (LPS). The chemical composition of LPS units determines the structure and physical-chemical properties of the bacterial wall. OBJECTIVES: In this work, we have investigated via atomistic computational simulations the role of lipid composition and spatial distribution on the structural properties of the Gram-negative bacterial membranes. MATERIALS AND METHODS: We have performed molecular dynamics simulations of the Lipid-A moiety of the LPS and performed extensive structure characterization with the SuaVE code.1 Lipid-A is the hydrophobic region of the LPS molecule responsible for the endotoxicity of the Gram-negative bacteria. DISCUSSION AND RESULTS: We have simulated a total of six different bilayers by combining four distinct chemotypes. The systems exhibit different molecular packing and fluidity as shown by curvature analysis and area per lipids. CONCLUSION: Our preliminary results indicate that Lipid-A composition has an important role modulating the structural dynamics of the OM of Gram-negative bacteria.
Keywords: Gromos force-field, Number of acyl chains, Number of phosphorylation sites
Supported by: FACEPE, CNPq and CAPES.

D.14 - In Silico Analysis of Mutations in Human FXN Protein Related to Friedreich Ataxia.

Loiane Mendonça Abrantes da Conceição [1]
[1]Departamento de Genética e Biologia Molecular; Instituto Biomédico; Universidade, Bioinformatics and Computational Biology Group, Federal University of the State of Rio de Janeiro (RJ, Brazil). (RJ, Brasil)

INTRODUCTION: Friedreich's Ataxia (FRDA) is the most frequent type of hereditary ataxia. FRDA is caused by mutations in human frataxin (FXN), a mitochondrial protein that plays a key role in intracellular iron homeostasis. OBJECTIVES: Obtain in silico a complete structure of human FXN, which is not yet fully elucidated, and characterize the functional effects of missense mutations in FXN protein. MATERIALS AND METHODS: Missense mutations of human FXN were obtained from the dbSNP database. The effects of missense mutations on the protein function, stability, aggregation tendency, amyloid propensity, and chaperone binding were predicted using 12 algorithms like SIFT, I-Mutant3.0, and SNPEffect4.0. A theoretical model of FXN protein was constructed using the Robetta server. TM-Align was used to align the theoretical model with the crystallographic structure of human FXN (PDB ID: 3S4M). The model had its quality assessed using the validation algorithms: ProSa-Web, QMEAN, PROCHECK, Verify3D, and ERRAT. ConSurf was used to estimate the evolutionary conservation for each amino acid of FXN. DISCUSSION AND RESULTS: : Two hundred and twenty-six mutations were compiled for FXN. Fifteen percent of the mutations were classified as deleterious by at least nine functional algorithms. The stability prediction indicated that most mutations reduce protein stability. Seventeen mutations were predicted to increase aggregation tendency and amyloid propensity. A complete model was generated for FXN, which is structurally similar to the crystallographic structure 3S4M and had its quality affirmed by all the validation algorithms used. Furthermore, approximately forty percent of the mutations analyzed were estimated to occur at evolutionarily conserved positions. CONCLUSION: In this work, we generated a complete, accurate, and unprecedented model of human FXN protein. The predictive analysis indicated that a considerable number of FXN mutations were predicted as deleterious and destabilizing, in addition to affecting conserved positions of the protein, which may have harmful consequences for the protein.
Keywords: Frataxin, Friedreich's Ataxia, In Silico / **Supported by:** FAPERJ, CNPq, CAPES, UNIRIO, NVIDIA e FINEP.

D.15 - Aging Affects Threshold Transmembrane Polarization Induced by External Electric Fields in Ventricular Cardiomyocytes

Hugo Fernando Maia Milan[1], **Ahmad Almazloum** [1], José Wilson Magalhães Bassani[1,2], Rosana Almada Bassani[1,2]
[1]Department of Electronics and Biomedical Engineering, School of Electrical and Computer Engineering - UNICAMP (Sao Paulo, Brazil), [2]Center for Biomedical Engineering, State University of Campinas (UNICAMP) (Sao Paulo, Brazil)

INTRODUCTION: External electric fields (*E*) are applied to the heart for pacemaking and reversal of arrhythmias, which require that the transmembrane electric potential (V_m) undergo a variation sufficiently large to reach its threshold value (ΔV_T). Although therapeutic *E* stimulation is more frequent in the elderly, little is known on how aging affects the myocardial membrane response to *E*. OBJECTIVES: To estimate ΔV_T by mathematical modeling from experimental excitation threshold *E* values (E_T) and geometrical data in ventricular myocytes isolated from young and aging Wistar male rats. MATERIALS AND METHODS: yocytes were enzymatically isolated from weaning, adult and old rats with ages of 28-32, 150-180 and >540 days, respectively. Myocyte geometry was modeled with a tridimensional numerical model based on the 2D cell microscopic image and with a prolate spheroid analytical model based on cell dimensions (Milan *et al.* 2019, doi:10.1007/s11517-019-02054-2). E_T was obtained at 0.5 Hz in perfused cells for different angles between *E* direction and the cell major axis. Linear mixed effects and generalized least squares models were used to assess the influence of age, cell dimensions and *E* angle on log E_T (for normal distribution) and ΔV_T estimated with both models. Statistical significance was considered for $p < 0.05$. DISCUSSION AND RESULTS: Cell dimensions increased with age. E_T increased not only with *E* angle, but also with cell length, as previously reported, and thus with age. Age also increased ΔV_T, but only in cells from old rats (~37 mV vs. ~25 mV in weanlings and adults at 0°; $p < 0.01$). This was observed with both models. CONCLUSION: Because ΔV_T was enhanced, larger E_T values in myocytes from aging rats cannot be attributed only to passive polarization phenomena, but seem to be due to the requirement of greater membrane depolarization to attain the threshold V_m, which characterizes decreased myocardial excitability with aging.

Keywords: excitability, myocardium, aging / **Supported by:** CNPq , FAPESP and CAPES

D.16 - Molecular docking consensus scoring function by the use of artificial intelligence

Artur Duque Rossi [1], Pedro Geraldo Pascutti[1], Pedro Henrique Monteiro Torres[1]
[1]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro (RJ, Brasil)

INTRODUCTION: Nowadays, the use of molecular docking is a paramount step during the process of drug discovery and it is evaluated through the use of scoring functions (SFs). There are many kinds of SFs and they behave differently across the multitude of complexes, having no linear correlation between them. This behaviour makes some SFs perform better than others, depending on the complex. One way of getting around this problem is to use a consensus among a set of different SFs. Here, we present a workflow written in python3, which is capable of using Artificial Intelligence (AI) to perform a consensus across various SFs and the molecular descriptors of the complexes. OBJECTIVES: Thus, our goal is to determine the AI algorithm which best suits the problem; along with the most relevant descriptors to feed the AI algorithm with. Subsequently, we also intend to improve and further evaluate the performance of the AI algorithm; MATERIALS AND METHODS: Complexes from the PDBbind database were downloaded, then, more than 400 descriptors for ligand, protein and complex were extracted and the complex has been submitted to diverse docking software such as Autodock Vina, PLANTS and SMINA. The full set of extracted data was fed to a supervised AI algorithm having as a goal, the real value of the interaction provided by PDBbind. DISCUSSION AND RESULTS: The preliminary results obtained during this project show that it is a promising way to perform virtual screening by using the consensus of the SFs combined with the molecular descriptors of the protein and ligand. CONCLUSION: Since this project is in its early steps, it is still being improved, so, it is expected that the AI deliver results with better accuracy in future versions. Furthermore, the library, allows the users to import the submodules separately to perform docking automation in a simple way.

Keywords: Molecular docking, Artificial intelligence, Computational biology / **Supported by:** CAPES

D.17 - Calibration and Validation of GROMOS Parameters for Sias and PolySias

Brisa Raíssa Bartellt Godoy [1], Hugo Verli[1]
[1]Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul (RS, Brasil)

INTRODUCTION: Sialic acids (Sias) are α-keto acids known as a structurally diverse family of carbohydrates, which play important modulatory and structural roles in cell biology and pathology. Still, this same chemical diversity creates a significant challenge for force-field-based calculations, requiring the previous development of parameters. OBJECTIVES: The present study aims to calibrate and validate torsional parameters for the description of Sias pseudo-rotational equilibrium and PolySias dynamics under GROMOS force field. MATERIALS AND METHODS: New torsional parameters for PolySia were developed by QM using Psi4 and fitted to molecular mechanics using RotProf, while the starting geometry for PolySia simulations was derived from a combination of metadynamics and MD. PolySias ensembles were analyzed using ConfID. To validate the GROMOS 53A6GLYC parameters for sialic acids, theoretical J3 values from 1 μs MD using GROMACS, based on Haasnoot-Altona equation, were compared to experimental values. DISCUSSION AND RESULTS: The GROMOS 53A6GLYC parameters were observed to accurately reproduce NMR ensembles, describing the 2C5 conformation for Sia residues, independently on the exocyclic groups. To combine these residues in PolySia, new torsional parameters for GROMOS were produced upon fitting to QM. Each linkage was sampled by metadynamics, and the main minima simulated by MD to identify the main populations PolySia glycosidic linkages reference geometries. These values were additionally compared to crystallographic information when available. Accordingly, accurate conformational models were produced for both Sia and PolySia derivatives. CONCLUSION: In the present work, we were able to expand and validate GROMOS 53A6GLYC parameters set for Sia and PolySia derivatives, paving the way for future studies on the biological role of such compounds.

Keywords: Sialic acids, GROMOS, Molecular dynamics
Supported by: CAPES

D.18 - Revisiting SAXS Data Analysis of Protein-Protein Interactions at Low Ionic Strength

Fernando Takeshi Tanouye [1], Jozismar Rodrigues Alves[2], Rosangela Itri[1]
[1]Departamento de Física Aplicada, Instituto de Física (São Paulo, Brasil), [2]-, Instituto Federal (, Brasil)

INTRODUCTION: Lysozyme protein-protein interactions are investigated under different conditions of density, temperature and NaCl concentration by means of small angle X-ray scattering (SAXS) and Monte Carlo (MC) simulations. OBJECTIVES: MATERIALS AND METHODS: The experimental data were fitted with a DLVO-like model (hard spheres interacting through a screened Coulomb repulsion and a Yukawa-like attraction) combined with the RPA closure relation. DISCUSSION AND RESULTS: Following the literature, this model proved to be adequate to explain the scattering curves with moderate salt concentrations, 50 and 150 mM. On the other hand, we argue, through MC simulations, that it presents serious problems when applied to dense and low-salt conditions, mainly due to distortions inherent to the RPA approximation. In addition, we also observed that: (i) the attraction between lysozymes is promoted by the addition of monovalent salt and/or by lowering the temperature; (ii) Density variations between 2-20 mg/ml do not seem to significantly alter protein-protein interactions; (iii) The effect of temperature on the scattering curves is more pronounced with increasing ionic strength, while the effect of density is greater at lower temperatures. CONCLUSION: Finally, we emphasize the importance of verifying the validity of interaction models in the analysis of SAXS data.

Keywords: Protein, Monte Carlo, SAXS
Supported by: Capes

D.19 - GRB2 Protein Folding Mechanisms: Intermediate States and Folding Routes

Raphael Dias[1], Renan Pedro[1,2], Murilo Sanches[1], Vitor Leite[1], Fernando Melo[1,2], Leandro Oliveira[1]
[1]Física, Instituto de Biociências, Letras e Ciências Exatas - Universidade Estadual Paulista "Júlio de Mesquita Filho" (São Paulo, Brasil), [2]Física, Centro Multiusuário de Inovação Biomolecular (São Paulo, Brasil)

INTRODUCTION: Growth Factor Receptor-Bound Protein 2 (Grb2), is composed of a single domain SH2 interspersed by two domains SH3. It is an essential adapter protein for a variety of cellular functions such as the activation of cell proliferation-related signaling pathways, responsible for the regulation of various types of cancers such as prostate, pancreatic, skin and breast. Recent studies revealed a balance between its monomeric and dimeric forms correlated with the communication between domains and its flexibility. Completely these features indicated importance for its stability, interaction with other proteins and performance. OBJECTIVES: Describing the folding mechanism of the GRB2 using simplified computational models, in order to highlight its folding routes and possible intermediate states. MATERIALS AND METHODS: MD simulations of the monomeric GRB2 were performed using the Structure-Based Model (SBM), in a temperature range that covers its folding and with times that allowed a good sampling of its conformational space. These trajectories were used to calculate the free energy profile and specific heat through WHAM, folding routes and intermediate states. DISCUSSION AND RESULTS: The Free Energy profile of the GRB2 protein shows a folding with intermediate states. A preferential pathway folding is calculated, where the protein core (SH2 domain) is formed first, followed by the formation of terminal portions (N-terminal and C-terminal SH3 domains). A second possibility where the ends are formed first is possible but less likely, However, both possibilities have biological relevance for GRB2 functionalities. CONCLUSION: Combining computational and experimental analysis, the folding mechanism of the GRB2 was evaluated indicating possible routes and intermediate states. These results are in agreement with the biological function associated with receptor proteins, where each domain has its specific behavior, such as flexible SH3s allowing anchoring with other proteins and flexibility in SH2, for interactions and phosphorylations.

Keywords: GRB2 Protein, folding mechanism, routes of folding
Supported by: CAPES

D.20 - Characterization of Thermodynamic Properties of Inosine Base Pairs in RNA

Gabriel Henrique Aguiar Schiess [1], Pâmella Miranda de Moura[1], Gerald Weber [1]
[1]Física, Universidade Federal de Minas Gerais (Minas Gerais, Brasil)

INTRODUCTION: The characterization of thermodynamic properties of biomolecules is essential for understanding processes like translation, transcription and replication function inside the cell. Stability of RNA and DNA molecules, especially at base pair level, has a fundamental role in the accurate manipulation and understanding of their dynamics, as well as in probe design and mismatch repair. Inosine is a naturally occurring nucleic acid which is found on vertebrate and invertebrate RNA. It has several roles in genetic variability, mismatch repair and is essential for the development of normal life. However, the thermodynamics of inosine RNA base pairs has not been fully explored. OBJECTIVES: In this work we have characterized the thermodynamic fundamental properties of inosine pairs in RNA, specifically inosine pairing to cytosine (IC) and uracil (IU). MATERIALS AND METHODS: We applied the Peyrard-Bishop mesoscopic model (PB) to model a published experimental melting temperatures set of 57 RNA sequences containing at least one inosine base pair (single or tandem). From the PB model, we obtained hydrogen bonds and stacking interactions parameters for IC and IU pairs. The model considers both parameters independently. DISCUSSION AND RESULTS: We found that IU base pair may have a similar behaviour to adenosine-uracil (AU) pair and IC base pair may be similar to cytosine-guanine (CG) pair even though it has only two hydrogen bonding. Our results also show that inosine base pairs interact with their neighboring bases in a similar way to canonical RNA pairs. Yet the presence of inosine may slightly decrease the stability of the interaction in some cases. CONCLUSION: The stability of a RNA strands is essential for the desing of RNA primers for PCR tests, the desing of micro-structures (such as circular RNA) and for further understanding dynamical processes and conformations of different RNA structures. Our work suggests that inosine pairs may maintain stability in an RNA duplex.

Keywords: inosine, RNA, mesoscopic models / **Supported by:** CNPq

D.21 - Magre II: Predicting aggregation region in proteins with machine learning, based on tertiary structure

Carlos Alves Moreira [1], Erich Philot[1], Ana Ligia Scott[1]
[1]CMCC, Graduate Program in Information Engineering - Federal University of ABC - Santo André, SP, Brazil (SP, Brasil)

INTRODUCTION: It is important to understand the processes related to the protein aggregation phenomenon, due to this several predictive bioinformatic tools have been developed in the past years. Computational models to predict regions prone to aggregate have been used to identify these regions and machine learning approach combined with experimental data becomes an important alternative to study this problem. OBJECTIVES: To contribute to this problem, we develop an aggregation prone region predictor based on protein conformation using a machine learning approach. MATERIALS AND METHODS: Magre-II considers the tertiary structure, i.e., 3D information of protein structure. It was accomplished using 3D structure, definition of distance cutoff between residues in the neighborhood sphere, the Relative Surface Area of the residues into the neighborhood sphere and the distance between residues. The Magre-II was developed in python and it used the Random Forest Regressor algorithm of the Scikit-Learn package. We validate the predictor with proteins in soluble and fibrillar forms. An Magre-II advantage is that we used experimental data from Amypro database to train and validate the machine learning model of the predictor. Magre is open to improving the model including more experimental data from literature. DISCUSSION AND RESULTS: The Magre shows good learning performance in the test set with AuC of 0.9659. Furthermore, we selected 4 disease associated proteins to validate the new methodology, considering each one in soluble and fibrillar forms and comparing the performance with two criteria: Soluble protein vs No expectation Aggregation, and Fibril Protein vs Amypro aggregation experimental annotation. Our approach was able to discriminate aggregation propensity of regions in soluvel and fibrillar conformations. CONCLUSION: The results indicate a good performance when comparing the prediction results with the experimental data of Transrethyn, Alpha-synuclein and insulin proteins, and also the difference in silico generated intermediate Prion structures.

Keywords: Prediction, Aggregation, Magre

D.22 - Conformational Study of the Complex Between the RBD of the Spike Protein of SARS-CoV-2 and the ACE2 Receptor by Molecular Dynamics Simulations

Thaisa Joanna Uzan [1], Alexandre Suman de araújo[1], Ingrid Bernardes Santana Martins[2]
[1]Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista (São Paulo, Brazil), [2]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brazil)

INTRODUCTION: The SARS-CoV-2 virus, which causes the respiratory disease COVID-19, is responsible for a pandemic that lasts since the beginning of 2020. It is a single-stranded RNA virus that encodes four structural proteins. The Spike protein is the most important for this work as it has the receptor binding domain (RBD), which recognizes the cellular receptor ACE2 in the process of the virus entry into human cells. In addition, with the widespread of the disease, several mutations have emerged, giving adaptive advantages to the virus, facilitating its transmission. One example is the P.1 variant, which was identified in December 2020. OBJECTIVES: In this work, we study the structural conformations of the complex between the RBD of the Spike protein of SARS-CoV-2 and the ACE2 receptor, for both the wild-type RBD and the P.1 variant, using Molecular Dynamics simulations. MATERIALS AND METHODS: The initial structures were found in the PDB database, water and ions were added in order to mimic the physiological environment. DISCUSSION AND RESULTS: The results indicate a stability of the secondary structure in the two viral strains, both for the isolated RBD and for the complex between RBD and ACE2. The simulations showed that the RBD of the P.1 variant is more flexible and some residues present greater mobility, although the differences in relation to the wild-type RBD are small. The results also indicate that there are more residue interactions between RBD and ACE2 in the wild-type protein than in the P.1 variant. CONCLUSION: Although the analyzes indicate more intense interactions between the ACE2 and the wild-type protein, energetic studies found in the literature suggests that the P.1 variant has greater affinity with ACE2, being necessary to carry out more extensive analyses for a better description of the system.

Keywords: ACE2, Molecular Dynamics Simulations, SARS-CoV-2

D.23 - Computational and experimental study of the interaction betweem Bromo Cresol Purple and Bovine Serum Albumin.

Tainá Pereira dos Reis [1], Bruna Pastrello[1], Maurício Ikeda Yoguim[1], Valdecir Farias Ximenes[1], Aguinaldo Robinson de Souza[1]
[1]Departamento de Química, Universidade Estadual Paulista "Júlio de Mesquita Filho" (São Paulo, Brasil)

INTRODUCTION: Serum albumin is the most abundant protein in blood plasma. These proteins are also responsible for the disposition and effectiveness of medicines. Although there are several types of albumin, the most used in research are Human Serum Albumin (HSA) and Bovine Serum Albumin (BSA). The latter is widely used due to its structural similarity to HSA, easy accessibility and low cost. BCP is a dye used as a pH indicator and the change in its UV-Vis absorption spectrum promoted by BSA may be due to the phenomenon of solvatochromism. OBJECTIVES: In this work, our objective to study the interaction between BCP and BSA via the association constants obtained by optical spectroscopy and to use these results in molecular docking simulations. MATERIALS AND METHODS: The binding constants were obtained by Rawel's method on BSA-BCP complex in the absence or presence of warfarin, ibuprofen, diazepam,digitoxin. According to the literature, the drugs warfarin, ibuprofen, diazepam and digitoxin are ligands of sites I, (II and FA6), II and III in albumin, respectively. The structure of BSA complexed with BCP (PDB: 4OR0, resolution: 2.58 Å) was used in molecular docking studies. The simulations were performed using GOLD 5.2 , a software based on a genetic algorithm to explore the spatial conformation of a ligand and on a GoldScore function. DISCUSSION AND RESULTS: The binding constant of the BCP-BSA complex was calculated as 6.8 x 104⟨sup⟩4⟨/sup⟩M⟨sup⟩-1⟨/sup⟩. The binding constant between BPC and BSA in the presence of the drugs warfarin, ibuprofen, diazepam and digitoxin were 4,18 x 10⟨sup⟩4⟨/sup⟩M⟨sup⟩-1⟨/sup⟩; 2,10 x 10⟨sup⟩4⟨/sup⟩M⟨sup⟩-1⟨/sup⟩; 2,10 x 10⟨sup⟩4⟨/sup⟩M⟨sup⟩-1⟨/sup⟩; 14,4 X 10⟨sup⟩4⟨/sup⟩M⟨sup⟩-1⟨/sup⟩; and 3.6 x 10⟨sup⟩4⟨/sup⟩M⟨sup⟩-1⟨/sup⟩, respectively. The molecular docking results indicated that sites I and FA6 are more likely to interact with BCP and BSA. CONCLUSION: In summary, the experimental results corroborate the results obtained computationally. This work was supported by FAPESP #2015/22338-9.

Keywords: Bovine Serum Albumin , Bromocresol Purple , Molecular Docking / **Supported by:** CAPES, FAPESP

D.24 - In Silico Characterization of Genetic Variants in Human SOD1 Protein Using Molecular Dynamics Simulations and Machine Learning

Gabriel Rodrigues Coutinho Pereira [1], Bárbara de Azevedo Abrahim Vieira[2], Joelma Freire De Mesquita[1]
[1]Genetics and Molecular Biology, Federal University of the State of Rio de Janeiro (Rio de Janeiro, Brazil), [2]Faculty of Pharmacy, Federal University of Rio de Janeiro (Rio de Janeiro, Brazil)

INTRODUCTION: Amyotrophic lateral sclerosis (ALS) is the most frequent motor neuron disorder in adults. Missense mutations in superoxide dismutase 1 (SOD1) are a major cause associated with the development of ALS, affecting approximately 9% of all patients. Among them, the variants A4V, H46R, D90A, and I113T account for approximately half of all ALS-SOD1 cases in the United States, Japan, Europe, and United Kingdon, respectively. OBJECTIVES: Characterize in silico the structural effects of A4V, H46R, D90A, and I113T variants on human SOD1 protein. MATERIALS AND METHODS: Three-dimensional structures for the variants were modeled using the VMD-1.9.3 package to induce the corresponding mutation in the crystallographic structure of wild-type SOD1 (PDB ID: 2C9V). Molecular dynamics (MD) simulations for the SOD1 wild-type and variants were performed in triplicates using the GROMACS-2018.8 package. AMBER99SB-ILDN was selected as the force-field of the simulations, which occurred inside a dodecahedral box using TIP3P water models. The systems were neutralized, minimized, and had their temperature and pressure equilibrated at 1atm and 300K before the start of the simulations, which lasted 300ns. The MD trajectories were analyzed using GROMACS distribution programs and the Bio3D package. DISCUSSION AND RESULTS: The establishment of a plateau in the values of root-mean-square deviation, radius of gyration, and solvent accessible surface area after approximately 150ns indicates system equilibration. All variants presented alterations in the overall essential dynamics during the simulations. The root-mean-square fluctuation contribution to the first two principal components pointed to local dynamics alterations at the electrostatic and metal-binding loops, especially for A4V and D90A. A well-accepted hypothesis suggests that dynamics and structural alterations at these functional loops of SOD1 could lead to ALS through a toxic mechanism involving aberrant protein interactions triggering aggregation. CONCLUSION: Our findings pointed to alterations in the A4V, H46R, D90A, and I113T variants that may have harmful implications for SOD1.

Keywords: Amyotrophic Lateral Sclerosis, Superoxide Dismutase 1, In Silico / **Supported by:** FAPERJ, CAPES, DAAD, FINEP, NVIDIA, CNPq, and UNIRIO

D.25 - MOLECULAR SIMULATIONS OF THE NUCLEOTIDE CAPTURE BY THE POLYMER BRUSH PDMAEMA

Vinicius Firmino dos Santos [1], Denys Ewerton da Silva Santos[2], Thereza Amélia Soares[3]
[1]Depto de Química, Universidade de São Paulo (SP, Brasil), [2]Depto de Química Fundamental, Univ. Federal de Pernambuco (Pernambuco, Brasil), [3]Departamento de Física, Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: It has been previously reported that the poly(dimethylaminoethyl methacrylate) (PDMAEMA) polymer brush can capture high loads of small nucleic acid sequences. It is particularly puzzingly that the PDMAEMA exhibits a much higher affinity, and thus higher load capture of RNA than DNA (Li. et al. Biomacromolecules. 2018), but the molecular basis for this preference is not well understood. OBJECTIVES: To investigate the influence of nucleotides chemistry and structure on this phenomenon MATERIALS AND METHODS: we performed molecular dynamics and metadynamics simulations of the PDMAEMA brushes in presence of the nucleotides deoxyadenylate and deoxythymidylate present in the DNA, and adenylate and uridylate present in RNA. The metadynamics simulations were performed to estimate the energies needed to dissociate the nucleotides from the polymer brush structure. DISCUSSION AND RESULTS: The dissociation free energies calculated for adenylate (-21,45 ± 4.94 kJ/mol) and deoxyadenylate (-13,74 ± 1,17 kJ/mol) suggests that the presence of an extra hydroxyl group in the RNA nucleotides contributes to a greater interaction with the polymeric brush, which may explain the greater loading of RNA. The high standard deviation values obtained made it difficult to find a significant difference between dissociation free energies for uridylate (-20,00 ± 5,54 kJ/mol) and deoxythymidylate (-23,66 ± 8,87 kJ/mol). CONCLUSION: So a higher precision methodology is needed to infer the influence of these nucleotides in the loading of nucleic acids by the PDMAEMA polymer brush. Determination of dissociation free energies for the remaining nucleotides present in DNA (deoxycytidylate and deoxyguanylate) and RNA (cytidylate e guanylate) will also be performed to confirm the role of the extra hydroxyl group in greater loading of RNA.

Keywords: Molecular dynamics, Metadynamics, Polymer brush / **Supported by:** FNDE, CAPES, CNPq

D.26 - Tracking the deactivation mechanism of 6-Thioguanine after UV excitation

Danielle C. Teles-Ferreira[1,5], Cristian Manzoni[2], Lara Martínez-Fernández[3], Giulio Cerullo Giulio Cerullo[2,4], Rocío Borrego-Varillas[2], **Ana Maria de Paula** [1]
[1]Depto do Física, Instituto Federal de Minas Gerais (MG, Brazil), [2]Dipartimento di Fisica, Istituto di Fotonica e Nanotecnologie (, Italy), [3]Facultad de Ciencias and Institute for Advanced Research in Chemistry, Universidad Autonoma de Madrid (, Spain), [4]Depto di Fisica, Politecnico di Milano, Milano (, Italy), [5]Depto de Física, Universidade Federal de Minas Gerais (BH, Brazil)

INTRODUCTION: Due to the effective population of their reactive triplet states upon excitation in the UV range, sulfur-substituted DNA and RNA bases are a class of biomolecules with important applications as phototherapeutic agents. The first stages after UV photoexcitation are crucial because they determine chemical rearrangement over longer time scales. However, most investigations have been limited to the exploration of ultrafast dynamics in thiobases on time scales ranging from hundreds of femtoseconds to nanoseconds due to technological limitations linked to the challenge of creation of ultrashort UV pulses. OBJECTIVES: Here we track the ultrafast decay mechanisms that lead to the electron trapping in the triplet manifold for 6-thioguanine in aqueous solution. MATERIALS AND METHODS: We perform broadband Transient Absorption Spectroscopy (TAS) experiments with sub-20 fs pump pulses and probe in a broad UV/visible spectral window. DISCUSSION AND RESULTS: At early times we observed a negative band, which is assigned to stimulated emission (SE) from the S2 state. At later times, we observed the rise of a photoinduced absorption that comes from the triplet state. We obtain two time constants: 81 fs and 522 fs, and a long lived value. The mismatch between the time constants delivers the experimental evidence of the involvement of a dark S1 mediating the intersystem crossing process. CONCLUSION: In conclusion we show experimental evidence of the fast internal conversion from S2 to the S1 state that takes places in about 80 fs and demonstrate that the S1 state act as a doorway to the triplet population.

Keywords: biomolecule, Thiobase, ultrafast spectroscopy

D.27 - There and Back Again: Evolution-Based Functional Switching Between 5-Hydroxyisourate Hydrolases and Transthyretins

Rafael Pereira Lemos [1], Flávio Antônio de Carvalho Júnior[1], Lucas Carrijo de Oliveira[1], Lucas Bleicher[1], Mariana Torquato Quezado de Magalhães[1]
[1]Bioquímica e Imunologia, Universidade Federal de Minas Gerais (MG, Brazil)

INTRODUCTION: Transthyretins are a protein family that exhibits binding and distribution of thyroid hormones as primary functions. Despite being exclusive to vertebrates, a major part of their evolution process occurred before the stages of amphibians and fishes, originating via duplication of purine catabolic pathway genes. Those encode the 5-hydroxyisourate hydrolase (HIUase) enzyme, which acts in the conversion of 5-hydroxyisourate (HIU) to 2-oxo-4-hydroxy-4-carboxy-5-ureidoimidazoline (OHCU). Differently from transthyretins, the HIUase subfamily is ubiquitous in both prokaryotes and eukaryotes, with hominids being an important exception. *In vivo* , both subfamilies are found in a homotetrameric form, with each monomer being formed by eight beta-strands connected by seven loops, and one alpha-helix. Tetramers are made stable by hydrophobic interactions between each dimer pair, leading to the formation of an internal charged catalytic cavity. OBJECTIVES: Considering these proteins' intricate evolutionary history, as well as their high primary and quaternary structural similarity, we hypothesized that specific *in silico* substitutions would be able to switch their functions. MATERIALS AND METHODS: We proposed two putative protein sequences, where one subfamily representative sequence was substituted in specific and correlated locally conserved positions by the other, and vice-versa. Applying computational modeling, molecular dynamics and docking, together with structural biology techniques, we aimed to better understand the neofunctionalization process of these paralogues. Using Modeller and AlphaFold, we generated 3D homology structural models, while also employing a chimeric manual alignment to further improve the results. The best models were refined, validated, and their cavities were analyzed using CAVER. DISCUSSION AND RESULTS: Our results indicate that, as expected, the volumes and topologies differ from one another, due to size and physicochemical differences between their ligands. The observed changes in polarity and large residue side chains fit the subfamilies' structural conservation. CONCLUSION: As a proof of concept, the recombinant proteins were expressed, and the enzymatic activity was assessed.
Keywords: Computational modeling, Protein engineering, Transthyretin / **Supported by:** CAPES, FAPEMIG, CNPq

D.28 - Identification Of Molecular Mechanisms Involved At The Chronification Of Chagas Disease

Victor de Barro Serrano Neves [1], Macambira Simone[2], Pablo Ramos[2]
[1]Biofísica e bioquímica, Universidade Federal da Bahia (BA, Brasil), [2]LETI, Fundação Gonçalo Muniz (BA, Brasil)

INTRODUCTION: Introduction Chagas disease is caused by infection with the *Trypanosoma cruzi*. For many years, it was considered a tropical disease, but due to global migration, almost 500000 carriers outside endemic areas. The acute phase of the disease is mostly asymptomatic or with mild unspecific symptoms. However, the chronic phase, manifested in almost 30% of patients, leads to cardiac, digestive and neurological disturbances. The chronic chagasic cardiomyopathy (CCC) is the main cause of heart failure in Latin America. Mechanisms underlying the chronic manifestation are not yet fully understood. OBJECTIVES: This study aims to identify molecular mechanisms involved in the acute phase that lead to chronic disease. MATERIALS AND METHODS: Host-pathogen interactions will be conducted accordingly to Davis protein interactions by structure. Acute and chronic mouse infection transcriptomes will be acquired via the GEO dataset (NCBI). Transcriptomes are going to be assessed for differentially expressed genes (DEG). DEGs from both disease phases are going to be compared to identify the DEGS common to these phases and then the molecular processes related to cardiac dysfunction pathways. A PPI analysis will be performed on DEG and the results will be compared to host-pathogen interaction to better understand how the protozoan influences the molecular mechanisms of the disease. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) will be infected with trypomastigote forms of Colombian strain T. cruzi or will be treated with proinflammatory cytokines. C57Bl6 mice will be infected trypomastigote forms of Colombian strain T. cruzi. The cardiac function evaluation by ECHO, EKG and treadmill test and morphometric analysis will be done during acute an chronic phases. mRNA will be extracted from hiPSC-CMs and the heart to determine gene expression by RTqPCR DISCUSSION AND RESULTS: Determine the molecular mechanisms due to host-pathogen interaction that leads to chronic phase manifestations in order to aid the identification of therapeutic targets to avoid and treat CCC. CONCLUSION:
Keywords: Host-pathogen interaction, Genes differentially expressed, Chronification / **Supported by:** FAPESB

D.29 - Study of Electronic Properties of the Antibiotic Linezolid

Giulia Saneti Grandini [1]
[1]Depto de Química, Universidade Estadual Paulista (São Paulo, Brasil), [2]Departamento de Físico- Química, Universidade Estadual de Campinas (São Paulo, Brasil), [3]Departamento de Química, Universidade Estadual Paulista (São Paulo, Brasil)

INTRODUCTION: Linezolid is a hospital-grade antibiotic that complexes with the functional bacterial ribosome, preventing its protein synthesis. Having a chiral carbon, only the S enantiomer has antibacterial activity. The nitrogen in the acetamide group, of the molecule, undergoes interconversion, showing no chirality signal in the experimental electronic circular dichroism (ECD) spectra. However, in ECD simulations, interconversion is not considered. OBJECTIVES: The objective is to study, through an TD-DFT molecular modeling, the signals of ECD spectra considering the structures: (R)-C(R)-N, (R)-C(S)-N, (S)-C(S)-N and (S)-C(R)-N –Linezolid. MATERIALS AND METHODS: Trough the software GaussView 6.0.16, the four structures were constructed and submitted to calculations in the software Gaussian 09 or 16. Ultraviolet (UV), ECD spectra and molecular orbital calculations were performed at PBE0/6-311++G(3df,2p) level of theory. Crystallographic coordinates of a complexed form of Linezolid, with the ribosome of the bacterium Haloarcula marismortui, were obtained from the PDB, ID: 3CPW, in order to compare the results. DISCUSSION AND RESULTS: The UV spectra of the Linezolid forms resulted in maximum absorption around ~200 nm and ~265 nm, which are in good agreement with the experimental results. At ~ 200 nm and ~265 nm, the molecular orbitals indicated electronic transitions: $\pi \rightarrow \pi^*$ in the aromatic ring and $n \rightarrow \sigma^*$ in the morpholine ring, respectively. In the ECD spectra, a signal from the acetamide nitrogen was observed around ~190 nm as expected. Another characteristic observed in the circular dichroism spectra was the inversion in relation to symmetrical - (R)-C(R)-N, (S)-C(S)-N) and asymmetrical - (R)-C(S)-N, (S)-C(R)-N) forms, suggesting that the ECD is sensitive to molecular geometry. CONCLUSION: It can be observed that the calculated electronic spectra (UV-Vis and ECD) and molecular orbitals are in good agreement with the experimental data found in literature. And are in good agreement with a system with an acetamide nitrogen that plays a significant role in ECD spectra of the Linezolid molecule.
Keywords: linezolid, electronic circular dichroism, molecular orbitals / **Supported by:** FAPESP

D.30 - Unveiling Mutation Effects on the Structural Dynamics of the Main Protease from SARS-CoV-2 with Hybrid Simulation Methods

Patricia Gasparini [1], Eric Philot Allison[1], Angelo Magro[3], Jean Carlos de Mattos[1], Natanael E. Souto Maior Torres Bonfim[1], André Kliousoff [1], Roberto Carlos N. Quiroz[1], David Perahia[4], Rafael Plana Simões[2], Ana Ligia B. Scott [1]
[1]Computational Biology and Biophysics Laboratory, Federal University of ABC (São Paulo, Brazil), [2]Department of Bioprocesses and Biotechnology, School of Agriculture (FCA), Unesp (São Paulo, Brazil), [3]Institute of Biotechnology (IBTEC), Unesp (São Paulo, Brazil), [4]LBPA, École Normale Supérieure Paris-Saclay (Paris, France)

INTRODUCTION: The main protease of SARS-CoV-2 (called Mpro) is essential for processing polyproteins encoded by viral RNA. Macromolecules adopt several favored conformations in solution depending on their structure and shape, determining their dynamics and function. Integrated methods combining the lowest- frequency movements obtained by Normal Mode Analysis (NMA) and the faster movements from Molecular Dynamics (MD), as well as data from biophysical techniques, are necessary to establish the correspondences between complex structural dynamics of macromolecules and their function. OBJECTIVES: The aim characterizes the structural dynamics and global motions of WT SARS-CoV-2 Mpro and 48 mutations, including mutations found in P.1, B.1.1.7, B.1.351, B.1.525 and B.1.429+B.1.427 variants, using a hybrid simulation method to sample the conformational space. MATERIALS AND METHODS: A hybrid approach was applied to compare the wild-type SARS-CoV-2 Mpro WT and its mutations using a normal modes-based protocol involving the analysis of simple parameters as Cα flexibility, potential energy, hydrophobic and electrostatic contributions to free energy, solvent accessible surface areas, and catalytic dyad distance. A machine learning analysis was performed combining susceptibility to Sγ (C145) deprotonation and Nε (His41) protonation (named as $CATALYSIS_{SUSCEP}$). DISCUSSION AND RESULTS: Four mutants (K90R, N151D, P108S and P99L) showed energetically stable structures, by alter the dimeric interface contacts and the functionality of the SARS-CoV-2 Mpro. Machine learning analysis indicated the chains A of the WT and mutant SARS-CoV-2 Mpros present higher catalytic reactivity values than chains B. The mutation P108S (located at the domain II) is found in structures with higher enzymatic reactivity and stability of the catalytic site. CONCLUSION: The results suggested that a single surface amino acid substitution can induce significant dimeric interface changes, however the specific conformational behavior of the wild-type and some energetically stable mutated (K90R, P99L, P108S, and N151D mutations) structures are exclusively due to surface amino acid residues.
Keywords: Molecular Dynamics, Normal Mode Analysis, Protease Mpro

D.31 - The Role of Platelet-activating Factor-Acetyl Hydrolase (PAF-AH) Levels Regulation in Zebrafish Development

Agatha Manzi De Carli [1], Bruno Paes De Carli[2,1], Isabelly Annunciato[1], Igor Nicodemo Oliveira[1], Mariana Novo Belchor[3,1], Caroline Ramos da Cruz Costa[3,1], Laila Lucyane Ferreira de Moraes[1], Airam Roggero [1], Marcos Antonio Oliveira[4], Marcos Hikari Toyama[1]

[1]Instituto de Biociências, São Paulo State University (SP, Brasil), [2]Instituto de Ciências da Saúde, Paulista University - Campus Rangel (SP, Brasil), [3]Postgraduate Program in Biotechnosciences, Universidade Federal ABC (SP, Brasil), [4]Instituto de Biociências, São Paulo State University (São Paulo, Brasil)

INTRODUCTION: Cytosolic PLA2 (cPLA2) can be found in the zebrafish and is related to the mobilization of arachidonic acid (AA), which is relevant to vertebrate development. AA production by cPLA2 also generates platelet-activating factor (PAF), a mediator in the pathogenesis of several disorders with an inflammatory component. Therefore, platelet-activating factor-acetyl hydrolase (PAF-AH) or lipoprotein-associated phospholipase A2 hydrolysis PAF can be considered an important biomarker. The organophosphorus malathion used commercially for the arbovirus control at microgram concentrations could imply the inhibition of normal vertebrate development with teratogenic effects. OBJECTIVES: The aim of this study was to evaluate the importance of the PAF-AH enzyme of Danio rerio exposed to malathion by *in silico* and biochemical studies. MATERIALS AND METHODS: *In silico* molecular docking was performed using Chimera, Autodock Vina, Discovery Studio, LIGPLOT, and Pymol. Phospholipase A2 biochemical tests were performed by enzymatic assays in 96 well plates. For this purpose, tissue sample extract was prepared with liquid nitrogen, and the supernatant was used for analyses. DISCUSSION AND RESULTS: *In silico* data showed that malathion binds strongly to the active site of PAF-AH but cannot interact with the active site of cPLA2. Using 4-Nitro-3-(octanoyloxy) benzoic acid as a substrate, kinetic enzymatic tests show a decrease in PAF-AH activity of more than 50%. CONCLUSION: Malathion at lower concentrations can be a potentially dangerous agent to inflammatory mediation. Our data suggest among several factors that AA generation and PAF control are two crucial factors for zebrafish ontogenetic development. Acute and chronic exposition by malathion can strongly contribute to impairing zebrafish larval development by inhibiting the PAF-AH enzyme, which cannot control PAF levels. - **Keywords:** Platelet Activating Factor Acetyl Hydrol, Malathion, Cytosolic PLA2

D.32 - Desenho racional de inibidores ortostéricos e alostéricos de tripanotiona redutase assistido por técnicas computacionais.

Guilherme Ian Spelta [1], Mariana Ferreira[1], Ingrid Martins[1], Pedro Pascutti[1]

[1]Instituto de Biofísica Carlos Chaga Filho, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: Chagas disease is an underreported, neglected, tropical-endemic, parasitic infectious disease, estimated to affect around 8 million people over the american continent, of which at least 1.9 million are brazilian. The currently available treatment shows undesired toxicity, low efficacy on late stages of the disease and rising parasitic resistance. Enduring the host immune system's oxidative burst is a key to guarantee the viability of *T. cruzi* parasites and the success of the infection, especially in its early stages, hence, enzymes that are involved in its oxidative stress homeostasis, such as trypanothione reductase, come into sight as targets of interest. OBJECTIVES: To characterise the interaction site of trypanothione reductase via Molecular Dynamics (MD). MATERIALS AND METHODS: MD was used to explore the protein conformational behaviour in its biological medium both in its apo and ligand-complex forms with Amber18. Both allosteric and orthosteric sites interactions were studied through Virtual Screening method. Alongside that, interactions between the orthosteric site and inhibitors already available on PDB were mapped and aligned using Discovery Studio Visualizer. DISCUSSION AND RESULTS: MD simulations of the apo structure of trypanothione reductase were done and some of its energetic and conformational parameters were analysed for the sake of discriptional analysis and future comparison with the ligand-complex form. Radius of gyration (RG) analysis showed progressive volume shrinkage, RMSD analysis showed poor conformational stabilisation but increasing values over the trajectory. The fluctuation of the internal portion of the protein, around the prosthetic group, showed lower values and external alpha-helices next to the substrate binding regions showed higher values, probably being stabilised in the presence of the ligand. Conformational Probability Density analysis through variable coupling showed prevalence of high RMSD and low RG conformations over the trajectory. CONCLUSION: Conclusions have not yet been made as the work is still in progress.

Keywords: Desenho racional de fármacos, Bioinformática, Doenças parasitárias tropicais

D.33 - Search for Novel Human Glutathione Peroxidase 4 (GPX4) Activators by Virtual Screening

Lucas Gasparello Viviani [1], Alex Inague[1], Erika Piccirillo[2], Antonia Tavares do Amaral[3], Sayuri Miyamoto[1]
[1]Bioquímica, Instituto de Química da Universidade de São Paulo (SP, Brasil), [2]CQMED, Centro de Química Medicinal da Univ. Estadual de Campinas (São Paulo, Brasil), [3]Química Fundamental, Instituto de Química da Univ. de São Paulo (SP, Brasil)

INTRODUCTION: Glutathione peroxidase 4 (GPX4) catalyzes the reduction of H_2O_2 or organic hydroperoxides to water or the corresponding alcohols, respectively. Due to its importance to ferroptosis inhibition, membrane cell repair, and inflammation, human GPX4 is recognized as a potential biological target for several diseases. However, only few activators/inhibitors of human GPX4 have been reported in the literature so far. OBJECTIVES: The aim of this study was to search for novel potential human GPX4 activators, using a virtual screening (VS) approach. MATERIALS AND METHODS: The structure of a known GPX4 activator was used to build a shape-based model ("query"), using ROCS. The generated "query" was applied to the ZINC (~$8x10^6$ compounds), ZDD (~$1,5x10^3$ compounds), and Pathogen Box (400 compounds) databases. Next, selected compounds were docked into a possible GPX4 allosteric binding site, using GOLD. Finally, a visual inspection of the predicted binding modes was performed, using LIGANDSCOUT. DISCUSSION AND RESULTS: In the shape-based screening, 516, 200, and 100 compounds with the highest "Tanimoto Combo" values (0.65-1.11) were selected from ZINC, ZDD, and Pathogen Box, respectively. The compounds were subsequently docked into a possible GPX4 allosteric binding site, and those with the highest docking score values were submitted to a visual inspection analysis. The following selection criteria were applied: (i) presence of hydrophobic, hydrogen-bonds, π-cation, and/or ionic interactions with the residues D21, V27, K90, A93, and/or D101; (ii) docking "poses" reproducibility; and (iii) surface complementarity with the protein binding site. Based on those criteria, 29, 13, and 2 compounds were selected (originally from ZINC, ZDD, and Pathogen Box, respectively), which represents ~99.9%, ~99.1%, and ~99.5% reduction in the number of compounds from each database. CONCLUSION: Using a combination of shape-based screening and docking approaches, our VS protocol has selected 44 structurally diverse compounds as human GPX4 activator candidates, which will be acquired and tested in cell-based assays for VS experimental validation.

Keywords: Glutathione peroxidase 4 (GPX4), Virtual Screening, Ferroptosis / **Supported by:** FAPESP (Proc. 2021/10514-8; 2017/13804-1; and 2013/07937-8).

D.34 - "Hidden jewels in fish toxins": Prediction of cell penetrating and antimicrobial peptides from the venomous toadfish Thalassophryne nattereri

Gabrielle Lupeti de Cena [1], Bruna Vitória Vicente Scavassa[1], Katia da Conceição[1]
[1]Ciência e Tecnologia, Universidade Federal de São Paulo (São Paulo, Brasil)

INTRODUCTION: The therapeutic success of venom-derived molecules, such as bioactive peptides (BAPs), is linked to their increased specificity, stability and assessment of pharmacokinetics properties. The BAPs, such cell penetrating peptides (CPPs) and antimicrobial peptides (AMPs), share several physicochemical characteristics and have been investigated as potential alternatives to drug-delivery systems and antibiotic-based therapies, respectively. OBJECTIVES: In the current study, CPPs and AMPs were predicted from the toxin natterins of the fish *T. nattereri*, using *in silico* analyses. MATERIALS AND METHODS: Using CAMP, AMPA, C2Pred and CellPPD web servers fifty eight sequences were generated (twenty AMPs, eight CPPs and thirty AMPs and CPPs). The physicochemical properties were analyzed, submitting the sequences to ProtParam and PepCalc tools. The probability of membrane binding potential and cellular location of each peptide were estimated using the Boman index by APD3, and TMHMM web servers. All CPPs and two AMPs analyzed showed high membrane binding potential. Among all peptides analyzed, fifty five had intra-membrane cellular localization. Peptides were examined for the immunogenicity, toxicity, allergenicity, and ADMET parameters using different web servers. Antiviral and anticancer potential was predicted using the web servers Meta-iAVP and AVPred, returning 17 potential AVP and 37 ACPs. Finally, the secondary helix structure and the helical wheel projections were predicted by the PEP-FOLD3 and Heliquest web servers. DISCUSSION AND RESULTS: Based on the parameters analyzed, 15 peptides are potential lead compounds and were selected to be further synthesized and tested experimentally *In vitro* to validate the *in silico* screening. Computer-aided design for predicting activity of peptides not only saves time and money, but also facilitates the designing of improved therapeutic peptides. CONCLUSION: Overall, this study confirms that toxins form a natural biotechnological platform still underexplored in drug discovery and the presence of CPPs/AMPs sequences among toxin families opens new roads in toxin biochemical research.

Keywords: antimicrobial peptides, cell penetrating peptides, in silico prediction / **Supported by:** FAPESP

D.35 - Insights into the porcine circovirus type 2b (pcv2b) capsid assembling using in silico tools

Angelo Jose Magro [1,2], Antoniel Augusto Severo Gomes[2], João Pessoa Araújo Jr.[3,2], David Perahia [5]
[1]School of Agriculture, São Paulo State University (Botucatu/SP, Brazil), [2]Institute of Biotechnology, São Paulo State University (Botucatu/SP, Brazil), [3]Institute of Biosciences, São Paulo State University (Botucatu/SP, Brazil), [4]Carlos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro (Rio de Janeiro/RJ, Brazil), [5]Laboratory of Biology and Applied Pharmacology, Université Paris-Saclay (Gif-sur-Yvette, France)

INTRODUCTION: Swine breeding has achieved a high development based on genetic improvement, nutrition, management and sanity. Among the most important pathogens that affect the swine world industry is the porcine circovirus 2 (PCV2), a small, icosahedral, non-enveloped virus, ambisense single-stranded circular DNA. This virus is highly resistant to environmental variations and disinfecting agents, endemic worldwide and has been associated to several distinct clinical manifestations related to important economic losses. One of the factors implicated in PCV2 pathogenicity and immune response is the Cap protein, a structural protein codified by ORF2 from PCV2 genome. Cap protein is also essential for viral replication, since this molecule composes the PCV2 capsid. Therefore, a better comprehension of the Cap protein key interactions for capsid assembling is very interesting for potential biotechnological applications. OBJECTIVES: To identify amino acid residues possibly related to the assembling of the PCV2 capsid using in silico simulations involving molecular dynamics and radial excitation of the capsid structure. MATERIALS AND METHODS: The PCV2 capsid model was generated based on the crystallographic structure determined by Khayat et al. (2011). Subsequently, the model was minimized using cojugated gradients and harmonic restraints and equilibatred with the program Charmm v.c41b1. Finally, the radial expansion of the model was performed with energy inputs (10 to 40 K) applied at 1 ps after each excitation. DISCUSSION AND RESULTS: The radial expansion of the PCV2 capsid revealed that Phe56, Phe3, Phe8, Phe187 and Pro192 are essential for hydrophobic interactions between the protein cap monomers. Additionally, Arg11, Arg14 and Asp191 are key components of the hydrogen bond network found between capsid units. CONCLUSION: The simulations indicated that certain interactions seem to drive capsid cohesion and, possibly, PCV2 assembling and replication in host cells. These data are crucial for further experimental investigation and, potentially, they could be important for the development of future vaccines and antiviral drugs.

Keywords: CIRCOVIRUS, VIRAL ASSEMBLING, PROTEIN CAP / **Supported by:** FAPESP

D.36 - *In vitro* Monitoring of IAPP Aggregation Using Luminescent Ru(II) Complexes

Lorena Maria Borges Pereira [1], Rose Maria Carlos[1]
[1]Departamento de Química, Universidade Federal de São Carlos (São Paulo, BRAZIL)

INTRODUCTION: Type 2 Diabetes Mellitus (DM2) is a metabolic disease characterized by the elevated level of glucose in the blood. The aggregation of the islet amyloid peptide (IAPP) is associated to the development of DM2, given that the formation of IAPP amyloid species are cytotoxic to insulin producing pancreatic β cells and lead to a disruption in the glicemic control. OBJECTIVES: Develop luminescent metal complexes to monitor the aggregation process of IAPP *invitro*. MATERIALS AND METHODS: The Ru(II) complexes were obtained by reacting the precursor complex *cis*-$[Ru(phen)_2(Cl)_2]$ with 3,4-Apy and pNDIp (naftalenodiimide derived phenanthroline) ligands under reflux. Human IAPP was obtined commercialy. The luminescence studies were done in phosphate buffer (pH=7,4; 50 mM). DISCUSSION AND RESULTS: The complexes' absobance spectra showed a characteristic absorption in the ultraviolet (λ_{max}=280nm) and a broad absorption band in the visible region *cis*-$[Ru(phen)_2(3,4\text{-}Apy)_2]^{2+}$ (λ_{max}=450nm) e $[Ru(phen)_2(pNDIp)]^{2+}$ (λ_{max}=480nm). The luminiescent properties of both compounds are characterized by an emission maxima at 650 nm. These spectrocopic properties indicate the biological application potential of these complexes, given that there's no overlap with the emission of aromatic aminoacids of IAPP. The aggregation process of IAPP was investigated in different incubation times, both in the absence and presence of the complexes. In the studies, an increase in the complexes emission in the presence of IAPP was observed. CONCLUSION: With these results, we can conclude that the complexes are potential luminescent probe candidates to monitor IAPP aggregation.

Keywords: Ru(II) complexes, luminescence, IAPP
Supported by: FAPESP (Processo 2021/04675-9), CNPq and CAPES

D.37 - Molecular modeling of iron transportation by OSVIT2: A target for biofortification in rice

Laís Arend [1], Fernando Biedermann[1], Mauricio Costa[2], Felipe Ricachenevsky[1], Hugo Verli[1]
[1]Departamento de Biologia Molecular e Biotecnologia, Universidade Federal do Rio Grande do Sul (RS, Brasil), [2]Programa de Computação Científica, Fundação Oswaldo Cruz (RJ, Brasil)

INTRODUCTION: Iron deficiency is our most prevalent and widespread nutritional shortfall. While half of the world's population depends on rice *(Oryza sativa)* as a staple food, this cereal cannot provide a sufficient amount of that micronutrient to meet people's nutritional needs. In this context, the Vacuolar Iron Transporters (VIT) become important objects of study, representing central targets for iron biofortification in rice. OBJECTIVES: We intend to model the transportation mechanism associated with the vacuolar intake of iron by OsVIT2 using a combination of molecular dynamics (MD), normal modes analysis (NMA) and metadynamics. MATERIALS AND METHODS: OsVIT2 was modeled by AlphaFold, while the protein-tonoplast system was generated with the aid of CHARMM-GUI. After equilibration, MD and NMA were employed to explore the plasticity of the protein's pore, while metadynamics was used to access the passage of Fe^{2+} across OsVIT2 utilizing the ion's position along the z axis as the collective variable. DISCUSSION AND RESULTS: Both MD and NMA point to a lack of major conformational changes on the OsVIT2 pore, indicating that the passage of Fe^{2+} occurs through subtle and localized conformational changes. Such adjustments were further explored by metadynamics, which was able to generate a free-energy profile associated with the ion's passage through the protein's pore. Our ongoing results suggest a transport regulated by an acidic filter on the transporter's vacuolar side. CONCLUSION: The preliminary results start to paint a picture of the iron transportation mechanism, which we expect to employ in future engineering efforts to increase OsVIT2's transport rate. This increase could both impact the iron content in rice seeds and aid in soil decontamination efforts.

Keywords: iron transportation, molecular simulations, OsVIT2

D.38 - Virtual Screening of ANVISA Approved APIs in SARS-CoV-2 PLpro

Bruce Veiga Andriolo [1], Caio Felipe de Araujo Ribas Cheohen[2], Diego Henrique da Silva Silvestre[3], Manuela Leal da Silva[1,2]
[1]Programa de Pós-Graduação em Biotecnologia, Instituto Nacional de Metrologia, Qualidade e Tecnologia (Rio de Janeiro, Brazil), [2]Programa de Pós-Graduação Multicêntrico em Ciências Fisiológicas, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brazil), [3]Programa de Pós-Graduação em Nutrição, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brazil)

INTRODUCTION: In 2019 cases of pneumonia led to the discovery of the SARS-CoV-2, responsible for COVID-19 pandemic. As the pandemic progresses several variants have been discovered. One of its most important proteins is the PLpro for its ubiquitin and deISGylating capabilities. Considering the costs of developing new drugs, repositioning already approved drugs is an excellent strategy in fighting COVID-19. We used the drugs approved by the Brazilian Health Regulatory Agency ANVISA using in silico techniques to predict interaction between these compounds and the PLpro. The importance of this work is based on the lower cost of money and time needed reposition drug when compared to developing, and the possible obsolescence of vaccines by mutations on the virus. OBJECTIVES: Perform Virtual Screening and use different in silico strategies to determine possible candidates to inhibit SARS-CoV-2 PLpro. MATERIALS AND METHODS: Virtual Screening was performed by AutoDock Vina using parameters defined in redocking with the exception of generation of 20 poses. After VS the candidates whose energy was lower than -8.0 kcal/mol were selected. For ADMETox prediction, SwissADME, admeSAR and pkCSM were used with PAINS and AMES being considered as limiting factors. The distance between the molecules and Y268 from PLpro was measured using PyMOL and molecular weight was used like a filter. The compounds were ranked based on these criteria. DISCUSSION AND RESULTS: From the original 336 compounds, 44 had the correct energy range. From those, 36 passed the AMES and PAINS test who were ranked and organized in accordance with their usage. Vitamins, unpromising or problematic drugs were excluded. This resulted in 19 APIs with possible Antiviral capabilities. CONCLUSION: Using Virtual Screening, ADMETox prediction and information about distance, pocket volume and literature we organize possible drugs to be tested *In vitro* against PLpro of SARS-CoV-2 and HEK-293 lineage cells.

Keywords: SARS-CoV-2, PLpro, Virtual Screening
Supported by: CNPq, CAPES and FAPERJ

D.39 - Enhanced sampling simulations of active-site protonation in respiratory complex I

Caroline Simões Pereira [1], Guilherme Arantes[1]
[1]Biochemistry, University of São Paulo (SP, Brazil), [2]MRC Mitochondrial Biology Unit, University of Cambridge (, UK)

INTRODUCTION: Respiratory complex I plays a central role in energy metabolism by catalyzing NADH oxidation in concert with ubiquinone (Q) reduction and transmembranar proton pumping. In the proposed reaction mechanism, Q receives two electrons from the N2 iron-sulfur cluster and two protons from catalytic His, Tyr and Asp residues (H59, Y108 and D160 in the NDUFS2 subunit of the mouse mitochondria enzyme) to form the product ubiquinol (QH2). Cryo-EM structures show that Q binds the active-site in a pre-reactive state when N2 is oxidized and no reactive hydrogen bonds are formed between the Q head and the catalytic residues. OBJECTIVES: Here, we aim to correlate the Q/QH2 head binding position with the protonation state of the catalytic residues H59, Y108 and D160. MATERIALS AND METHODS: We applied classical molecular dynamics simulations with sampling enhanced by metadynamics to explore the activation mechanism and protonation states of catalytic residues before and after the reaction, corresponding to reactant and product (Michaelis) complexes. DISCUSSION AND RESULTS: Free energy profiles indicate that Q binding is modulated by the charge-states of catalytic residues. Tyr, His and Asp should be in neutral form in the experimental pre-reactive state. After the product is formed, Tyr should be re-protonated before QH2 can efficiently dissociate and leave the active-site. CONCLUSION: The agreement found here between our simulations and experimental results suggests that we are approaching a fundamental understanding of Q binding and reactivity in this essential enzyme.
Keywords: Electron transport chain, Molecular dynamics, Free energy methods / **Supported by:** FAPESP

D.40 - Interaction of Sulfasalazine with Human Serum Albumin: a theoretical (TD-DFT) and experimental (ECD) approach

Valdecir Farias Ximenes[1], Nelson Henrique Morgon[2], Maurício Ikeda Yoguim[1], **Aguinaldo Robinson de Souza[1]**
[1]Chemistry, São Paulo State University (São Paulo, Brasil), [2]Physical-Chemistry, Campinas State University (São Paulo, Brasil)

INTRODUCTION: Sulfasalazine, a conventional drug, is widely utilized in treating inflammatory diseases, rheumatoid arthritis, and Crohn´s disease. This drug induces ferroptotic cell death in head and neck cancer and promotes human glioblastoma cell death through the intracellular oxidative response imbalance. Therefore, the study of the interaction of sulfasalazine with human proteins is a required subject. Human Serum Albumin (HSA) is the most abundant protein in the human blood, approximately 30 – 50 g/L. It is responsible for transporting hormones, fatty acids, drugs, and other compounds, among other functions. OBJECTIVES: We present initial results about the interaction of the drug sulfasalazine and HSA from a theoretical (TD-DFT) and experimental (ECD) approach in the search for the interaction site MATERIALS AND METHODS: The electronic circular dichroism spectra of SFZ (30.0 mmol L-1) were recorded in the absence and presence of HSA at 250 C. The electronic circular dichroism spectra were recorded in a Jasco J-815 spectropolarimeter equipped with a thermostatically controlled cell holder. The theoretical ECD spectra were obtained at the CAM-B3LYP/6-311++G(2d,p) TD(FULL,SINGLET,NSTATES=15) level of theory. The RMSD between the structures inside the protein with PDB ID 6r7s was obtained using the Discovery Studio Visualizer software.DISCUSSION AND RESULTS: The higher value of the RMSD showed that the three structures have different conformations in the DS1, DS2, and DS3 sites in HSA, as well as the theoretical ECD spectra. The experimental ECD spectra showed two prominent bands at ~325, and ~440 nm; these findings were corroborated by the theoretical calculation. CONCLUSION: The experimental and theoretical ECD spectra of SFZ in the presence of HSA are in good agreement when considered that the ligand are positioned at DS1 site.
Keywords: Sulfasalazine, Human Serum Albumin, Electronic Circular Dichroism

D.41 - Computational Design of Protein-based Inhibitors for Blocking the Dimerization Process of the Main Protease of SARS-CoV-2

Emerson Goncalves Moreira[1,2], Bruno Henrique de Sousa Leite[2], Danilo Fernandes Coêlho[1,2], Carlos Henrique Bezerra da Cruz[2], Roberto Dias Lins[1,2]
[1]Department of Fundamental Chemistry, Federal University of Pernambuco (PE, Brazil), [2]Aggeu Magalhães Institute, Oswaldo Cruz Foundation (PE, Brazil)

INTRODUCTION: The main protease (Mpro) of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a key enzyme for the cleavage process of the viral polyprotein and has a catalytic activity as a homodimer. As a consequence of its conserved and vital role in the replication cycle of the virus, Mpro has been used as a target for drug development. The Mpro has two main sites for drug targeting: 1) the catalytic pocket and 2) the dimerization interface. OBJECTIVES: Our goal is the computational design of protein-based inhibitors that bind to the dimerization interface with high binding affinity and block the formation of the active catalytic structure of SARS-CoV-2 Mpro. MATERIALS AND METHODS: The design protocol of potential protein-based inhibitors was carried out in the following steps: 1) identification of a key motif with high binding affinity for homodimer formation; 2) MotifGraft protocol for transplanting the key binding motif to a carrier protein; 3) protein interface design to optimize binding affinity; 4) sequence design of the scaffold based on phylogenetic constraints. Protein-based inhibitors designed were selected based on protein-protein interface metrics, folding, and binding energy. All design steps were carried out in the Rosetta software. We performed molecular dynamics (DM) simulations of the protein-based inhibitors isolated and complexed with Mpro to assess the stability of these systems. DISCUSSION AND RESULTS: In this study, we obtained 15 protein-based inhibitors designs that bind to the Mpro dimerization interface, with binding energies ranging from -53 to -71 REU (Rosetta Energy Unit), which have potential for nanomolar-order affinity constants. Our analysis of DM simulations supports that the protein-based inhibitors designs are stable proteins and behave similarly to the native carrier proteins. CONCLUSION: These stable designs provide starting points for the development of therapeutic agents capable of blocking the dimerization process of Mpro SARS-CoV-2. Experimental binding affinity measurements between selected designed proteins and the SARS-CoV-2 Mpro enzyme are underway.

Keywords: Protein Design , Protease, Inhibitors / **Supported by:** Fiocruz, FACEPE, CAPES, and CNPq.

D.42 – *In vitro* Antiviral Activity and FRET-Based Inhibition of SARS-Cov-2 N-NTD Double-Stranded RNA Unfolding Activity by Computationally Identified Natural Compounds

Isabela Brunozi de Oliveira [1,3], Peter Reis Bezerra[2,3], Victor Augusto Roncaglia Pereira[2], Marcella Moreno Caldeira[2], Vanessa Lopes de Azevedo Braga[2], Andrea Cheble do Oliveira[2], Andre Marco de Oliveira Gomes[2], Fátima Pereira de Souza[1], Fabio Ceneviva Lacerda de Almeida[2,3], Ícaro Putinhon Caruso[1,2,3]
[1]CMIB e Depto de Física, Universidade Estadual Paulista (São Paulo, Brasil), [2]IBqM e CENABIO, Univ. Federal do Rio de Janeiro (Rio de Janeiro, Brasil), [3]Rio BioNMR Network, Centro Nac. de Ressonância Magnética Nuclear (Rio de Janeiro, Brasil)

INTRODUCTION: The antiviral drugs currently available against SARS-CoV-2 mostly target the spike protein (S) or viral proteases, being ineffective due to the frequent mutations that the S protein presents or because they cause cell toxicity when focused on the proteases. On the other hand, the viral nucleocapsid (N) protein is the most abundant protein in infected cells and antibodies produced against it have greater persistence and specificity. OBJECTIVES: The computational selection of natural compounds that target the N-terminal domain of the SARS-CoV-2 nucleoprotein and a subsequent evaluation of the inhibition of the double-stranded RNA unfolding activity of the N-NTD promoted by these ligands through FRET experiments and plaque assays. MATERIALS AND METHODS: A virtual screening of 270,530 natural compounds taken from the ZINC15 database was performed, with subsequent docking calculations, molecular dynamics simulations, FRET experiments and plaque assays. DISCUSSION AND RESULTS: A final computational selection of 17 natural compounds with higher binding potential to the N-terminal domain of the N protein (N-NTD) of SARS-CoV-2 was obtained. 15 of these underwent FRET experiments in which they showed inhibition of the double-stranded RNA unfolding activity promoted by N-NTD. Plaque assays were performed with 16 of the 17 selected compounds and showed an inhibition in the viral replication for most of them. CONCLUSION: The results obtained highlight the method's capacity to facilitate the identification of potential drugs and reduce costs with experimental approaches.

Keywords: SARS-CoV-2 nucleoprotein, N-terminal domain, natural compounds / **Supported by:** CNPq, CAPES, FAPESP, FAPERJ, INCT, Finep

D.43 - Conformational and Energetic Analysis by Molecular Docking of Mycotoxins with α-Hemolysin Protein Nanopore

Maria Isabel dos Santos Cavalcanti [1], Débora Brígida Moura de Freitas[1], Catarina Victoria Nascimento de Oliveira[1], Artur Alves Rodrigues da Silva[1], Claudio Gabriel Rodrigues[1]
[1]Biofísica e Radiobiologia, Universidade Federal de Pernambuco (Pernambuco, Brasil)

INTRODUCTION: Mycotoxins (MTXs) are toxic secondary metabolites produced by fungi of the phylum *Ascomycota*. They are able to affect specimens of food crops, impacting the health of animals due to contamination of grains and cereals, as well as human health, causing mycotoxicosis. The techniques for detecting, quantifying and discriminating these toxins are time-consuming and expensive. OBJECTIVES: In this research, a predictive analysis of MTXs interaction with a protein nanopore of α-hemolysin (PN) from *Staphylococcus aureus* was performed through a receptor-ligand molecular docking. MATERIALS AND METHODS: The analyzed ligands were ochratoxin A (OTA) and fumonisins B1 (FB1), B2 (FB2) and B3 (FB3), obtained from PubChem. The energy was minimized by Avogadro 1.1.3. Furthermore, the crystallographic structure of α-hemolysin PN was obtained from the Protein Data Bank (PDB), code 7AHL, and the solvent removed. The molecular docking was performed using Dockthor and GOLD (in duplicate), both with the same PN coordinates (Å): 39,188, 32,853 and 31,429 for X, Y and Z. The results were analyzed in Discovery Studio Visualizer, generating a graphic representation of the receptor-ligand in addition to a 2D diagram of interactions. DISCUSSION AND RESULTS: The Dockthor results showed affinity scores (Kcal/mol) of -6,288, -6,786, -7,289 and -7,156 for OTA, FB1, FB2 and FB3, respectively. It also displays many hydrogen interactions for all molecules, beyond pi-sulfur, alkyl, salt bridges and charge attraction interactions. The average of the GOLDScore Fitness (Kcal/mol) results were: 32.1 (OTA), -61.36 (FB1), -62.02 (FB2) and -38.93 (FB3) with less hydrogen interactions. CONCLUSION: Mycotoxins anchor in the constriction region of the α-hemolysin nanopore forming molecular complexes stabilized mainly by hydrogen interactions.

Keywords: molecular docking, mycotoxins, α-hemolysin
Supported by: FACEPE

D.44 - Structural study of biological targets COX-1, COX2 and TMEM176B involved in inflammatory processes of Covid-19

Simone Pantaleão [1], Eric Philot[1], Angelo Magro[2], Ana Ligia Scott[1]
[1]Centro de Matemática, Computação e Cognição, Universidade Federal do ABC (São Paulo, Brasil), [2]Faculdade de Ciências Agronômicas, Universidade Estadual Paulista "Júlio de Mesquita Filho" (São Paulo, Brasil)

INTRODUCTION: Patients infected with SARS-CoV-2 may develop a phenomenon commonly called “cytokine storm”, which is characterized by a high production of inflammatory molecules during the course of COVID-19 infection.There are different potential targets related to the infection and inflammatory process related to SARS-CoV-2.The proteins Cyclooxygenase-1 (COX-1), Cyclooxygenase-2 (COX-2) and Transmembrane Protein 176B (TMEM176B) are mainly related to inflammatory processes in the human organism. OBJECTIVES: The analysis of structural dynamics and conformational space of the COX-1, COX-2, TMEM176B was performed with Hybrid methods aiming for a better understanding of these possible receptors and their mechanism. MATERIALS AND METHODS: The systems investigated were: COX-1, COX-2 and TMEM176B (wild-type, PHE203 mutated by ALA, ALA134 by phosphorylated THR). For the Cox-1 and Cox-2, we calculated the ANM (Anisotropic network model) normal modes of vibration in the Anisotropic Network Model 2.1 Web Server. For TMEM176B, we built the nuclear membrane in CHARMM GUI, equilibrated the system and calculated the normal modes with all atoms. The conformational space was sampled with Molecular Dynamics with Normal Modes Excited and the stability and transition of these metastases was investigated. DISCUSSION AND RESULTS: The systems investigated were: COX-1, COX-2 and TMEM176B (wild-type, PHE203 mutated by ALA, ALA134 by phosphorylated THR). For the Cox-1 and Cox-2, we calculated the ANM (Anisotropic network model) normal modes of vibration in the Anisotropic Network Model 2.1 Web Server. For TMEM176B, we built the nuclear membrane in CHARMM GUI, equilibrated the system and calculated the normal modes with all atoms.The conformational space was sampled with Molecular Dynamics with Normal Modes Excited and the stability and transition of these metastases was investigated. CONCLUSION: We were able to assess the functional motions of the proteins of interest, which aids in comprehending their function and will be useful for the drug design phase of drug design.

Keywords: COX-1, COX-2, TMEM176B / **Supported by:** CNPq

D.45 - Using mesoscopic models to identify intra-molecular interactions in metal mediated DNA

Luciano Gabriel Silva [1], Gerald Weber[1]
[1]Departamento de Física, Universidade Federal de Minas Gerais (Minas Gerais, Brasil)

INTRODUCTION: Mismatch is a DNA defect that occurs when two non-complementary bases (different from AT and CG) like TT and CC are aligned. Metal mediated DNA has metal ions bridging the base pairs. TT and CC mismatches in the presence of Hg2+ and Ag+, respectively, form this type of structure. OBJECTIVES: Here, we study, T-Hg2+-T which is known to be stabilized by hydrogen bonds, and C-Ag+-C, whose stabilization is partially understood. The main goal is to determine if the interactions are direct (base-metal-base) or inter-planar (metal interacts with the adjacent bases as well). MATERIALS AND METHODS: We use mesoscopic models and published melting temperatures of sequences containing CC or TT mismatches in the presence and absence of the metal ions. The nearest-neighbor model was used to normalize the salt and duplex concentrations extracted from different articles. The Peyrard-Bishop model was used to determine the values of stacking and hydrogen bonds, and to determine if the interactions are direct or inter-planar. First we found the best set of parameters, stacking and hydrogen bond, for the sequences without the metal ion. To find the best set of parameters for the metal dataset, we grouped the sequences in the presence and absence of metal. The sequences without metal acts as reference, while we only calculate the parameters of the metal sequences. DISCUSSION AND RESULTS: For T-Hg2+-T we found a large hydrogen bond value, 56 meV, which is approximately 90% of a CG bond (70 meV). While for C-Ag+-C we found a weaker hydrogen bond, 22 meV, that is roughly 66% of a AT bond (34 meV). However, the metal mediated stacking interactions are mostly very similar to non-metal DNA. Only two stacking configurations are larger than usual. CONCLUSION: CC-CC and TT-TT stacking in the presence of metal ions are very weak, which exclude inter-planar interactions. Therefore, the main interaction is direct.
Keywords: Metal-mediated DNA, metal-base-pair, Peyrard-Bishop DNA model / **Supported by:** CNPq, Capes, Fapemig

D.46 - Antimycobacterial activity prediction using machine learning and molecular docking: a ligand and structure-based virtual screening approach.

Diego de Souza Lima [1], Maria Aparecida Fernandez[2], Flávio Augusto Vicente Seixas[1]
[1]Technology, Univ. Estadual de Maringá (PR, Brazil), [2]Biotechnology, Genetics and Cell Biology, Univ.e Estadual de Maringá (PR, Brazil)

INTRODUCTION: Tuberculosis (TB), a disease caused by the gram-positive bacteria Mycobacterium tuberculosis (Mtb), remains a major cause of death worldwide. An estimated 1.3 million TB deaths were reported in 2020, and more than 150,000 were caused by drug-resistant TB. Therefore, there is an urgent need for novel treatments. Computational methods are an interesting approach to finding new candidates, due to their relatively low-cost and less time required in comparison with traditional methods. Recently, machine learning (ML) has also become a promising method for molecular activity prediction. OBJECTIVES: Our work had the goal of combining a ligand-based method, using ML models, and a structure-based method, using molecular docking, to virtually screen libraries of natural compounds for novel antimycobacterial candidates. MATERIALS AND METHODS: Molecules tested against Mtb were obtained from ChEMBL and were labeled as “active” or “inactive” based on a pMIC threshold of 7, resulting in 6,242 active and 8,743 inactive molecules, which were used to train models with different ML algorithms. Ligands were encoded as Morgan fingerprints. For molecular docking, the target was enoyl-ACP reductase (inhA), and its structure obtained from PDB Database (2X23). Docking protocols were validated by redocking the crystallographic ligand using Autodock Vina. 177,213 natural compounds were virtually screened. DISCUSSION AND RESULTS: Hyperparameters were selected by 10-fold cross-validation. Support Vector Machines was the chosen algorithm, achieving 0.89 accuracy and 0.85 F1-score on the test set. After screening, 5,364 molecules were predicted as actives, which were docked against inhA. 6 molecules with docking scores and binding modes similar to the crystallographic ligand were found and will be further investigated. CONCLUSION: Natural compounds were virtually screened in search for novel antibacterial candidates. Starting from 177,213 molecules, 6 were selected for further in silico (molecular dynamics simulations) and *In vitro* (growth inhibition assays) studies, to validate our results and contribute to the discovery of novel antimycobacterial natural compounds.
Keywords: Machine learning, Drug discovery, tuberculosis
Supported by: CNPq,Capes and Fundação Araucária

D.47 - Virtual Screening Of Novel Human 15-Lipoxygenase-2 (15-LOX-2) Inhibitors

Thais Satie Iijima [1], Lucas Viviani[1], Antonia Tavares do Amaral[2], Sayuri Miyamoto[1]
[1]Bioquímica, Instituto de Química, Univ. de São Paulo (SP, Brasil), [2]Química Fundamental, Instituto de Química da Univ. de São Paulo (SP, Brasil)

INTRODUCTION: 15-Lipoxygenase-2 (15-LOX-2) converts arachidonic acid into 15-hydroperoxyeicosatetraenoic acid. Human 15-LOX-2 is overexpressed in atherosclerotic plaques, and its activity is linked to the differentiation of macrophages into foam cells. Despite its relevance as a biological target for atherosclerosis and other diseases, only few 15-LOX-2 inhibitors/activators have been reported. OBJECTIVES: To search for novel potential human 15-LOX-2 inhibitors, using a virtual screening (VS) approach. MATERIALS AND METHODS: The structures of two known 15-LOX-2 inhibitors were used to build a shape-based model ("query"), using ROCS. The generated "query" was applied to the ZINC (~8x10^6 compounds), ZDD (~1,5x10^3 compounds), and Pathogen Box (400 compounds) databases. Next, selected compounds were docked into the 15-LOX-2 active site, using GOLD. Finally, a visual inspection of the predicted binding modes was performed, using LIGANDSCOUT. DISCUSSION AND RESULTS: In the shape-based screening step, 1037, 404, and 100 compounds with the highest shape-Tanimoto values (0.422-0.565) were selected from ZINC, ZDD, and Pathogen Box, respectively. The compounds were subsequently docked into the 15-LOX-2 active site, and those with the highest docking score values were submitted to a visual inspection analysis of their predicted binding modes. The following criteria were applied: (i) presence of hydrophobic contacts with at least 5 active site residues; (ii) presence of at least one hydrogen-bond and/or another polar interaction with an active site residue or the catalytic Fe2+; (iii) docking "poses" reproducibility; and (iv) surface complementarity with the protein binding site. Based on those criteria, 39, 28, and 17 compounds were selected (originally from ZINC, ZDD, and Pathogen Box, respectively), which represents ~99.9%, ~98.1%, and ~95.8% reduction in the number of compounds from each database. CONCLUSION: By combining shape-based screening and docking approaches, our VS protocol has selected 84 structurally diverse compounds as human 15-LOX-2 inhibitor candidates, from which 32 are being acquired and tested in enzymatic assays for VS experimental validation.

Keywords: 15-lipoxygenase-2 (15-LOX-2), virtual screening, inhibitors / **Supported by:** CAPES, FAPESP.

D.48 - Exploring Structural Dynamic Signatures in Intrinsically Disordered Proteins

André Kliousoff Junior [1], Angelica Nakagawa Lima[2]
[1]Programa de Pós-Graduação em Engenharia de Informação, Universidade Federal do ABC (SP, Brazil), [2] Laboratório de Genética e Cardiologia Molecular, InCor/HCFMUSP, Universidade de São Paulo (SP, Brazil), [3]Centro de Matemática, Computação e Cognição, Universidade Federal do ABC (SP, Brazil)

INTRODUCTION: There is a class of proteins that can be found in the form of intrinsic disorder proteins (IDPs) or protein hybrids that contain both intrinsically disordered protein regions (IDPRs) and ordered regions. IDPs and IDPRs are found in all organisms and, normally, play vital roles in various biological processes. A better understanding of these proteins and their functional importance is an important challenge. OBJECTIVES: Propose an unprecedented protocol to study of the evolution of structural dynamics in IDPs and to analyze the structural dynamics signature of IDPs and compare the results with non-disordered proteins. MATERIALS AND METHODS: We combine existing databases and tools such as Disprot, CATH, PDB and SignDy. The combination of this data was stored in a new SQL-based database. From this dataset three CATH superfamilies were selected. We also defined datasets with non-disordered structures for each of three superfamilies and each containing the same amount of structures from its respective disorder set. The protein dynamics data from these sets were processed using Elastic Network Models through SignDy. DISCUSSION AND RESULTS: We obtained the signature data, mode conservation, mode collectivity and spectral overlap data. The results indicated that the conservation, collectivity and upper spectral overlap for global and collective movements in IDPs may be related to the larger conservation behavior of residues in IDPs. CONCLUSION: The results indicate that there are different dynamic patterns in disordered proteins when compared to structures of the same structural superfamily and that such patterns that differentiate them are repeated in the three analyzed sets. The proposed protocol is effective and, when automated and turned into an open solution, it may become an integrative tool for the study of dynamics in disordered proteins.

Keywords: dynamics signature, Intrinsically disordered proteins, molecular dynamics / **Supported by:** UFABC

D.49 - Structural Proteome for Pseudomonas aeruginosa's Multidrug-Resistant Strain CCBH4851

Camila Rodrigues Chaves [1], Pedro Henrique Monteiro Torres[1]
[1]Insituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: It is well established that changes in the protein sequences that impact their structures may lead to a gain or loss of function. Resistance to antimicrobial drugs is an example of a gain of function observed in many bacterial strains and poses a critical healthcare issue. In Brazil, a Pseumononas aeruginosa's multidrug-resistant strain (CCBH4851) had its genome and proteome sequenced by Silveira, M. et.al, 2008. However, the 3D structures for most of these proteins are still lacking. In the last decades, computational methods to predict protein's 3D structure have been widely employed, and provides us with a way to generate data that can be employed in computer-aided drug design. OBJECTIVES: Thus, the aim of this project was to identify interesting targets for CCBH4851 through metabolic network analysis and through the modeling and analysis of the 3D structures of its entire proteome. MATERIALS AND METHODS: To accomplish that, we obtained the CCBH4851 proteome and built the models using ProtCHOIR, a software developed by our team which provides monomeric and homo-oligomeric structural models from sequences in FASTA format. ProtCHOIR relies on well-established tools such as PSI-Blast, MAFFT, PISA, Molprobity and MODELLER to construct models based on homologous sequence templates. In order to identify essential proteins for CCBH4851 metabolism, we accessed its metabolic map (available at http://pseudomonas.procc.fiocruz.br) and performed essential genes and choke point analysis using COBRApy library. DISCUSSION AND RESULTS: ProtCHOIR generated structural models for 5473 proteins, approximately two thirds of the whole proteome, from which 1901 are monomeric structures and 3474 are predicted homo oligomers. The models were submitted to druggability analysis to assess their potential as novel drug candidates. CONCLUSION: Finally, we identified around 10 proteins as being potential targets for drug design by means of metabolic network and druggability analysis.

Keywords: Proteome-scale modelling, metabolic network modelling, druggability
Supported by: FAPERJ, CNPq, CAPES

D.50 - Search for the Origins of Isomalto-Oligosaccharides Ring Distortion in Crystallographic Complexes

Vinicius Ávila Cabral [1], Hugo Verli[1]
[1]Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul (RS, Brasil)

INTRODUCTION: α-1,6-Isomalto-Oligosaccharides are short chain, digestion-resistant, carbohydrates considered as potential targets for dextran-based drug delivery systems. These molecules also show promise in bacteriophages in the use against multi-drug resistant bacteria. While the conformational preferences of the torsion angles from the α-1,6-isomalto-oligosaccharide isomaltotetraose have already been characterized by NMR and MD-simulation studies, on crystallographic complexes to glucoamylase we observe a distortion on its first and fourth residues rings in relation to its expected, solution state. OBJECTIVES: We aim to assess the structural determinants for ring distortion upon complexation to glucoamylase, so we can have a broader view of its behavior to provide possible aid to drug design strategies. MATERIALS AND METHODS: In order to mimic the crystallographic environment and to include electronic effects on hexopyranose ring conformational preferences and so circumvent possible force field parameterization limitations, metadynamics simulations of the ring puckering of a isomalto-oligosaccharide in both complexed and uncomplexed states were performed in vacuum employing QM/MM simulations methods in the CHARMM36m force-field, using the Cremer-Pople puckering coordinates as collective variables. We also performed metadynamics using classical mechanics (MDMC) and the same parameters. DISCUSSION AND RESULTS: Preliminary data of QM/MM presented us a free energy landscape (FEL) in which the distorted residue adopt a skew conformation as its most stable conformation. In comparison, the uncomplexed sugar promptly adopts a chair conformation as its more stable structure. MDMC provided similar results although with a less detailed description of the FEL. These results show that the ring torsions observed in the crystallographic complex are probably due to crystal packaging effects. CONCLUSION: This preliminary result reveals how QM/MM metadynamics could be used for the purpose of exploring the conformational itineraries of sugar rings in more detail when compared to MDMC. Therefore, we aim to expand this method further to other carbohydrates and ligands.

Keywords: QM/MM, metadynamics, conformational analysis / **Supported by:** CAPES

D.51 - Investigating the potential cofactor binding of Sox9 - a transcription factor involved in pancreatic development.

Zeyaul Islam [1], Prasanna R Kolatkar[2]
[1]Diabetes, Qatar biomedical research institute (, Qatar), [2]Diabetes, Qatar biomedical research institute (, Qatar)

INTRODUCTION: Induced pluripotent stem cell (iPSC) development is a key process being studied widely due to the potential for modulating these pathways towards specific differentiation outcomes such as cardiac or pancreatic tissues.Among the TFs the family of Sox proteins plays significant roles in diverse pluripotency and differentiation programs despite having highly conserved sequences within this family. Sox9, a member of the SoxE family, is known to be important in pancreatic development. Dimerization of Sox9 was observed to be important for its biological function and A76E mutation was shown to disrupt dimerization and its function. OBJECTIVES: We investigated the cofactor binding in the purified protein and its implication in Sox9 function. MATERIALS AND METHODS: We have clone the constructs in expression vector, expressed and purified it. Purification was carried out by affinity as well as gel filtration chromatography. Site directed mutagenesis by primer extension was employed to introduce mutation at specific position. Uv-Vis spectroscopy was employed to understand the binding of cofactor. Identification was confirmed by mass spectrometry. DISCUSSION AND RESULTS: We have purified Sox9 HMG plus N-terminal construct (1-173) using affinity and gel filtration chromatography. After purification, surprisingly, we observed a clear yellowish color. We measure the Uv-Vis spectra and it shows a lamda max around 420 nm (yellow range of visible light). Mass spectrometry suggests the binding of FAD. Several mutations in the HMG domain as well as dimerization domain highlights the unique nature of Sox9. CONCLUSION: We present here a unique cofactor, Flavin Adenine Dinucleotide (FAD), which interacts with Sox9 and promotes pancreatic progenitor development. Using a combination of data from mass spectrometry of purified Sox9, as well as absorbance spectroscopy and several mutations of FAD binding residues, we show the mechanism of Sox9-FAD binding.

Keywords: Sox9, Transcription factor, Stem cell / **Supported by:** NPRP

D.52 - Energy Landscape of Amyloid-β Oligomers Simulations

Murilo Nogueira Sanches [1], Kaitlin Knapp[2], Rafael G. Viegas[1], José Nelson Onuchic[2], Peter G. Wolynes[2], Vitor Barbanti Pereira Leite[1]
[1]Department of Physics, São Paulo State University (UNESP), Institute of Biosciences, Humanities and Exact Sciences (São Paulo, Brasil), [2]Center for Theoretical Biological Physics, Rice University (Texas, United States)

INTRODUCTION: Amyloid-βs (Aβ) are intrinsically disordered peptides that appear with 40 or 42 residues, and that are able to aggregate leading to Alzheimer's disease. This mechanism however it's still not clear, with studies showing that the shortest and dominant Aβ40, seems to play a more important role in later stages of plaque formation, as evidenced by a strong correlation between the detection of Aβ40 and the maturity of these plaques. OBJECTIVES: The main objective of this work is the study of the energy surfaces of Aβ aggregation simulations, as well as the conformational changes they undergo. MATERIALS AND METHODS: This work analyzed the coarse-grained AWSEM simulations of Aβ40 and Aβ42 monomers, along with Aβ40 dimers, trimers and tetramers. The dynamics were performed at five temperatures and examined using the Energy Landscape Visualization Method (ELViM), a new methodology that does not need a specific reaction coordinate to analyze energy surfaces. DISCUSSION AND RESULTS: Results showed that for Aβ40 aggregation to occur, it needs first to assume a prefibrillar state, which corresponds to the dimer's formation. From this, a restriction of the phase space occurs within the trimers, which leads to the formation of fibers as tetramers. We can also indicate the most likely path to the formation of fibers in each case, as well as the amino-acid contacts characterizing each ensemble. CONCLUSION: Through the results, we were able to understand the conformational change mechanism driving the aggregation process, as well as confirm the need for a prefibrillar state for the Aβ 40 to assume its fiber conformations.

Keywords: Energy Landscape Theory, Amyloid-β, Fiber formation / **Supported by:** CAPES

D.53 - Study of atypical structures on protein energy landscape visualization

Juliana Bueno de Camargo [1], Murilo Nogueira Sanches[1], Rafael Giordano Viegas[2], Vitor Barbanti Pereira Leite[1]
[1]Departamento de Física, Unesp - Instituto de Biociências , Letras e Ciências Exatas (São Paulo, Brasil),
[2]Departamento de Física, Instituto Federal de Educaçao, Ciencia e Tecnologia de Sao Paulo (São Paulo, Brasil)

INTRODUCTION: The protein folding process is a problem that explains how a protein reaches its native and functional state. The energy landscape theory, an approach based on principles of statistical mechanics, led to the accepted description known as the protein folding funnel. However, a detailed analysis of the high-dimensional phase space is challenging. The energy landscape visualization method, ELViM, is a candidate for tackling this problem. Nevertheless, rare conformations in the data set seem to hinder the performance of the method. OBJECTIVES: The main objective of this work is to study the energy surfaces of the SH3 domain and protein A using the ELViM, as well as to identify the atypical structures that appear on these surfaces. MATERIALS AND METHODS: This work analyzed simulations of the SH3 domain of tyrosine kinase c-Src and the protein-A obtained with a structure-based model. The dynamics were performed at the folding temperature of each system and examined using ELViM. DISCUSSION AND RESULTS: The results allowed the visualization of the energy landscape of the target proteins, identifying the parallel folding routes. However, in a two-dimensional representation, some configurations often behave as outliers, that is, they are significantly distant from all neighboring points in the 2D projection. Such "islands" may distort the overall landscape representation. Upon analysis, we observed no major difference between outliers and conformations around them, which indicates that these shapes arise in the energy surface due to a limitation of the multidimensional projection method. CONCLUSION: We were able to visualize the energy landscape of the SH3 domain and the protein-A, confirming their funnel-like aspect. The analysis of the structures found in island locations revealed that they do not have any singular characteristics, and it only occurs due to the multidimensional reduction method. It seems appropriate to introduce filters to avoid the appearance of large islands that may distort the energy landscape representation.

Keywords: Protein folding, Folding funnel, Energy Landscape
Supported by: Cnpq

D.54 - Molecular modeling of a drug target Mura enzyme in Rickettsia prowazekii

Shabir Ahmad[1,1], Hugo Verli[1,1]
[1]PPGBCM, Federal University of Rio Grande do Sul (Rio Grande do Sul, Brazil)

INTRODUCTION: Rickettsia prowazekii, the agent of epidemic typhus, is an obligate intracellular bacterium that is transmitted to humans through the body louse. UDP-N-acetylglucosamine 1-carboxyvinyltransferase (MurA) catalyzes the formation of peptidoglycans in R. prowazekii cell wall by adding enolpyruvyl to UDP-N-acetylglucosamine through an addition and elimination process. The peptidoglycan layer is a component of the bacterial cell wall that sits on top of the plasma membrane. As a result, disrupting their formation by inhibiting the MurA enzyme should result in a reduction in R. prowazekii cell synthesis OBJECTIVES: In this context, we intend to study the inhibitory or suitability effect of Fosfomycin drug against Mura enzyme MATERIALS AND METHODS: Since there's no crystal structure of this enzyme for R. prowazekii therefore, to explore its exact structural properties, we used a comparative modeling approach to estimate the three-dimensional (3D) structure using the template (PDB ID 1UAE). Based on this strategy, we have designed this study where the substrate from the template PDB file was first docked using DockThor against our modeled MurA structure and then refined the docking result through molecular dynamic simulations DISCUSSION AND RESULTS: Triplicate molecular dynamics (MD) simulations of R. prowazeki Apo-MurA and Holo-MurA exhibited stability and useful insights into the folding pattern. MD simulations of the Apo-MurA and Holo-MurA revealed identical folding patterns and trajectories. Throughout the 500 ns simulations, the MurA enzymes remained compact and stable. CONCLUSION: The results of the analysis revealed that the both Apo and Holo-MurA triplicate systems show stability during the 500ns MD simulation and the preliminary results show that the complex is stable and the ligand did not dissociate during the 500ns duration. Further we see a little conformational change in the loop of the enzyme located at the active site as we still did not dock and simulate the drug Fosfomycin in the enzyme which is the perspective of our work

Keywords: MurA, Modeling, Molecular dynamic simulation / **Supported by:** CAPES

D.55 - Prediction of Protein-Peptide Docking Viability by a Convolutional Neural Network Aproach

Ramon Hernany Martins Gomes [1], Rafael Plana Simões[1], Lucas Hecker Vasques[1], Angelo José Magro[1]
[1]Departamento de Bioprocessos e Biotecnologia, Faculdade de Ciências Agronômicas, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP) (São Paulo, Brasil)

INTRODUCTION: Several softwares for molecular docking are available and have been used for research purposes. The main problem of the docking techniques is the low correlation between the docking solution scores and the energies empirically determined. In addition, it should be considered that the classical methodologies do not allow inference about the viability (or existence) of the docking solution in a binary way: "EXIST" or "NOT EXIST". OBJECTIVES: The main aim was to develop a method for validating the molecular complexes inferred by conventional docking methods using a Convolutional Neural Network (CNN). MATERIALS AND METHODS: The initial development was focused only on the protein-peptide complex Nuclear Localization Signal (NLS)/Importin-alpha (Impα). Two datasets were created: one containing experimentally validated NLS/Impα complexes and the other containing molecular structures with a low probability of forming complexes. This set of instances was expanded using molecular dynamics simulations and converted into two-dimensional images that contain relevant information about the molecular complex interaction. These images constituted the training and test sets. The training set was applied for inference of a CNN model aiming the binary prediction (EXIST or NO EXIST) of solutions obtained by conventional docking methods. Metrics of predictive performance of the trained CNN on the test set were determined through the variables from the confusion matrix. DISCUSSION AND RESULTS: The accuracy and loss functions of the training and validation sets (referring to cross-validation) showed good convergence, revealing that the measured CNN has good generalization capacity. The performance metrics were: Matthews Correlation Coefficient = 0.7999, Accuracy = 0.8892 and F1 score = 0.8784. These results indicate that the images used to train the neural network successfully encoded relevant information about the molecular complex interactions. CONCLUSION: The results show that the measured CNN has high predictive performance and can be applied to infer the feasibility of molecular docking results.
Keywords: proteins interaction, protein-peptide docking, deep neural network
Supported by: FAPESP

D.56 - Screening of antivirals by mapping of potential binding sites in the Dengue Virus capsid thought Structure-based Drug Design approach

Philipe de Oliveira Fernandes [1], Marcelo Andrade Chagas[2], Vinícius Gonçalves Maltarollo[1], Adolfo Henrique de Moraes Silva[3]
[1]Depto de Produtos Farmacêuticos - FaFar, Universidade Federal de Minas Gerais (MG, Brasil), [2]Depto de Ciências Exatas, Univ do Estado de Minas Gerais (MG, Brasil), [3]Depto de Química - ICEx, Universidade Federal de Minas Gerais (MG, Brasil)

INTRODUCTION: Dengue fever is a mosquito-borne prominent tropical disease caused by the Dengue Virus without a specific antiviral or vaccine against it. The glycoprotein E, the main component of the viral particle, plays an essential role in the host cellular recognition/infection, and, due to its exposure and structural and functional roles, is a prominent target for developing antivirals. OBJECTIVES: Identify potential antivirus against DENV using a structure-based approach targeting glycoprotein E. MATERIALS AND METHODS: A trimeric binding site was identified and extracted from the DENV protein viral particle surface (PDB 3J27), edited, and submitted to 10 ns molecular dynamics in AMBER16. Then, four frames from the trajectory were chosen to be mapped using FTMap, FTSite, and molecular interactions fields (Autogrid). Those results were used to build a pharmacophoric model with UNITY/SYBYL-X to filter molecules in NuBBE, DrugBank, and ZINC drug-like databases. Finally, two molecular dockings runs (FRED) were performed for each database, one using the pharmacophore restriction and the other without restrictions. DISCUSSION AND RESULTS: In this study, critical residues for ligand interaction with protein E in the particle surface were identified. Based on the interaction frequency, residues MET297, SER298, and GLU172 were chosen as the pharmacophore model used to filter the databases. The different Docking runs resulted in a distinct rank of hit compounds for the DrugBank (190) and ZINC (4,097). CONCLUSION: Additional binding sites in glycoprotein E were identified when a quaternary structure of the DENV surface was used. This result shows the importance of using new cryo-EM structures for the Structure-Based antivirus design approach. The important residues for molecular interactions were identified, including the LYS291, an essential residue whose role in DENV host recognition and infection was experimentally observed. Finally, the hits identified by docking simulations will be further evaluated.
Keywords: Dengue virus, protein E, antiviral / **Supported by:** CNPq, CAPES e FAPEMIG

D.57 - PyAutoFEP: An Automated Free Energy Perturbation Workflow for GROMACS Integrating Enhanced Sampling Methods

Luan Carvalho Martins [1], Elio Anthony Cino[2], Rafaela Salgado Ferreira[2]
[1]Graduate Program in Bioinformatics. Institute for Biological Sciences., Federal University of Minas Gerais (MG, Brazil), [2]Biochemistry and Immunology Department, Institute for Biological Sciences, Federal University of Minas Gerais (MG, Brazil)

INTRODUCTION: Free energy perturbation (FEP) calculations are now routinely used in drug discovery to estimate the relative FEB (RFEB) of small molecules to a biomolecular target of interest. Using enhanced sampling can improve the correlation between predictions and experimental data, especially in systems with conformational changes. Due to the large number of perturbations required in drug discovery campaigns, the manual setup of FEP calculations is no longer viable. OBJECTIVES: Develop and validate an automation tool for setup, run and analysis of FEP calculations. MATERIALS AND METHODS: PyAutoFEP is a flexible and open-source tool to aid the setup of RFEB FEP, written in Python3. The program automates the generation of perturbation maps, dual topologies, and system building and running of molecular dynamics, and analysis of results. PyAutoFEP supports multiple force fields, incorporates Replica Exchange with Solute Scaling (REST2) enhanced sampling method, and allows flexible λ values along perturbation windows. DISCUSSION AND RESULTS: To validate PyAutoFEP, it was applied to a set of 14 Farnesoid X receptor ligands. An 88% mean correct sign prediction was achieved, and 75% of the predictions had an error below 1.5 kcal/mol. Results using Amber03/GAFF, CHARMM36m/CGenFF, and OPLS-AA/M/LigParGen had Pearson's r values of 0.71 ± 0.13, 0.30 ± 0.27, and 0.66 ± 0.20, respectively. Applying REST2 improved the results using CHARMM36m/CGenFF (Pearson's r = 0.43 ± 0.21) but had little impact on the other force fields. CHARMM36-YF and CHARMM36-WYF modifications did not yield improved predictions compared to CHARMM36m. CONCLUSION: Our results demonstrate that PyAutoFEP is a useful tool for tackling drug discovery problems, and has gained a user community both from academia and industry since its release. The program is available on GitHub at https://github.com/lmmpf/PyAutoFEP. We are actively developing and adding new functionalities to the code.
Keywords: Free energy of binding, Free energy perturbation, Small molecule drug Discovery / **Supported by:** CAPES, FAPEMIG, and CNPq

D.58 - *In Silico* Analysis of Post-translational modifications and Molecular Dynamics of a-Syn protein variants associated with Parkinson Disease

Aloma Nogueira Rebello Da Silva [1], Gabriel Rodrigues Coutinho Pereira[1], Tiago Fleming Outeiro[2,3,4], Joelma Freire De Mesquita[1]
[1]Department of Genetics and Molecular Biology, Federal University of the State of Rio de Janeiro (Rio de Janeiro, Brazil), [2]Department of Experimental Neurodegeneration, University Medical Center Göttingen (Göttingen, Germany), [3]Translational and Clinical Research Institute, Newcastle University (, UK, [4]Experimental Neurodegeneration, Max Planck Institute for Experimental Medicine (, Germany)

INTRODUCTION: Parkinson's disease (PD) is a threatening neurodegenerative disorder that seriously affects patients' life quality. PD is characterized by typical motor symptoms that include resting tremor, postural instability, bradykinesia. 5-10% of the cases of PD have a familial origin, with mutations in the *SNCA* gene, which encodes the a-Syn. Several of a-Syn's residues are subject to post-translational modification (PTM), the most amply studied one being the phosphorylation of the 129 serine (S129). Studies have shown the relation between S129's phosphorylation and a-Syn's selective degradation, emphasizing this PTM's relevance. OBJECTIVES: In this work, we aim to evaluate the effects caused by mutations and PTM related to PD's in the a-Syn protein. MATERIALS AND METHODS: Effects of mutations on the protein function were predicted using 10 algorithms. Information about proteins interacting with human a-Syn were obtained from Cytoscape software using the interactome. The structural evolutionary conservation analysis of a-Syn were performed with the ConSurf algorithm. The PTM were predicted using 14 algorithms. The *in silico* mutagenesis was performed using VMD 1.9.3 mutator plugin. The molecular dynamics simulations (MD) were performed using the GROMACS 5.0.7 package with an AMBER99SB-ILDN force field and a TIP3P water cubic box. The production simulations were performed at 300K for the duration of 300ns. DISCUSSION AND RESULTS: The A30P mutation was predicted to be deleterious by all algorithms. The interactome showed an interaction with 109 proteins. MD analysis pointed to flexibility decrease at the N-terminal region of the analyzed variants and flexibility increase in their C-terminal regions compared to the WT. The secondary structure analysis suggested alterations in the G51D variant, particularly by increasing the number of β-sheets formed, could be increases its tendency to form toxic protein aggregates. CONCLUSION: This work suggests that mutations affects the structure and function of the protein, which could be related to the development of PD.
Keywords: Parkinson disease, in silico, alpha-synuclein / **Supported by:** CAPES, DAAD, FINEP, NVIDIA, CNPq, FAPERJ and UNIRIO

D.59 - An Algorithm For The Generation And Electronic Structure Calculations Of Complexes Between Amino-Acids And Copper Ions

André Luís Pesquero de Melo [1], Maurício Coutinho[1]
[1]Ciência e Tecnologia - Química, Universidade Federal do ABC (, Brazil)

INTRODUCTION: Considering the growing need to understand mechanisms of chemical reactions to increase their yield and efficiency under milder conditions, catalysts have a pivotal role. This study relevance is given by the fact that many biomolecules have potential catalytic activity with industrial applicability, so that in this project the properties of several sequences of amino acids and small peptides are studied using computer simulations. Studies were carried out on the generation of structures, thermodynamic properties, geometries and binding sites present in small sequences of amino acids and peptides when complexed with copper ions, in order to propose possible structures with catalytic activity for REDOX reactions. Among the properties studied are the redox potential, the energy of reorganization of different configurations of some amino acids and small copper complexing peptides, like GHK, DAHK and GGH in vacuum and aqueous solution. OBJECTIVES: Setup a semi-automatic procedure for structure generation and subsequent redox potential and reorganization energy calculation for copper peptides. MATERIALS AND METHODS: The methodology for generating structures in this project is done through a sequential protocol that employs the use of SMILES strings, generation of 3D structures, semi-empirical calculations and the Density Functional Theory in an ordered fashion. Initial structures were first optimized with the semi-empirical GFN2-xTB method and later a DFT method, the PBE functional, belonging to the GGA class (generalized gradient approximation) and the 6-31G(d,p) base, which provides results in good agreement with experimental data. DISCUSSION AND RESULTS: The results obtained reveal that the resulting geometries, like the square planar ATCUN-site, reorganization energies and redox potentials are in good agreement with experimental data CONCLUSION: We show here that the protocol used to produce the geometries of copper complexes with amino acids and peptides is efficient and amenable to automation. Data obtained from the calculations also converged when compared to experimental literature.

Keywords: copper, DFT, peptides / **Supported by:** CAPES

D.60 – Computational characterization of transthyretin-like hiuase and its evolution in vertebrates

Paulo Henrique G. dos Santos [4], Lucas Bleicher[1], José Fernando R. Bachega[3], Mariana Torquato Q. de Magalhães[4]
[1]Bioquímica e Imunologia, Universidade Federal de Minas Gerais (MG, Brasil), [2]Bioquímica e Imunologia, Universidade Federal de Minas Gerais (MG, Brasil), [3]Farmacociências, niversidade Federal do Rio Grande do Sul (SP, Brasil), [4]Bioquímica e Imunologia, Universidade Federal de Minas Gerais (MG, Brasil)

INTRODUCTION: Purine metabolism in humans results in the production of uric acid, which is excreted in urine. However, in some organisms, the pathway continues to the formation of (S)-Allantioin, and a known transthyretin (TTR) homolog has an important role by catalyzing the conversion of 5-Hydroxysourate (5-HIU) into 2-Oxo-4-hydroxy-4-carboxy-5-ureidoimidazoline (OHCU). Previous works showed that TTR and TTR-like proteins have an evolutionary history that included a duplication event followed by neofunctionalization. Older members of this protein family are hydrolases, converting 5-HIU to OHCU; a gene duplication during the evolution of vertebrates created a new class of proteins that binds to thyroid hormones (T3 and T4). Through the reconstruction of ancestral sequences, 3 points in the evolution of the TTR-like proteins were inferred, and three resurrected functional proteins were obtained. OBJECTIVES: This work will investigate the impacts of the evolution in the HIUase function of those TTR like proteins in vertebrates. MATERIALS AND METHODS: Using two available 3D structures 7KCN (Resurrected HIUase) and 2H1X (a modern HIUase from zebrafish), we will investigate their catalytic mechanism using QM/MM methods. Hybrid calculations will be performed using pDynamo/EasyHybrid, and a potential curve will be obtained. DISCUSSION AND RESULTS: The results on those calculations will be compared, and the catalytic properties and active site structures of both enzymes will be evaluated. To better understand the changes in the evolution between both proteins modeling of intermediate sequences can be performed for the investigation of catalytic properties in other points during their evolution. Simulations will be compared to experimental data from isothermal titration calorimetry. CONCLUSION: We expect to understand the importance of residue substitutions in the changes of the catalytic properties, and therefore the role of evolution in this protein family.

Keywords: Structural Bioinformatics, Evolution, Hybrid Calculations / **Supported by:** CAPES

D.61 - Surfactant-induced Unfolding of Tetanus and Diphtheria Anatoxins

Pedro Leonidas Oseliero Filho [1], Barbara Bianca Gerbelli[2]
[1]Instituto de Física, Univ de São Paulo (SP, Brasil), [2]Centro de Ciência Naturais e Humanas, Univ Federal do ABC (SP, Brasil)

INTRODUCTION: Thermal and chemical induced unfolding of proteins can be applied to understand their folding mechanisms. Nevertheless, some proteins are quite resistant to those effects, for instance tetanus (TT) and diphtheria (DT) anatoxins, the major components of the corresponding vaccines. In such cases, the surfactant-induced unfolding could be used instead. OBJECTIVES: Herein we propose the investigation of TT and DT unfolding by the anionic surfactant sodium dodecyl sulfate (SDS), a potent and well-known denaturant agent. Modifications in the protein secondary and tertiary structures were monitored by circular dichroism (CD) and tryptophan fluorescence (Trp-F), while information on thermodynamic changes and overall structure were assessed by isothermal titration calorimetry (ITC) and small-angle X-ray scattering (SAXS), respectively. MATERIALS AND METHODS: Anatoxins were produced at the Butantan Institute in Brazil and donated to us. SDS was purchased from Sigma-Aldrich and used as received. Sample preparation and experiments followed the protocols described previously in the literature and already used in the group. DISCUSSION AND RESULTS: From ITC data, the enthalpy variation is negative in all range of surfactant concentration, indicating the anatoxins unfolding is majorly governed by electrostatic interactions. Trp-F intensity decreases with the increase of SDS amount, suggesting restriction of tryptophan to the hydrophobic environment only. Changes in the CD profiles for each protein can be assigned to the gain and loss of α-helix and β-sheet secondary structures. According to SAXS, this is followed by the emergence of core-shell complexes, characterised by a bump at $q \sim 0.2$ Å^{-1} , in agreement with previous results reported in the literature for similar systems. CONCLUSION: In this work, the surfactant-induced unfolding of DT and TT was systematically monitored by a combination of different biophysical methods that yielded to the unprecedent correlation of thermodynamic and structural changes as well as the full characterization of the unfolding process, thus contributing to the overall knowledge about the investigated proteins.

Keywords: Diphtheria and tetanus anatoxins, protein unfolding, surfactant-induced unfolding / **Supported by:** FAPESP

D.62 - Generation of Datasets for Protein Docking Prediction by a Deep Learning Approach

Rafael Simões[1], Ramon Gomes[1], Lucas Vasques[1], Angelo Magro[1]
[1]Departamento de Bioprocessos e Biotecnologia, Universidade Estadual Paulista "Júlio de Mesquita Filho" (São Paulo, Brazil)

INTRODUCTION: There are several software and approaches for predicting protein-protein docking. Recently, artificial intelligence (AI) techniques have also been employed in searching for docking solutions, and their results are promising. However, one of the great challenges associated with the use of AI is the generation of a dataset that represents the conformational space of the proteins under study. Specifically for deep learning (DL) techniques, it is also necessary to convert a three-dimensional molecular complex into two-dimensional images for the model inference using classical DL algorithms. OBJECTIVES: The objective was to create two-dimensional images representing a vast conformational space of a protein-protein complex and use/evaluate these images as a dataset to infer deep learning models for protein-protein docking prediction. MATERIALS AND METHODS: Molecular dynamics (MD) simulations were performed to create docking solutions that represent a large protein conformational space and, thus, can adequately model the flexibility of the molecules. Additionally, a novel methodology was proposed for converting a molecular complex (protein-protein) into a two-dimensional image (matrix of RGB pixels) that contains the most representative information about the interaction between the proteins. These images were used as training and test datasets for DL algorithms. DISCUSSION AND RESULTS: The results showed the following parameters as representative for generating arrays of pixels to compose the dataset: *i*) components from the conventional force field that model interactions between non-bonded atoms (non-bonded interactions), more specifically, the Van der Waals and Coulomb potentials and; *ii*) structural data from the proteins interaction surface, such as distance between atoms, atom types and charges. Two-dimensional images were generated to compose the dataset using the cited parameters, and the inferred DL model resulted in AUC = 0.90. CONCLUSION: All these results denote a high predictive performance and potential to be used as an alternative approach for protein-protein docking.

Keywords: protein-protein docking, docking techniques, docking with artificial intelligence

D.63 - Characterization of the oral virome of healthy individuals and those with periodontits

Pâmela Natasha M. Bentes [1], Cristiane Saito[1], Anderson Basbosa[1], Fabiana Canavan[2], Siu Tsai[2], Daniel Saito[1]
[1]Escola Superior de Ciências da Saúde, Universidade Estadual do Amazonas (, Brasil), [2]Centro de Energia Nuclear na Agricultura, Universidade de São Paulo (, Brasil)

Periodontitis is microbial-triggered disease characterized by deleterious inflammation of the periodontium, causing significant morbidity at the national and global scales. The oral cavity encompasses a highly complex microbiome, composed of a wide variety of microbial types (bacteria, fungi, protozoa and viruses). In this sense, this project aims to study the viral genetic material (virome) of the oral cavity of healthy individuals and those with periodontitis, in order to highlight possible differences in composition between the groups. To this end, saliva samples were collected from healthy individuals (n = 13) and periodontitis patients (n = 14) attended at the Dental Clinic of the State University of Amazonas, Manaus, Amazonas. The genetic material of the samples was extracted, quantified and sequenced via a Next Generation Sequencing platform. The generated sequences were classified and annotated using the Kaiju program coupled with the NCBI Refseq virus reference database. Quantification and tabulation of taxonomic units were performed using R software packages. Analysis of relative abundances of health and disease study groups revealed BeAn 58058 virus as the most prevalent. Venn diagram analysis depicted over 1200 commonly shared viruses between both groups. Bacteriophages of the Siphoviridae family were significantly more prevalent in health, whereas Myoviridae phages and Mimiviridae viruses were more commonly detected in periodontitis. Nonetheless, ANOSIM and NMDS analyses revealed that both groups did not present significant differences in overall virome composition. The results helped us better understand the oral virome diversity, unveiling previously unreported viruses associated with disease establishment and homeostasis. In summary, we believe that the taxonomic units denoted here may represent important biomarkers for future research in oral microbial ecology.

Keywords: Periodontits, microbiome , phage

D.64 - Molecular Dynamics (MD) study of the mobility and conformational patterns of SARS-CoV-2 Spike Receptor-Binding Domain (RBD) mutants with phenotypes of increased infectivity and/or reduced immunogenicity

Yan Jerônimo Gomes Lobo [1]
[1]Departamento de Ciências Exatas e Biológicas, Universidade Federal de São João del-Rei (Minas Gerais, Brasil), [2]Departamento de Química, Universidade Federal da Paraíba (Paraíba, Brasil), [3]Departamento de Ciências Exatas e Biológicas, Universidade Federal de São João del-Rei (Minas Gerais, Brasil)

INTRODUCTION: SARS-CoV-2 has mutated since its inception, originating variants such as Delta, Gamma, and Omicron. This is concerning because some variants have greater infectivity and/or decreased immunogenicity compared to the Wild type (wt) variant. Mutations in RBD of the Spike glycoprotein are of particular interest due to its pivotal role in infection. OBJECTIVES: Evaluate the impact of mutations with different phenotypical groups: neutral; increased infectivity (+-); reduced immunogenicity (-+); increased infectivity and reduced immunogenicity (++) phenotypes, in the mobility of the RBD, through the analysis of productive MD simulations of 36 RBD mutants. MATERIALS AND METHODS: The wt Spike structure was collected from the Protein Data Bank, we manually selected the residues that correspond to the RBD, and modelled comparatively various (36) mutants. Protonation states for all residues in each structure were estimated for a pH of 7.4 and salinity of 0.15 M. All systems were parameterized and solvated in water boxes, and were submitted to a multistep equilibration protocol and to productive 100 ns MD simulations in triplicates. The last 50 ns of the obtained trajectories were analyzed by per residue root-mean-square fluctiation and cluster analysis. DISCUSSION AND RESULTS: Analysis of the obtained trajectories indicate that the most variable regions in the RBD are the beta1-beta2 loop of the receptor-binding motif (RBM) and the 360-374 residues. There is also a tendency of stabilization of the RBM to more favorable binding conformations in (+-), while (-+) shows greater conformational diversity to avoid detection by the immune system. (++) shows greater mobility than neutral mutants, but less mobility when compared to (-+). The 360-374 residues showed greater mobility in (+-) and lesser mobility in (++), possibly in order to respectively reduce detection and increase stability. CONCLUSION: Phenotypical differences among SARS-CoV-2 mutants are reflected coherently by the conformational patterns of the RBD.

Keywords: MD, RBD, Spike / **Supported by:** FAPEMIG

D.65 - Study of the Relationship Between Chemical Properties and Biological/ Toxicological Activity of Endocrine Disruptor Using Computational and Experimental Techniques

Rafaela Molina de Angelo [1], Vanessa da S. Antonin[2], Renata Colombo[1], Mauro C. dos Santos[2], Káthia M. Honório[1,2]

[1]Química Computacional, Escola de Artes, Ciências e Hum. (SP, Brazil), [2]Eletroquímica, Univ. Fed. do ABC (SP, Brasil)

INTRODUCTION: This doctoral project aim to understand, at a molecular level, mechanisms of degradation and biological/toxicological action related to chemical substances classified as endocrine disruptors (ED) to minimize the impacts they may cause on the environment and human health OBJECTIVES: MATERIALS AND METHODS: An exploratory chemical-biological study will be carried out on three hormone receptors (glucocorticoid, estrogen alpha and beta receptors) and main interactions between several selected substances from a filtration study and also with 5 endocrine disruptors (glyphosate, acetochlor, ethinylestradiol, RB5 dye and orange II) initially studied by the collaborative group. To carry out the prediction of reactive sites and electronic properties of endocrine disruptors, the following approaches will be used: (i) virtual screening assays from a database of endocrine disruptors to discover which compounds interact with the biological targets of interest; (ii) construction of 2D (HQSAR) and 3D (CoMFA and CoMSIA) models to predict toxicity values of the substances under analysis; (iii) molecular docking to evaluate the molecules at the receptors' active sites, analyzing the types of interactions and the possible conformations of the compounds at the binding site of the targets; (iv) molecular dynamics simulations to assess the dynamic behavior of endocrine disruptors at target action sites as a function of time; (v) calculations of binding free energy using MM-GBSA and MM-PBSA methods to evaluate the stability of the complexes formed between EDs and the biological targets under analysis. Finally, the experimental validation of the theoretical results will be performed using electrodegradation and by-product characterization techniques, with a collaborative group < br> DISCUSSION AND RESULTS: CONCLUSION: In this way, the use of the computational methodologies mentioned above and the experimental validations will be fundamental for the electronic and chemical-biological study of endocrine disruptors, as well as the construction of prediction models of toxicity and mechanisms of oxidation/degradation of several endocrine disruptors of great environmental and public health importance. - **Keywords:** Endocrine disruptors, Molecular Dynamics, Electrochemistry

E – Structural Biology

E.01 - Production of the Human Hsp70-Escort Protein (hHep1) for Structural Analysis by NMR

Samile Bezerra de Aguiar [1], Carolina Oliveira Matos[2], Noeli Soares Melo da Silva[1], Carlos Henrique Inacio Ramos[2], Júlio Cesar Borges[1]
[1]Instituto de Química de São Carlos, Universidade de São Paulo (São Paulo, Brasil), [2]Instituto de Química, Universidade Estadual de Campinas (São Paulo, Brasil)

INTRODUCTION: Heat shock proteins (HSP) are molecular chaperones involved in several processes in the protein homeostasis. An important family of heat shock proteins is the Hsp70, that participate in the aggregation prevention, protein disaggregation, protein targeting for clearance and protein folding/refolding, among others. However, several co-chaperones assist Hsp70 to perform such multitude of functions. One of them is hHep1 (human Hsp70-escort protein 1) that stimulates its ATPase activity and prevents the aggregation of human mitochondrial Hsp70 (mtHsp70 or HSPA9) as well as client proteins. The structural study of this human co-chaperone is important to gain further insights about how it regulates mtHsp70 and help in the functions of the later protein. hHep1 has a zinc-finger domain and poorly folded N- and C-terminal regions of unknown functions. The yeast Hep1 (yHep1) is the best-known orthologue, but yHep1 and hHep1 are 30% identical and have some divergent properties and are not functionally equivalent. OBJECTIVES: Express hHep1 in M9 minimal medium to purify it in the folded state for NMR structural characterization. MATERIALS AND METHODS: The pQE2::hHep1 expression vector was transformed in *Escherichia coli* BL21 (DE3) cells and induced in M9 minimal medium. The expressed hHep1 was purified by Ni^{2+} -affinity and preparative size exclusion chromatographies. Such protein was characterized by circular dichroism, intrinsic fluorescence spectroscopy and SDS-PAGE. DISCUSSION AND RESULTS: hHep1 was satisfactorily expressed in M9 minimal medium which allowed to purify it as monomer and to be obtained in high concentrations (around 600 µM of hHep1). Its spectroscopy signatures were confirmed by the biophysical tools. CONCLUSION: hHep1 protein was successfully obtained in minimal medium with a good yield. This result opens the possibility to it be structural characterized by NMR

Keywords: hHep1, Molecular chaperones, NMR
Supported by: CAPES

E.02 - Development of Nanostructured Matrices Based on Antidiabetic Peptides

Ana Beatriz Caliari Souza [1], Emerson Rodrigo Silva[1], Clovis Ryuichi Nakaie[1]
[1]Biofísica, Universidade Federal de São Paulo (São Paulo, Brasil)

INTRODUCTION: Diabetes has high prevalence in Brazil and all around the world. The number of people with diabetes is expected to be near to 366 million by 2030. Therefore, the search for anti-glycemic agents is a relevant matter with a potential impact in the public health. The development of therapies based on nanostructures is at the frontline of nanomedicine, representing a revolutionary way to treat different health conditions. Our objective was to develop nanostructured materials with anti-glycemic properties. The production of nanostructures was based on liraglutide, a peptide with 31 residues, and desmopressin, an ultrashort cyclic peptide. These compounds are available in the pharmaceutic market, and they are currently used to treat diabetes mellitus and diabetes insipidus, respectively. OBJECTIVES: To develop nanoparticles based on anti-glycemic peptides and provide information about their structure, from the molecular level to the nanometer range. MATERIALS AND METHODS: The structural analyses were performed through spectroscopic techniques (UV-Vis, fluorimetry and circular dichroism), whereas morphology assays were carried out using atomic force microscopy. DISCUSSION AND RESULTS: Both liraglutide and desmopressin were found to form nanometric aggregates at critical concentrations near to 0.5 mg/ml, with b-sheet conformations dominating the secondary structure of the aggregates. Quenching of tryptophan (or tyrosine) emission indicated a major role of hydrophobic domains in the self-assembly. The interaction of peptides with lipid membranes was also demonstrated, showing that their secondary structure is modified in the presence of membranes, and interaction with lipid bilayers being driven by hydrophobic domains. CONCLUSION: We demonstrated the fabrication of ordered nanoassemblies based on liraglutide and desmopressin, which are stabilized by b-sheets. It was also verified that these nanoparticles interact with lipid membranes, suggesting they can associate to cell membranes. To our knowledge this is the first study reporting the production of nanostructured materials from peptides endowed with anti-glycemic activity.

Keywords: Diabetes, Liraglutide, Desmopressin

E.03 - Structural Characterization Of SARS-CoV-2 Non-Structural Protein 8 (NSP8)

Flávia Duarte Maia [1], Deborah Kimie Yonamine[1], Taísa Magnani Dinamarco[1], Daniel Junqueira Dorta[1]
[1]Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto (São Paulo, Brasil)

INTRODUCTION: The ongoing global pandemic of the coronavirus disease of 2019 (COVID-19) has caused a huge number of deaths. Knowledge of protein structures, such as NSP8 of the disease-causing SARS-CoV-2 virus, can provide further information on pathogenicity and even treatment given that NSP8 and NSPs are involved in genome replication regulation. OBJECTIVES: This study focuses on the SARS-CoV-2 NSP8 protein structure. MATERIALS AND METHODS: The pET28a(+)::NSP8 recombinant vector was transformed and maintained in *E. coli* Rosetta™(DE3)pLysS and expressed with 0.5 mM IPTG at 37 °C for 5 h. Expression was confirmed by Western blotting and SDS-PAGE. After expression, the protein was purified by nickel stationary phase affinity chromatography by elution with different imidazole concentrations. The structure of the purified protein was analyzed by circular dichroism (CD) and tryptophan fluorescence (ITFE). To characterize changes in structural conformation under different conditions, the purified protein was analyzed after incubation for 30 min under different temperatures (4, 30, 40, 50, 60, or 70 ºC). DISCUSSION AND RESULTS: The CD and ITFE spectra showed denaturation temperature (Tm) of 51.1 and 56.6 ºC, respectively. The CD spectrum revealed that the NSP8 secondary structure is mainly α-helix. Increasing temperature changed the protein structural conformity, indicating more intense denaturation from 50 ºC. CONCLUSION: Analysis of the results provided greater understanding of the NSP8 structure, which may help in the development of a treatment for COVID-19.

Keywords: SARS-CoV-2, non-structural protein, circular dichroism

E.04 - Significant Increase in Enzyme Activity of the Thermophilic 3 β-glucosidase (GH3) from Clostridium thermocellum

Leonardo Rodrigues de Almeida[1], Wanius José Garcia da Silva[2], **João Renato Carvalho Muniz** [1]
[1]Física e Ciência Interdisciplinar , São Carlos Institute of Physics, University of Sao Paulo (São Paulo, Brasil),
[2]Center for Natural and Human Science, Federal University of ABC (São Paulo, Brasil)

INTRODUCTION: The production of bioethanol, sugars and carboxylic acids through the hydrolysis of lignocellulosic biomass by cellulases has received great attention and with a competitive cost in the long term. The β-glucosidase (BGl) family 3 (CtBgl3B) from *C. thermocellum* is involved in the degradation of biomass, hydrolyzing the β-glycosidic linkage between two adjacent molecules in dimers and glucose oligomers. Also, increased levels of BGls in cellulase mixtures may benefit the conversion of cellulose to glucose. OBJECTIVES: Structural, biochemical, and biophysical characterization of the cellulosomal enzyme *Ct*Bgl3B. MATERIALS AND METHODS: We expressed *Ct*Bgl3B in *E. coli* (BL21), and purified by affinity and size-exclusion chromatography. We conducted biophysical analyzes using circular dichroism (CD), differential scanning fluorimetry (DSF), SEC-MALS and SAXS. The macromolecular structure was determined by X-ray diffraction in single crystals. Kinetic parameters with the Michaelis-Menten model were determined using the substrates where it was hydrolytically active *p*NPG, *p*NPX, and C*p*NPG2. DISCUSSION AND RESULTS: Both CD and DSF confirmed its thermophilic characteristics with optimal activity at pH 5.5 and Tm ≈70 °C. We evaluated its enzymatic activity and kinetic parameters with pNPG: Sp. act. = 124 U mg-1, *KM* = 0.37 (mM), catalytic constant *kcat* = 173 (s-1), and catalytic efficiency *kcat/KM* = 469 (mM-1 s-1). The enzyme also showed the ability to act against synthetic substrates, such as *p*NPX (≈40%) and C*p*NPG2 (≈25%). A boost of 70% in enzyme activity was reached in the presence of Triton X-100 and 60% with Tween20. The crystallographic structure of *Ct*Bgl3B was determined at an anisotropic resolution of a* 3.47, b* 2.20, and c* 1.77 Å, demonstrating a dimer arrangement, corroborated by SEC-MALS and SAXS experiments. CONCLUSION: The increased thermal stability, activity, and superior biochemical characteristics of *Ct*Bgl3B make it an excellent candidate for addition to enzyme mixtures designed to operate at higher temperatures.

Keywords: β-glucosidase, cellulosome, Clostridium thermocellum /**Supported by:** FAPESP

E.05 - Binding Specificity Assessment Of SH2 Domains Present In PLCgamma For Peptides Derived From Tyrosines Kinases

Noemi Jacó de Souza [1], Allan Pradelli Roldão[1], Helder Veras Ribeiro-Filho[2], Fernanda Aparecida Heleno Batista[2], Ana Carolina Migliorini Figueira[2], German Gustavo Sgro[3], Paulo Sergio Lopes de Oliveira[2], Deborah Schechtman[1]
[1]Biochemistry, Institute of Chemistry, University of São Paulo (São Paulo, Brazil), [2]Brazilian Biosciences National Laboratory, Brazilian Center for Research in Energy and Materials (São Paulo, Brazil), [3]Biomolecular Sciences, School of Pharmaceutical Sciences of Ribeirão Preto (São Paulo, Brazil)

INTRODUCTION: Inflammation generated by a tissue injury leads to secretion of pro inflammatory factors, amongst them the nerve growth factor (NGF), which binds to and activates its high affinity receptor, TrkA - a tyrosine kinase coupled receptor. Upon TrkA activation, autophosphorylation of the kinase creates protein anchoring sites enabling phosphorylation of Phospholipase C gamma (PLCγ) by one of its two SH2 domains. There are studies supporting PLCγ anchoring to either N-SH2 or C-SH2 domains of PLCγ. Additionally, *In vivo* studies conducted by our group indicated that the inhibition of the interaction between PLCγ and TrkA through a decoy peptide (TAT-pQYP) led to analgesia. OBJECTIVES: To study the binding affinity of TAT-pQYP and of peptides derived from other tyrosine kinases to each SH2 domain. MATERIALS AND METHODS: Molecular dynamics simulation of peptides derived from TrkA and B binding to N and C-SH2 domains of PLCγ. Affinity chromatography and gel filtration to purify N-SH2, C-SH2 and N-SH2 fused to C-SH2. Determination of binding affinity of peptides derived from TrkA and B using fluorescence anisotropy. DISCUSSION AND RESULTS: Molecular dynamics simulations suggest that the affinity of peptides derived from TrkA and B are greater for the N-SH2 than for the C-SH2. Ideal conditions for the expression and purification filtration of N-SH2 and C-SH2 constructs were established. The affinity constant of TAT-pQYP for N-SH2 was determined by fluorescence anisotropy. CONCLUSION: In simulation studies, PLCγ has a greater affinity for N-SH2 than C-SH2. Determining the affinity of different peptides to PLCγ SH2 domains will help us understand the specificity of TAT-pQYP and how PLCγ is activated.

Keywords: PLCgamma, SH2, protein-peptide interaction / **Supported by:** FAPESP

E.06 - Structural Characterization of Histone Deacetylase 2 of Toxoplasma gondii

Caroline de Moraes de Siqueira [1], Julia Zamith Schwartz[1], Mariana Sayuri Ishikawa Fragoso[1], Vanessa Rossini Severo[1], Tatiana Arruda Campos Brasil de Souza[1], Sheila Cristina Nardelli[1]
[1]Laboratório de Pesquisa em Apicomplexa, Instituto Carlos Chagas - Fundação Oswaldo Cruz (PR, Brasil)

INTRODUCTION: Within the epigenetic mechanisms of gene expression control, histone post-translational modifications are one of the most studied, and in addition to transcription, these modifications are markers for many cellular functions, such as DNA replication and repair. Histone deacetylation is associated with gene silencing since removing acetyl groups by histone deacetylases (HDACs) results in a more condensed chromatin. In Toxoplasma gondii little is known about these enzymes, but it is believed they are an essential part of gene expression regulation. T. gondii has 7 HDACs, including HDAC2 (TgHDAC2). TgHDAC2 is a typical HDAC class I zinc-dependent; nevertheless, this protein has two amino acid insertions inside the HDAC domain, which is unique to Toxoplasma, and whose structure and function remain unknown. In silico homology modeling revealed no similar structure in the database. OBJECTIVES: For this reason, this work aimed to analyze the structural function of these insertions. MATERIALS AND METHODS: Two forms of TgHDAC2 were constructed, one with the complete sequence (TgHDAC2 CDS) and another without the amino acid insertions (TgHDAC2Δ74-276) DISCUSSION AND RESULTS: Circular dichroism analysis indicated that TgHDAC2 CDS is less stable and less structured than TgHDAC2Δ74-276, suggesting a more functional than a structural role of this region. To confirm this hypothesis, we performed pull-down experiments with both proteins to understand if there are different protein interactions with TgHDAC2. In addition, we also performed the cross-linking mass spectrometry (XL-MS) of TgHDAC2 CDS to evaluate the tertiary structure and unravel the conformation of this domain. CONCLUSION: So far, we have concluded that amino acid insertions in the HDAC domain have no structural gain for the protein and may function in the protein-protein interaction or post-translational regulation.

Keywords: Protein structure, Epigenetics, Histone deacetylase / **Supported by:** Fiocruz; CAPES; CNPq; Inova-fiocruz; Fundação Araucária

E.07 - NMR study of sugar interaction to the N-terminal domain of Spike protein from Sars-cov-1

Julie Carvalho Lopes [1,2], Francisco Felipe Bezerra[1], Paulo A. S. Mourão[1], Adriane Regina Todeschini [3], Beatriz Penna[1,2], Ana Paula Valente[1,2]

[1]Institute of Medical Biochemistry , Federal University of Rio de Janeiro (Rio de Janeiro , Brasil), [2]National Center of Nuclear Magnetic Resonance (CNRMN), Federal University of Rio de Janeiro (Rio de Janeiro, Brazil), [3]Biophysical Institute Carlos Chagas Filho (IBCCF), Federal University of Rio de Janeiro (Rio de Janeiro, Brazil)

INTRODUCTION: The envelope protein of coronavirus, called Spike is responsible for the process that allows the virus to enter the host cell. The N-terminal of the Spike protein shares similarity with galectins and may help the attachment process by interacting with carbohydrates from the cellular extracellular matrix. OBJECTIVES: Our aim is to study the N-terminal galectin-like (NTD) domain of Spike protein from Sars-CoV-1. MATERIALS AND METHODS: The NTD domain of the Spike protein was obtained by recombinant DNA technique, which was inserted into the BL21 (DE3) strain of Escherichia coli in plasmid pET3a. The purification was obtained by ion exchange liquid chromatography. We used Nuclear Magnetic Resonance (NMR) and intrinsic fluorescence spectroscopy for structural studies. DISCUSSION AND RESULTS: Intrinsic Fluorescence thermal denaturation assays showing a Tm ≅ 60 ºC. 1H/15N HSQC experiments gave us good spectra with good signal visualization. The titration results using intrinsic fluorescence showed fluorescence intensity and maximum lambda variation for low molecular weight porcine heparin, N-acetylneuraminic acid and chondroitin sulfate. CONCLUSION: Our results indicated interaction between NTD-domain and carbohydrates. Perspectives for this work are the analysis of the protein-sugar interaction by NMR (STD, Tr-NOE, etc) and the domain labeling with deuterium for further analysis by NMR.

Keywords: Sars-Cov-1, Spike protein, Sugar interaction

Supported by: FAPERJ, CNPq, PIBIC/UFRJ

E.08 - Grb2 Biophysical Profile upon Cancer Cell's pH boundaries

Jéssica Andrade Tedesco [1], Raphael Vinícius Rodrigues Dias[1], Giovana Casteluci[1,2], Renan Pereira Pedro[1,2], Leandro Cristante de Oliveira[1], Ícaro Putinhon Caruso[1,2,3], Fernando Alves de Melo[1,2]

[1]Departamento de Física, Universidade Estadual Paulista "Júlio de Mesquita Filho" (São Paulo, Brasil), [2]Centro Multiusuário de Inovação Biomolecular, Universidade Estadual Paulista "Júlio de Mesquita Filho" (São Paulo, Brasil), [3]Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: Carcinogenic cells present a dysregulated pH as a hallmark. The higher intracellular pH enables adaptive characteristics such as increased replicative potential. Grb2, an adapter protein, is largely involved in a variety of cancers due to its role on signaling pathways responsible for cell growth and proliferation. OBJECTIVES: In this study we aim to characterize thermodynamics and conformational changes on dimeric Grb2 related to the pH difference from 7 to 8. MATERIALS AND METHODS: Studies of DSC, DLS, ^{15} N HSQC NMR, CD and computational analysis were carried out with dimeric Grb2 to determine structural and thermodynamic parameters for both pHs. DISCUSSION AND RESULTS: At pH 7, we observed a more compact structure with high cooperativity within domains. Whereas, at pH 8, a more loosen structure is shown, with relatively independence between domains characterized by higher values of melting temperatures and lower values of enthalpy correlated to each domain. CD spectra showed a higher percentage of disordered structures at pH 8, conferring more flexibility into the protein. This flexibility can be correlated to the lower cooperativity at higher pH, as well as the higher hydrodynamic diameter. ^{15} N HSQC chemical shift differences show an expressed shift of several amino acids when both pH spectra are compared. Assignments of SH2 and cSH3 domains show a larger shift at cSH3 domain, confirmed by computational data. Molecular dynamic analysis demonstrated that Grb2's movement (specific for both SH3 domains) at pH 7 is towards the center of the protein, whereas at pH 8 this movement is towards the outside, characteristic of a more elongated structure at higher pH. CONCLUSION: The pH alteration is an important hallmark of carcinogenic cells. Here, we show that Grb2 presents important thermo and structural modifications due to pH. These modifications could be essential to disrupt the stability of the signaling pathways and to understand mechanisms of aberrant signals.

Keywords: Grb2, NMR, pH / **Supported by:** CAPES, FAPESP

E.09 - Unraveling Proteins with Unknown Function: Search for New Therapeutic Targets for Diseases Caused by Trypanosomatids

Raíssa Santos de Lima Rosa [1], Manuela Leal da Silva[2,1]
[1]Graduate Program in Computational and Systems Biology of the Instituto Oswaldo C, IOC/Fiocruz (Rio de Janeiro, Brazil), [2]Institute of Biodiversity and Sustainability (NUPEM), Federal University of Rio de Janeiro (Macaé, Brazil)

INTRODUCTION: Neglected diseases are an international health problem, several are caused by the Trypanosomatidae family: Leishmaniasis, Chagas disease, and sleeping sickness (Human African Trypanosomiasis). Alarming epidemiological data and treatments use toxic drugs, and their interruption is common. OBJECTIVES: To use an integrative approach for functional prediction of proteins with unknown functions described in the proteomes of *Trypanosoma spp.* (*Tcr*, *Tbr*, and *Tbg*) and *Leishmania spp.* (*Lif*, *Ldo*, and *Lbz*) to find new protein targets for testing new drugs. MATERIALS AND METHODS: The following programs and databases were used for the functional prediction: Pfam, String, Psort, Smart, CDD, Prosite, TritryDB, HMMER, Uniprot, BlastP (NR), and MHOLline (comparative modeling with Modeller vs 10.0). From the data obtained in the MHOLline program, we used the SCOP, SCOPe, and CATH databases. For the analysis of metabolic pathways, we consulted the MetaCyc, Trypanocyc, KEGG, and Reactome databases. DISCUSSION AND RESULTS: From the proteomes deposited at the NCBI, proteins with unknown functions (hypothetical protein) were separated, totaling in *Trypanosoma cruzi* 11,171 protein (*Tc*11,171), *Tbr*5,711, *Tbg*6,611, *Lif*5,245, *Ldo*3,527, and *Lbz*3,373. The sequences were submitted to the MHOLline which built a 3D model and were selected based on identity and coverage (>35% and >70%, respectively). Subsequently, the programs/databases were used and the function of *Tc*26, *Tbr*13, *Tbg*16, *Lif*11, *Ldo*1, and *Lbz*2 was predicted. CONCLUSION: Our strategy breaks new ground by combining methodologies using sequence and structure information to unravel the function of proteins. The consensus between this information led us to potential domains to be studied, such as the IFT70 protein that has the TPR domain with locomotor function. Studies have shown that inhibition of protein complex formation can compromise ciliogenesis viability. Another potential domain is the ACBP responsible for carrying the Acyl-CoA molecule, if the membrane of the parasites is compromised, it is susceptible to lysis.

Keywords: Chagas Disease, Leishmaniasis, Functional prediction / ***Supported by:*** *Capes*

E.10 - Evaluation of the Interaction Between PrP and α-Synuclein Using Spectroscopic Techniques

Paulo Leandro Garcia Meirelles Junior [2,1]
[1]Programa de Biologia Estrutural, Instituto Nacional de Ciência e Tecnologia de Biologia Estrutural e Bioimagem (Rio de Janeiro, Brasil), [2]Programa de Biologia Estrutural, Instituto de Bioquímica Médica Leopoldo de Meis (Rio de Janeiro, Brasil)

INTRODUCTION: Introduction: Neurodegenerative diseases (NDs), such as Parkinson's disease (PD) and prion disease (PD), affect the central nervous system and are mainly related to aging. Protein aggregation is one of the causes of NDs that can lead to the death of neurons and the development of various symptoms. PD is related to the aggregation of α-synuclein (αSyn), which becomes abnormal and self-associates. Prion diseases are fatal and occur by the structural conversion of cellular prion protein (PrPC) into scrapie prion (PrPSc). It has been reported that PrP *In vivo* is involved in several NDs, such as a receptor for amyloid-β oligomer in Alzheimer's disease, and facilitating the internalization of αSyn fibers in PD. OBJECTIVES: Objectives: We aimed to investigate the interaction between αSyn fibers and oligomers with PrP using spectroscopic techniques. MATERIALS AND METHODS: Material and methods: We used fluorescence spectroscopy, static light scattering, and fluorescence polarization. To understand the influence of the PrP N-terminal domain on the interaction with αSyn, we used full length (PrP23-231) and truncated PrP (PrP90-231). DISCUSSION AND RESULTS: Results and discussion: Our results showed that the PrP N-terminal domain enhances PrP:αSyn interaction and that interaction with oligomers is more significant than with fibers and monomers of αSyn. We observed an increase in light scattering, suggesting an oligomerization process between these proteins. CONCLUSION: Conclusions: Our data show the interaction and association between these proteins, but a more detailed investigation is essential. Assessing the interaction between PrP and αSyn can provide information about the pathological mechanisms involved in these diseases and associated comorbidity.

Keywords: Prion, Synuclein, neurodegenerative diseases

E.11 - Interaction Features Between the Recombinant disintegrins, Jararacin and Jarastatin with Platelets: NMR studies and *In vivo* Activities.

Jorge Eduardo Chang Estrada [1], Ariana Azevedo Vasconcelos[1], Victor Da Conceição David[1], Russolina Benedeta Zingali[1], Fabio C. L. Almeida[1]
[1]Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brazil)

INTRODUCTION: Disintegrins are small cysteine-rich peptides found in snake venom, capable of modulating a broad range of transmembrane receptors known as integrins, heterodimeric receptors that have a key role in mediating physiological and pathological processes, such as platelet aggregation and thrombosis. Integrins, thus, have become a therapeutical target and disintegrins can be used as tools to provide new information about physiopathologies related to this receptor. OBJECTIVES: The objective of this study was to express and analyze the effect of recombinant disintegrins, jararacin (rJarc) and jarastatin (rJast), upon platelets. MATERIALS AND METHODS: For this, we expressed disintegrins on a Pichia pastoris system. The yeast was transformed with the vector pPIC9 containing the synthetic gene of disintegrins. The disintegrins were expressed and secreted in the cultured media and then purified by molecular exclusion chromatography. Also, 15N-rJarc and 15N-rJast were expressed and purified. We confirmed the molecular mass by mass spectrometry and their folding by 1H nuclear magnetic resonance spectra and circular dichroism. NMR studies, in the presence or absence of platelets, revealed changes in rJarc and rJast structure and the residues involved in the platelet-disintegrins interaction. We, then, generate interactions models based on experimental data. DISCUSSION AND RESULTS: The data reveal that rJast interact with both subunits of the Integrins, making interactions with the N-terminal domain, the C-terminal domain and the RGD binding motif. These models helped us to better understand how our disintegrins interact with platelets integrins, simulating the interaction in a more real way. Following these data we evaluate the ability of rJarc on an arterial thrombosis model, ex vivo platelet aggregation and bleeding assays revealed the ability to prevent thrombus formation and impaired platelet aggregation at 5mg/kg dose. CONCLUSION: In conclusion, rJarc and rJast have distinct structure-affinity features toward integrins, allowing us to reveal the potential anti-thrombotic activity of rJarc.
Keywords: Disintegrin, Integrin, Platelet aggregation
Supported by: FAPERJ, CNPq and CAPES

E.12 - Impact of Subchronic Exposure to Aripiprazole on Sperm Parameters and Fertility of Adult Male Wistar Rats

Renata Gleysiane De Sousa Felix[1], Maria Joana Nogueira De Moura[2,1], Aline Gabrielle Gomes Da Silva[2,1], Artemia Kelly Holanda Pereira[1], Caio Cesar Araujo Dos Santos[1], Ana Beatriz Silva Angelo[1], **Cibele Dos Santos Borges[1,2]**
[1]Department of Bioscience, Biological and Health Sciences Center - CCBS, Federal University of the Semi-Arid Region (RN, BRAZIL), [2]Multicenter Graduate Program in Biochemistry and Molecular Biology (PMBqBM), State University of Rio Grande do Norte (RN, BRAZIL)

INTRODUCTION: Numerous antipsychotics drugs have been used to treat mental disorders, such as Aripiprazole. Aripiprazole has high affinity for serotonin, dopamine and noradrenaline receptors, that could directly influence the reproductive function, due to its communication with the sex steroid system and also endocrine system. However, there are few reports of the aripiprazole effects on male reproductive system and fertility. OBJECTIVES: Evaluate the impact of subchronic aripiprazole exposure on male reproductive system, with emphasis on sperm parameters and fertility. MATERIALS AND METHODS: Adult male Wistar rats (n=12/group) from vehicle group (CTRL); group treated with 3.0mg/kg (EXP1) and 6.0mg/kg (EXP2) of aripiprazole were treated during 28 days. After the end, 7 animals/group were weighted and euthanized by decaptation. Vital organs (kidney, adrenal, heart, liver, brain, pituitary and thyroid) and reproductive organs (testis, epididymis, ventral prostate and seminal vesicle) were collected and weighed. The right testis and epididymis were fixed for further histopathological processing. The sperm samples from left epididymis was used to sperm motility and count. The remained animals (n=5/group) were used to perform sexual behavior and fertility test. Ethics Committee (UFERSA-23091.014948/2019-20). Statistical analysis: ANOVA ($p < 0.05$). DISCUSSION AND RESULTS: No alterations were observed in the final body and vital organ weights between all groups. However, there was a significant increased in the full seminal vesicle from EXP2 group when compared to CTRL group. EXP1 showed a significant reduction on progressive mobile sperm (57.7% x 37.7%) and a decrease of the mounts before the ejaculation (16.0+3.2 x 6.0+1.5) compared to the CTRL group. On the other hand, EXP2 showed a significant reduction on fertility potential compared to CTRL group (83.0+6.9 x 93.0+2.4). CONCLUSION: Based on this experimental model, the subchronic exposure to aripiprazole during adulthood can cause impairment on male sperm quality and fertility.
Keywords: antipsychotic drugs, sperm motility, fertility potential / **Supported by:** UFERSA/PROPPG

E.13 - Characterization of the structure and dynamics of domain III of the Zika virus envelope glycoprotein and its interaction with glycosaminoglycan mimetics and antibodies.

Gabriela de Lima Camara [1], Adolfo Moraes[2], Ana Paula Valente[1]
[1]Institute of Medical Biochemistry Leopoldo de Meis (IBqM)-CENABIO-CNRMN, Federal University of Rio de Janeiro (Brazil), [2]Department of chemistry, Federal University of Minas Gerais (, Brazil)

INTRODUCTION: The ZIKA virus (ZKV) has a propensity to infect nerve tissue cells and is related to neurological syndromes such as Guilláin-Barré and microcephaly. ZKV belongs to the flavivirus genus, and has three structural proteins: capsid, membrane protein, and envelope glycoprotein E. Glycoprotein E participates in cell receptor binding and has the fusion peptide. It is formed by three domains: DI, DII and DIII; studies indicate that DIII is conserved among flaviviruses and is related to the interaction of the virus with host cells and recognition by neutralizing antibodies. OBJECTIVES: The present study analyzes the structural properties and the interaction with the host targets of the DIII of the Glycoprotein E of the Zika virus (DIII-ZKV), using the technique of Nuclear Magnetic Resonance (NMR). MATERIALS AND METHODS: DIII-ZKV was expressed in Escherichia coli BL21-DE3 and purified on size exclusion chromatography (SEC). We used the domain III labeled with 15N, 13C and 2H. We performed measurements of relaxation parameters to map their dynamics such as T1, T2 and heteronuclear NOE DISCUSSION AND RESULTS: he results show the presence of flexible residues and in conformational exchange. In addition, NMR experiments were performed on the interaction of DIII-ZKV with glycosaminoglycans mimetics (GAGs), such as heparin and Fondaparinux, the results show a reduction in the signal intensity of the protein in the presence of both ligands, that suggest the complex formation. We also performed fluorescence assays of free DIII-ZKV and in the presence of heparin titration, which show a reduction in intensity with increasing heparin concentration. We also used free and bound DIII to the Fab antibody ZKA64 for epitope mapping. CONCLUSION: The results, show the residues with more variation in de chemical shifts in the interaction with antibody and GAGs. We will perform further experiments to confirm the features of the complexes.

Keywords: Zika virus, antibody, glycosaminoglycan / **Supported by:** FAPERJ, CNPq, CAPES, FINEP

E.14 - Structural Basis of Single-Stranded DNA Binding to the Cold Shock Domain of the Plant Glycine-Rich Protein AtGRP2

Karina de Carvalho Pougy [1], Marcelo Musa Ruas Filho[1], Clara Malizia Leal Ferreira da Motta[1], Luis Mauricio Trambaioli da Rocha e Lima[2], Gilberto Sachetto Martins[3], Anderson de Sá Pinheiro[1]
[1]Depto de Bioquímica, Univ. Federal do Rio de Janeiro (RJ, Brazil), [2]Departamento de Fármacos e Medicamentos, Univ. Federal do Rio de Janeiro (RJ, Brazil), [3]Departamento de Genética, Universidade Federal do Rio de Janeiro (RJ, Brazil)

INTRODUCTION: AtGRP2 (*Arabidopsis thaliana* glycine-rich protein 2) is a glycine-rich, RNA-binding protein that plays key roles in abiotic stress response and flowering time regulation in *Arabidopsis thaliana*. AtGRP2 consists of an N-terminal cold shock domain (CSD) and two C-terminal retroviral CCHC-type zinc knuckles interspersed with glycine-rich regions. Despite the wealth of information on AtGRP2 function, the molecular mechanisms underlying its biological roles are largely unknown. OBJECTIVES: Here, we investigated the structure and DNA binding properties of AtGRP2-CSD. MATERIALS AND METHODS: Two AtGRP2-CSD constructs (residues 1-79 and 1-90) were used throughout this work. Both constructs were cloned into RP1B and expressed in *Escherichia coli* BL21 DE3 as His_6-fusion proteins. Recombinant proteins were purified by nickel-affinity and size exclusion chromatography and further analyzed by NMR and fluorescence spectroscopy. DISCUSSION AND RESULTS: The 2D [^{1} H, ^{15} N] HSQC spectrum of AtGRP2-CSD_{1-79} revealed the presence of an unfolded state in equilibrium with the native, folded protein with exchange kinetics in the slow time regime. Addition of eleven residues at the C-terminus stabilized the folded conformation, suggesting that this C-terminal extension is part of the CSD. Despite that, a small population of the unfolded state is still observed for AtGRP2-CSD_{1-90}. Using multidimensional, triple resonance NMR, we unambiguously assigned 95% of the folded and 68% of the unfolded state resonances. Three-dimensional structure determination of AtGRP2-CSD_{1-90} is currently being performed. We investigated the binding specificity of AtGRP2-CSD_{1-79} by fluorescence spectroscopy using a set of 25 different 7-mer DNA oligonucleotides. DNA binding occurred with affinities ranging from low nM to µM, suggesting that AtGRP2-CSD_{1-79} selectively interacts with T-rich sequences. NMR titration results identified aromatic residues at strands β2 and β3, as well as loop β3β4 as critical for DNA binding. CONCLUSION: These results shed light on the mechanism by which AtGRP2-CSD specifically recognizes single-stranded DNA.

Keywords: AtGRP2, Structure, NMR / **Supported by:** FAPERJ, CNPq, and CAPES

E.15 - Features of the C-terminus domain of CDNF: Structural characteristics and its neuroprotective activity

Antonio dos Santos Silva[1], Cristiane Latgé de Almeida e Silva[2], Debora Foguel[1]
[1]IBqM, Univ. Federal do Rio de Janeiro (RJ, Brasil), [2]ECS, Univ. do Grande Rio Prof. José de Souza Herdy (RJ, Brasil)

INTRODUCTION: Parkinson's disease is characterized by the loss of dopaminergic neurons in the substantia nigra of the brain. There is no efficient available therapy to treat this disease, which is the second most prevalent neurodegenerative disorder worldwide. Neurotrophic factors promote survival, differentiation, and maintenance of neurons in the developing and adult vertebrate nervous system. A potent neurotrophic factor for dopaminergic neurons described is the cerebral dopamine neurotrophic factor (CDNF). Mature Full-Length CDNF (FL-CDNF) is composed of 161 amino acids, 7 alpha-helix, 4 disulfates bounds and is divided into two domains. N-terminus domain has the first 100 amino acids, 5 alpha-helix, and 3 disulfates bounds. C-Terminal has the last 57 amino acids with 2 alpha-helix, a 3_{10} helix, and a single disulfate bound. Both domains are connected by a flexible linker. Our group first presented FL-CDNF's complete structure and its action across some different tissues. Now we are engaged in understanding which domain is responsible for CDNF neuroprotection and their structural stability. OBJECTIVES: Compare the structural stability of FL-CDNF and its domains against structural stress, their possible neuroprotective role, and cysteine's functions in its structure. MATERIALS AND METHODS: Protein expression in the heterologous procaryotic system; fluorescence spectroscopy; circular dichroism; live/dead assays. DISCUSSION AND RESULTS: The C-terminus mass center presents an important right shift compared to FL-CDNF due to tryptophan natural exposition; however, it does not affect the protein resistance. Experiments using Bis-ANS showed low resistance to chemical and physical denaturation, in contrast, its alpha-helix is thermal resistant. Lost the disulfate bound decreases C-Terminus resistance to denaturation exhibiting the disulfate bond's importance to protein structure. CONCLUSION: C-terminus can protect cells N2A against stress caused by thapsigargin, on the other hand, N-terminus did not show the same activity, suggesting that the C-terminus domain is responsible for CDNF protection.

Keywords: Protein Structure, Neurotrophic factor, Parkinson's Desease / **Supported by:** CAPES

E.16 - Comparative Study of Phospholipase Activity of Crotalus durissus terrificus, Crotalus durissus cascavella and Crotalus durissus collilineatus Snake Venoms

Maria Gabriella Conceição [1], Lidia Jorge Tasima[1], Eduardo Oliveira Venancio de Lima[1], Sávio Stefanini Sant'Anna[1], Kathleen Fernandes Grego[1], Karen de Morais Zani[1], Anita Mitico Tanaka Azevedo[1]
[1]Laboratório de Herpetologia, Instituto Butantan (São Paulo, Brazil)

INTRODUCTION: *Crotalus durissus* is considered one of the most important species of venomous snakes in Brazil, due to the high mortality of its snakebites. Belonging to this only species present in Brazil, different subspecies are recognized in accordance with geographical distribution. Phospholipase A2 (PLA2) figures as the main bioactive component in crotalic venoms. So, the study of this enzyme is of medical/scientific interest given the important role that it plays in cell membrane degradation and in inflammatory diseases. OBJECTIVES: This study aimed to identify PLA2 by SDS-PAGE, HPLC and to compare the phospholipase activity of venoms from snakes of Crotalus durissus terrificus, *Crotalus durissus cascavella* and *Crotalus durissus collilineatus subspecies.* MATERIALS AND METHODS: Lyophilized venoms samples of each subspecies were analyzed by the following techniques: Bradford method, phospholipase activity, SDS-PAGE and HPLC. DISCUSSION AND RESULTS: Although few individual variations were observed in the venom samples of C.d. *terrificus*, C.d. *cascavella* and C. d. *collilineatus* with the presence of one or two bands on SDS-PAGE in the region corresponding to PLA2 (about 15 kDa). The results of PLA2 activity showed similar results between C. d. *terrificus* and C.d. *collilineatus*, with the average of 51.1 and 49.3 µ/min/mg, respectively. On the other hand, venom of the subspecies C.d. *cascavella* showed PLA2 activity about 2 times higher than the other two subspecies, with an average of 123.7 µ/min/mg. As a result, differences in phospholipase activity were noted between the different subspecies. CONCLUSION: The results presented by this work exhibit similar patterns regarding the electrophoretic profile of PLA2 by SDS-PAGE. However, it showed significant differences in PLA2 activity according to C.d. *ssp*. This difference may reflect on the envenomation caused by these snakes. As perspectives, we intend to analyze the quantitative differences between PLA2 of the venoms through the chromatographic profiles by HPLC.

Keywords: Venom, phospholipases A2, Crotalus durissus ssp

E.17 - Calcium interaction with HSPA1A Nucleotide Binding Domain

Amanda Helena Tejada [1,2], Carlos Sabino de Oliveira[2], Noeli Soares Melo da Silva[2], Júlio César Borges[2]
[1]Departamento de Genética e Evolução, Universidade Federal de São Carlos (São Paulo, Brazil), [2]Instituto de Química de São Carlos, Universidade de São Paulo (São Paulo, Brazil)

INTRODUCTION: The HSPA1A protein belongs to the Hsp70 family of molecular chaperones, which are involved in the protein quality control by participating in folding of nascent proteins, protein delivery to traffic through membranes, protein aggregation prevention, protein disaggregation and refolding and targeting of client proteins to proteolysis, among others. It has two domains: Nucleotide Binding Domain (NBD) and Peptide Binding Domain connected by a hydrophobic linker, which are regulated by a reciprocal heterotrophic allosteric mechanism. Hsp70s can be related to several human pathologies, such as: neurodegenerative diseases, diabetes and some cancers. Because of that, it is important to know their regulation mechanisms, including the interaction with Ca^{2+}, an important cellular messenger. OBJECTIVES: Purification of the recombinant NBD construct of the HSPA1A and the evaluation of calcium and magnesium ions effects on the domain structure. MATERIALS AND METHODS: Recombinant hHSPA1A_NBD was produced in a bacterial system and purified by 2 chromatographic steps: Ni^{2+} affinity and size exclusion chromatographies. Then, the secondary and the local tertiary structures were evaluated by Circular Dichroism (CD) and Intrinsic Fluorescence emission, respectively. These techniques were also used to evaluate the effect of Ca^{2+} and Mg^{2+} in the hHSPA1A_NBD structure. DISCUSSION AND RESULTS: The biophysical evaluations demonstrated that hHSPA1A_NBD was obtained pure, in the monomeric and folded states. Intrinsic Tryptophan Fluorescence emission indicates that Ca^{2+} or Mg^{2+} caused quenching. In addition, while a blue shift was observed in the presence of Calcium, a subtle red shift was registered in the presence of Magnesium. Furthermore, the presence of Calcium ions caused a significant increase in the midpoint of a temperature transition curve, monitored by CD at 222 nm. CONCLUSION: hHspA1A_NBD was obtained folded and the presence of the divalent ions led to different conformation changes, which can indicate that they interact with different binding sites.

Keywords: HSPA1A, Calcium, Magnesium / **Supported by:** CNPq

E.18 - Biophysical Characterization of the Interaction Between Grb2 Protein and DNA G-quadruplex

Larissa Fernanda Borges de Oliveira[1,2], Ahmed Zamal [3], Renan P. Pedro[1,2], Ícaro Putinhon Caruso[1,2,4], Fernando Alves de Melo[1,2]
[1]Department of Physics, São Paulo State University "Júlio de Mesquita Filho" (São Paulo, Brazil), [2]Multiuser Center for Biomolecular Innovation (CMIB), São Paulo State University "Júlio de Mesquita Filho" (São Paulo, Brazil), [3]Departments of Molecular and Cellular Oncology and Cancer Biology, The University of Texas MD Anderson Cancer Center (Texas, USA), [4]Institute of Medical Biochemistry (IBqM), Federal University of Rio de Janeiro (Rio de Janeiro, Brazil)

INTRODUCTION: Grb2 plays an important role in tyrosine kinase-mediated signal transduction, including binding of receptor tyrosine kinases to the Ras/MAPK pathway, which is implicated in oncogenic outcome. Due to its importance within the cell, is a potential target of studies to test and evaluate its interactions, such as the case of DNA G-quadruplex, composed of a guanine rich sequence and is present in DNA regions with high biological significance. Studies have described the presence of G-quadruplex in oncogene promoters, telomeres, and transcription start sites. The presence of these structures in such specific locations confirms that they play a crucial role in controlling a variety of cellular processes. OBJECTIVES: Considering the importance of DNA G-quadruplex, this work aimed to study its interaction with the Grb2 protein by fluorescence. MATERIALS AND METHODS: Grb2 was expressed in E. coli: BL21(DE3). It was realized an affinity purification with cobalt-affinity column IMAC-HiTrap-HP in buffer 500mM imidazole, 50mM Tris-HCl (pH 8.0), 100mM NaCl and 1mM β-ME. After, was applied in Sephacryl-100 resin in buffer 20mM Na_2HPO_4/NaH_2PO_4 (pH 7.0), 50mM NaCl and 1mM β-ME for size-exclusion chromatography. The binding of DNA to Grb2 was analyzed by fluorescence spectroscopy at temperatures of 288, 298 and 308K, in the presence and absence of DNA. DISCUSSION AND RESULTS: In a preliminary analysis, our results show that DNA interacts with Grb2, evidenced by fluorescence quenching of Grb2 as a function of increasing ligand concentration. Thermodynamic analysis of this interaction reveals to us a ΔH, $\Delta S < 0$ characteristic of Hydrogen bond or Van der Waals type interactions. Also, it was found that the protein-ligand binding is of the 1:1 type, that is, one ligand for each protein. CONCLUSION: This study allows us to understand at a molecular level the microenvironment in which Protein-DNA interacts and how it can be physically and structurally affected.

Keywords: DNA G-quadruplex, Fluorescence Spectroscopy, Grb2 / **Supported by:** FAPESP/Cnpq

E.19 - Using Brazilian Natural Products to Inhibit SARS-CoV-2 Replication

Marcos Freitas Parra [1], Fabio Albuquerque Marchi[2], Simone Queiroz Pantaleão[1], Antonio Sergio Kimus Braz[3], David Corrêa Martins Junior[1]

[1]Centro de Matemática, Computação e Cognição, Universidade Federal do ABC (SP, Brasil), [2]Hospital das Clínicas, Faculdade de Medicina, Univ. de São Paulo (SP, Brasil), [3]Centro de Ciências Naturais e Humanas, Univ. Federal do ABC (SP, Brasil)

INTRODUCTION: SARS-CoV-2 is the RNA virus that causes COVID-19, a disease that has infected 513,217,362 people worldwide, causing 6,260,235 deaths. A key factor for viral infection is the viral replication, with the involvement of polyprotein and non-structural proteins NSP7 and NSP8 with RdRp protein as part of the replication complex. To find inhibitors to block those interactions the molecular docking is a powerful tool. OBJECTIVES: Identify inhibitors for the SARS-CoV-2 replication complex. MATERIALS AND METHODS: The replication complex structure was obtained from Protein Data Bank (PDB), and we refine the model to build its loops through Modeller's DOPE protocol, which employs an optimized statistical potential for model evaluation. After, the chosen model was adopted as reference model for the in silico experiments. Using reference model, we applied FTMap to find the possible pockets for protein-ligands interactions using autodock Vina. The promising molecules were submitted to a molecular dynamics (MD) of 2000 ns. In the selected ligands, a bioisosteric substitution was be proposed for enhanced affinity and control of ADME-Tox. DISCUSSION AND RESULTS: The RNA-dependent RNA polymerase 7BTF, which contains the replication complex and is crucially involved with transcription machinery, was selected. Using Modeller, 2,000 possible models were modeled, and the reference was chosen. Using the NuBBE database of Brazilian natural products, we found inhibitors that possibly interact in the 2 different pockets in the regions of interaction with NSP7 and NSP8. Some molecules selected in docking already inhibit RdRp of other viruses. An MD was used to visualize the complex interaction of the chosen molecules. Finally, some modifications in the structure of the molecules were suggested to increase the affinity with the replication complex. CONCLUSION: With the refined model of RdRp we select possible inhibitors, which can be structurally improved and move towards the development of more accurate strategies for therapy.

Keywords: Docking, SARS-CoV-2, RdRp inhibitors / **Supported by:** FAPESP, CAPES e CNPq

E.20 - New Insights into the P186 Flip and Oligomeric State of Staphylococcus aureus Exfoliative Toxin E: Implications for the Exfoliative Mechanism

Carolina Gismene [1], Jorge Enrique Hernández González[1], Raghuvir Krishnaswamy Arni[1], Ricardo Barros Mariutti[1]

[1]Centro Multiusuário de Inovação Biomolecular, Instituto de Biociências, Letras e Ciências Exatas (São Paulo, Brazil)

INTRODUCTION: Staphylococcal exfoliative toxins (ETs) are glutamyl endopeptidases belonging to the serine protease family that cause the loss of cell-cell adhesion in the epidermis by cleaving the desmosomal protein desmoglein-1. These toxins exhibit complex action mechanism and exquisite specificities, being inactive against broad-spectrum SPase substrates. To date, four ETs, i.e., ETA, ETB, ETD and ETE, have been identified in different strains of *Staphylococcus aureus*. Of note, ETE is most recently reported ET, found in *S. aureus* isolates from ewe mastitis. The unusual properties of ETs have been traditionally linked to two unique structural features of ETs, namely, a highly-charged N-terminal alpha-helix and a 180° flip of the carbonyl oxygen (O) of the non-conserved residue at position 186, which points toward the catalytic serine thus rendering an inactive oxyanion hole. OBJECTIVES: From this description, the aim of this research was to structurally understand the specificities for activation of the action mechanism of ETs through an in-depth experimental and computational study of the ETE of *S. aureus*. MATERIALS AND METHODS: In this work, the expression, purification and crystallization of ETE ensured the resolution of the three-dimensional structure used in protein-protein docking simulations and umbrella sampling free energy calculations. DISCUSSION AND RESULTS: In the high-resolution crystal structure of ETE, two alternating conformations of P186 were observed exhibiting a 180° rotation of P186(O). This finding, together with free energy calculations conducted for monomeric ETE in solution, support the coexistence of both conformations under the tested conditions. Moreover, experimental results combining different techniques demonstrate that ETE forms homodimers in aqueous solution. Computational analyses identified the most likely structure of ETE homodimers and revealed the participation of non-conserved loops characteristic of the ETs in the interface formation. CONCLUSION: Finally, our predictions showed that the active site conformations can be influenced by ETE homodimerization, thus suggesting a link between the latter process and the exfoliative activity.

Keywords: Toxin exfoliative E , Staphylococcus aureus, Exfoliative mechanism / **Supported by:** FAPESP

E.21 - Quantification of the Interaction between Importin-alpha and Different Peptides by Fluorescence Spectroscopy

Hamine Cristina de Oliveira [1], Thiago R. Dreyer[1], Marcos R. M. Fontes[1]
[1]Departamento de Biofísica e Farmacologia, Instituto de Biociências de Botucatu - UNESP (São Paulo, Brasil)

INTRODUCTION: Biophysical methods have been used to increase the knowledge about molecular mechanisms applied to the study of interaction between biomolecules and ligands. Some techniques are able to experimently obtain the affinity between biomolecules, including fluorescence spectroscopy, a marker free technique. Importin-alpha is a protein responsible for recognizing process the molecules to be transported to cell nucleus. Fluorescence spectroscopy shows the presence and solvation of fluorescent amino acids in the sample, mainly tryptophans, changes in the concentration and environment of these amino acids generate a variation in the fluorescence spectrum. As importin-alpha has tryptophans at both binding sites, the presence of a ligand will cause a difference in signal, since binding will make this environment more hydrophobic. OBJECTIVES: The objective of this study was establishing a protocol for analyzing the interaction between the protein and different peptides. MATERIALS AND METHODS: The assays were performed using a Hitachi F7000 fluorimeter with 30µM protein and serial pipetting of 2µL ligand (300µM). The data analysis, considering the existence of dilution at each pipetting, was performed by subtracting the measurement from the isolated protein measurement and fitting a sigmoid curve to the data, thus obtaining the dissociation constant. DISCUSSION AND RESULTS: The adapted protocol was efficient in estimating of dissociation constant in all tested peptides, the values of this constant were compared with obtained by isothermal titration calorimetry (ITC). Although it is not possible to differentiate the binding at different sites, such as ITC, the values were very close to the average between the affinities in each of two sites. Data analysis highlighted the importance of subtracting the dilution effect at each point, as the decrease in concentration causes a depression in the signal. CONCLUSION: The protocol defined was effective for determination of affinity between the importin-alpha and peptides, indicating that fluorescence spectroscopy can be a useful tool to study the binding between biomolecules.

Keywords: affinity assays, fluorescence spectroscopy, importin alpha / **Supported by:** FAPESP

E.22 - CryoEM Structure of the *Xanthomonas citri* Type IV Pilus Secretin PilQ in Complex with TsaP

Gabriel Guarany Araujo[1], Edgar Enrique Llontop[2], Chuck Shaker Farah[2], **Germán Gustavo Sgro[3]**, Cristiane Rodrigues Guzzo[1]
[1]Departamento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo (SP, Brazil), [2]Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo (SP, Brazil), [3]Departamento de Ciências BioMoleculares, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo (SP, Brazil)

INTRODUCTION: Type IV pili (T4P) are bacterial retractable filaments that promote surface-associated twitching motility. The outer membrane channel through which the pilus emerges from the cell is made of an oligomer of PilQ, a member of the secretin superfamily which, in some bacteria, associates with the peptidoglycan-binding protein TsaP. We chose to study this complex in the phytopathogen *Xanthomonas citri*, where T4P also contributes to biofilm development and adherence to host leaves. OBJECTIVES: Determine the cryoEM structure of the T4P secretin complex from *X. citri*. MATERIALS AND METHODS: The native copy of PilQ was fused with msfGFP plus a StrepTag at its C-terminus by allelic exchange with a suicide vector. The mutant was grown in liquid KB medium supplemented with 2 mM $CaCl_2$ to induce the expression of the T4P machinery. Cells were lysed and the membranes collected by ultracentrifugation prior solubilization in DDM 1% and LDAO 0.8%. The sample was loaded onto a StrepTrap column and eluted in buffer with DDM 0.05% and desthiobiotin 2.5 mM. CryoEM samples were vitrified on Quantifoil R2/2 ultrathin carbon layer grids and the data was acquired in a Titan Krios G3i 300 kV cryoTEM coupled with a Falcon 3 DED at LNNano-CNPEM. Relion was used for single particle reconstruction and Coot and Phenix for atomic model building and refinement, respectively. DISCUSSION AND RESULTS: An initial refinement resulted in a 3.2-Å resolution map with C7 symmetry that allowed modeling the N0, N3, and Secretin domains of the tetradecameric PilQ core and both C-terminal domains of the surrounding heptameric TsaP ring. Further refinement and polishing enabled a 2.7-Å resolution map to be achieved, though the flexible N0 domain was lost during the reconstruction. CONCLUSION: Our results reveal the most detailed map yet of the PilQ-TsaP complex essential for T4P biogenesis.

Keywords: Type IV pili, Secretin, cryoEM / **Supported by:** FAPESP, CAPES

E.23 - Resistance profile of SARS-CoV-2 Mpro against Nirmatrelvir

Ellen de Sousa Silva [1], Gabriela Dias Noske[1], Isabela Dolci[1], Glaucius Oliva[1], Andre Schutzer Godoy[1]
[1]Instituto de Fisica de Sao Carlos, University of Sao Paulo (SP, Brasil)

INTRODUCTION: SARS-CoV-2 is the causative agent of COVID19. The viral Main protease (Mpro) is a key enzyme for viral cycle and one of the most promising targets for drug development. The recent emergency approved drug Paxlovid contains Nirmatrelvir, which is a Mpro inhibitor that had showed near 90% reduction in hospitalization during phase III clinical trials. Despite its high efficiency against wild-type Mpro, very little is still known about how Mpro active site mutations could generate resistance to Nirmatrelvir. OBJECTIVES: Therefore, we here identified from genomic databases sixteen mutation that exist in the radius of action of Nirmatrelvir and could affect its activity. MATERIALS AND METHODS: Using molecular biology and biochemical techniques, we produced the recombinant version of all these single mutants. All proteins were purified using standard techniques. We perform enzyme kinetic characterization for all the Mpro mutants and compared with wild-type. Furthermore, we tested the potency of Nirmatrelvir against each one of the mutants to identify possible resistant mutants. DISCUSSION AND RESULTS: As a result of the tests carried out, we observed that mutants M49I, A193V, R188K, M49T, M165I and N142S showed less than 2x fold increase in *Ki* when compared with the wild-type enzyme. Moreover, we see that mutants T190I, A191T, A193S, N142D, A191V, A193T and R188S showed between 2-3 fold increase in the *Ki* when compared with the wild-type. On the other hand, we saw that mutants Q189K and G143S showed significant resistance, with a *Ki* increase of 16.4 and 147.7, respectively, when compared with the wild-type. CONCLUSION: These results showed great advance in predicting resistance against Paxlovid. More in-situ studies are required for a better understanding the role of these mutations on Nirmatrelvir resistance.

Keywords: sars-cov-2, Mpro, nirmatrelvir
Supported by: FAPESP

E.24 - Structural Basis for the Interaction of Sis1 J-domain with the EEVD Motif of Hsp70

Carolina Oliveira Matos [1], Glaucia M. Squizato Pinheiro de Castro[1], Fabio Ceneviva Lacerda Almeida[2], Carlos Henrique Inacio Ramos[1]
[1]Institute of Chemistry, University of Campinas (São Paulo, Brazil), [2]National Center for Structural Biology and Bioimaging (CENABIO), Federal University of Rio de Janeiro (Rio de Janeiro, Brazil)

INTRODUCTION: Members of the co-chaperone Hsp40 (also known as J-proteins) family are characterized by the presence of the J-domain that is essential for the stimulation of the ATPase activity of Hsp70. Besides that, Hsp40s recognize and bind to unfolded or partially folded polypeptides and deliver them to the Hsp70. Hsp70s have a conserved EEVD tetrapeptide at the C-terminus, which is involved in ATPase activity and interaction with client proteins and Hsp40. Sis1, a type II Hsp40 from *Saccharomyces cerevisiae*, binds the EEVD motif, while Ydj1, type I, does not. Such Sis1-EEVD interaction is important to efficient partnership and is necessary for the ability of Sis1 to partner with Hsp70 in *In vitro* protein refolding. OBJECTIVES: The detailed mechanism of Sis1-EEVD interaction remains to be understood. Therefore, we started experiments aimed to solve the structure of the J-domain of Sis1 (residues 1-81) from S. cerevisiae bound to the EEVD motif peptide by Nuclear Magnetic Resonance (NMR). MATERIALS AND METHODS: Protein sample was enriched with 15N/13C and spectra were acquired on a Bruker 900 MHz at 25 °C. All NMR samples were prepared in 25 mM Tris-HCl (pH 7.5), 200 mM NaCl and 10 % D2O with 1:4 protein:peptide concentration. DISCUSSION AND RESULTS: The bound conformation of Sis1 1-81 displays 5 α-helices, 4 located in the J-domain (α1 to α4) and one at the G/F domain (α5). The α1 (residues 6-11), α2 (19-33), α3 (42-56), α4 (58-66) and α5 (69-74) folds in a similar way to free Sis1 1-81, but important differences were verified. The binding patch α2/α3 is open, when compared to the free state. α3 became twisted in the bound conformation, probably to accommodate the EEVD motif. CONCLUSION: Initial results are promising and more experiments are being conducted.

Keywords: HSP40 type II, J-domain, NMR
Supported by: Fapesp, Faperj, Capes and CNPq.

E.25 - Heparin modulates p53 aggregation, leading to an aggregation route with amyloidogenic properties

Natália Neves de Souza [1], Gabriela Assis de Lemos[1], Gileno dos Santos de Sousa[1], Giulia Diniz da Silva Ferretti[1], Caroline Augusto Barros[1,2], Tuane Cristine Ramos Gonçalves Vieira[1], Jerson Lima da Silva[1]
[1]Institute of Medical Biochemistry Leopoldo de Meis , [2]Department of Biochemistry and Molecular Biology, Federal Institute of Rio de Janeiro (Rio de Janeiro, Brazil)

INTRODUCTION: The p53 tumor suppressor protein is known as the guardian of the genome due to its functions of maintaining cell stability, responsible for DNA repair and blocking gene alteration. Mutations in the central domain of the protein are present in approximately 50% of human cancers, leading to alterations of p53 functions, promoting rapid proliferation of cells with mutated DNA, and forming intracellular aggregates. The propagation mechanism of these structural alterations has been studied and there are works that cite adjuvant molecules in this process. Among these molecules, glycosaminoglycans (GAGs), negatively charged carbohydrates such as heparan sulfate and chondroitin sulfate, are found in amyloid deposits of tumors, increasing their malignancy. However, the influence of these GAGs on the p53 aggregation process and tumor development are still unclear. OBJECTIVES: Our objective is to describe the effects of heparin on p53 aggregation (wild-type and mutant R280K), as well the thermodynamic parameters of this interaction. MATERIALS AND METHODS: To answer these questions, *in vitro*, we used spectroscopic techniques such as fluorescence, light scattering and circular dichroism. We also used tumor cell lines that express p53 (MDA-MB-231 and MCF-7) to investigate the cytotoxic effects of heparin. DISCUSSION AND RESULTS: By polarization, we observed that p53 and heparin interact with different binding affinity between wild-type and mutant R280K. At 25 °C, this interaction doesn't alter the secondary and tertiary structures of the protein, nor does it interfere with its thermal stability. However, at 37 °C, heparin modifies the p53 aggregation profile, promoting a slower kinetics and with influence of the ligand concentration. Regarding cell viability, there's no reduction relative to the treatment of cells with heparin. CONCLUSION: Considering the data obtained, heparin modulates temperature-induced p53 aggregation, leading to the formation of distinct aggregated species. Furthermore, the analysis of this interaction in tumor cell lines may elucidate the effect of this glycosaminoglycan on tumor development.
Keywords: amyloid, cancer, glycosaminoglycans / **Supported by:** CAPES, CNPq e FAPERJ

E.26 - Investigation of Hepatitis C Virus Core Protein Assembly and its Interaction with the Tumor Suppressor Protein p53

Fabiana Pestana Albernaz[1], **Antonio Leonardo Freitas Casalinho** [1], Vanessa Lopes de Azevedo Braga[1], Leandro Neves Thadeu Feitosa [1], Suellen Souza Barros de Moura[1], Luciana Pereira Rangel[2], Iaci Nunes Soares[3], Elio Anthony Cino[3], Katia Maria dos Santos Cabral[1], Theo Luiz Ferraz Souza[2], David Peabody[4], Jerson Lima da Silva[1], Andre Marco de Oliveira Gomes [1], Andréa Cheble de Oliveira[1]
[1]Instituto de Bioquímica Médica Leopoldo de Meis, Univ. Federal do Rio de Janeiro, Rio de Janeiro (RJ, Brasil), [2]Faculdade de Farmácia, Univ. Federal do Rio de Janeiro, Rio de Janeiro (RJ, Brasil), [3]Depto de Bioquímica e Imunologia, Universidade Federal de Minas Gerais (MG, Brasil), [4]Molecular Genetics and Microbiology, Unniversity of New Mexico (NM, U.S.A)

Hepatitis C virus (HCV) is a leading cause of chronic liver disease, cirrhosis, and hepatocellular carcinoma. Despite the advances, we still do not have a cure and effective treatment for all hepatitis C virus genotypes. HCV core protein (HCVcp) assembles the viral capsid and has several viral partners, among them the p53 tumor suppressor protein. The interaction between HCVcp-p53 has been attributed to the development of hepatocellular carcinoma during Hepatitis C but the molecular mechanisms involved are still poorly understood. In this work, we use Escherichia coli expression systems for biophysical characterization of the interaction between a 124 amino acids truncated form of HCVcp (C124) and p53 DNA binding domain (p53DBD) or between the entire p53 sequence (p53full). Mobilization assay, Nuclear Magnetic Resonance (NMR), Turbidity analyzes by spectrophotometry and Transmission Electron Microscopy were used to characterize the C124-p53 interaction. Binding assays by precipitation showed that, unlike what is seen with the p53DBD, C124 interacts more specifically with the p53full, showing the importance of the C-terminal region for interaction. 1H/15N HSQC NMR spectra of C124 presented chemical shift variation in the presence of p53DBD or p53full, possibly induced by the interaction. Turbidity analyzes demonstrated that C124 assembles into nucleocapsid-like particles (NLPs) in the presence of different and unspecific negative ligands, such p53 consensus DNA. Aggregates formed by the interaction between C124 and p53full were visualized by transmission electron microscopy. Studies that evaluate the characteristics of these aggregates are in progress.: Our data reveal a new approach to understand the nucleocapsid assembly and the HCVcp-p53 interaction, that can contribute for a better comprehension of the HCV infection.
Keywords: Hepatitis C virus, p53 tumor suppressor protein, nucleocapsid-like particles
Supported by: FAPERJ, CNPq, CAPES, INCT-INBEB

E.27 - Role of H310 in pH- and metal-sensitivities of Amydetes viviani firefly luciferase

Gabriel Felder Pelentir [2], Vanessa Bevilaqua[3], Vadim Viviani[1,2]
[1]Department of Physics, Chemistry and Mathematics, Universidade Federal de São Carlos (, Brazil), [2]Graduate Program of Biotechnology, Universidade Federal de São Carlos (, Brazil), [3]Faculty of Medical and Health Sciences, Pontifical Catholic University of São Paulo (, Brazil)

INTRODUCTION: Firefly luciferases emit yellow-green light, they are pH-sensitive, changing the bioluminescence color to red in the presence of heavy metals, pH and high temperatures. The Amydetes viviani luciferase has one of the lowest pH sensitivity among firefly luciferases, high bioluminescent activity, and spectral selectivity for cadmium and mercury, which makes it a promising analytical reagent. Recently, our group discovered the binding site of protons and metals, which consists of two salt bridges close to the phenolate group of oxyluciferin, E311/R337, and H310/E354. OBJECTIVES: The aim of this work is to better understand the role of residue H310 on pH and metal sensitivity and to improve Amydetes viviani luciferase for analytical purposes. MATERIALS AND METHODS: Amydetes vivianii luciferase mutants were performed using the Phusion™ protocol (Thermofischer), expressed in E.coli and purified by affinity chromatography on nickel matrix. The luminescence intensity was measured using a AB2200 luminometer (ATTO) in counts per second. The bioluminescence spectra was measured in ATTO Lumispectra spectroluminometer (ATTO). The KM values were calculated using Lineweaver−Burk plots taking the peak of intensity (I0) as a measure of V0. DISCUSSION AND RESULTS: Were obtained 7 mutants at position H310 (C, D, E, F, Q, T and R). There was no change in emission color among the mutants at pH 8.0. However, negatively charged mutants increased sensitivity to pH and metals, as well as having a spectral shift with zinc and lead. In contrast, H310F significantly reduced the spectral shift in acidic pH and divalent metals. The negatively charged mutants also highly affected bioluminescent activity, whereas only the threonine mutant increased it. There were no differences in bioluminescent activity with metals. CONCLUSION: The negative charge at this position breaks the salt bridge with the E354 residue and causes repulsion, changing the size of the cavity and increasing the polarity of this region, increasing the pH and metal sensitivity.
Keywords: Amydetes vivianii, Enzyme engineering, Metal biosensor / **Supported by:** CNPq , FAPESP and FAPESP

E.28 - Structural basis of Bacterial Type IV Pilus Biogenesis

Edgar Enrique Llontop Cornejo [1], Germán Gustavo Sgro[2], Shaker Chuck Farah[1]
[1]Departamento de Bioquímica, Instituto de Química (Sao Paulo, Brazil), [2]Departamento de Ciências Biomoleculares, Faculdade de Ciências Farmacêuticas de Ribeirão Preto (Sao Paulo, Brazil)

INTRODUCTION: Bacterial type IV pili (T4P) are flexible filaments found on the surface of a wide range of Gram-negative bacteria where they play a crucial role in in a variety of processes including motility, biofilm formation, and phage infection. T4P are dynamic structures that undergo cycles of extension and retraction powered by two hexameric ATPases. During extension, the T4P assembly ATPase PilB stimulates the polymerization of pilin monomers from the inner membrane, although the precise mechanism of polymerization is unknown. In both Pseudomonas aeruginosa and Xanthomonas citri, two proteins, FimX and PilZ, are involved in the regulation of T4P biogenesis by interactions with PilB. OBJECTIVES: Determine the cryoEM structure of the PilB-PilZ and PilB-PilZ-FimX complexes and understand the mechanism by which PilZ and FimX regulate T4P polymerization through their interactions with PilB. MATERIALS AND METHODS: Full length P. aeruginosa PilB, PilZ and FimX were co-expressed in E. coli cells and purified by standard chromatography techniques. Analytical size exclusion chromatography multi-angle light scattering (SEC-MALS) assays were also performed. CryoEM samples were vitrified in Quantifoil R2/1 grids and the images were acquired in a Titan Krios G3i 300 kV cryo-TEM coupled with a Falcon 3EC DED at the LNNano - CNPEM. Relion was used for single particle reconstruction. DISCUSSION AND RESULTS: Here, we show that FimX, PilZ and PilB form a stable ternary complex by SEC with a molecular weight of 697 kDa and PilB-PilZ complex with a molecular weight of 536-560 kDa. We also present the preliminary cryoEM map of PilB-PilZ and PilB-PilZ-FimX complexes. CONCLUSION: Our results show that FimX–PilZ–PilB interactions could be involved in the regulation of PilB function, where specific environmental signals sensed by FimX domains could be transmitted via PilZ to PilB. Also allow us to propose an internally consistent model for the PilB-PilZ-FimX complex and its interactions with the inner membrane platform of the T4P.
Keywords: Type IV Pilus, FimX-PilZ-PilB complex, c-di-GMP / **Supported by:** FAPESP

E.29 - Biophysical Studies of Monomeric Grb2 and its Interaction with Coumarin

Giovana Casteluci Moreira [1,2], Raphael Vinicius Rodrigues Dias[1,2], Jéssica Andrade Tedesco[1,2], Ícaro Putinhon Caruso[1,2,3], Fernando Alves de Melo[1,2]

[1]Department of Physics, São Paulo State University (São Paulo, Brazil), [2]Multiuser Center for Biomolecular Innovation, São Paulo State University (São Paulo, Brazil), [3]Institute of Medical Biochemistry Leopoldo de Meis, Federal University of Rio de Janeiro (Rio de Janeiro, Brazil)

INTRODUCTION: Growth factor receptor-bound 2 (Grb2) is an adaptor protein that plays a pivotal role in normal vs. oncogenic outcome, whereas only its monomeric state is capable of upregulating the MAPK signaling and the dimeric state is inhibitory to this process. Coumarin (1,2-benzopyrone) is a well-known molecule to have anticarcinogenic properties *In vivo* and it can be easily found in plants and seeds. Previous studies carried out by our group have shown that dimeric Grb2 interacts spontaneously with coumarin through Grb2-SH2 domain, driven majority by hydrophobic forces ($\Delta G < 0$, $\Delta H > 0$, $\Delta > 0$). OBJECTIVES: Therefore, the aim of this study was to characterize the structural and thermodynamic parameters of this interaction with the monomeric state in order to compare the results obtained to the dimer one. MATERIALS AND METHODS: Mutant Grb2 Y160F was chosen to prevent protein dimerization and was expressed in E. coli BL21(DE3). Fluorescence spectroscopy experiments were carried out at 291, 295, and 299K. Circular dichroism experiments were carried out at 0:1, 0.5:1, 1:1, and 3:1 concentrations of Coumarin:Grb2 to identify whether the interaction could provoke conformational changes in the protein's secondary structure. DISCUSSION AND RESULTS: Fluorescence suppression results showed that, like the dimeric version, monomeric Grb2 also interacts with coumarin in a spontaneous and entropically driven manner. The moderate binding constant of $K_b \sim 10^5\ M^{-1}$ was verified by different methodologies at a molar ratio of 1:1. CD spectra showed Grb2 secondary structure was not disturbed by the addition of coumarin. CONCLUSION: Given the importance of the protein and the molecule in the carcinogenic context, the results showed a moderate hydrophobic interaction that still requires further experiments to obtain a better understanding of the whole mechanism between the molecule and the protein in its monomeric state.

Keywords: Coumarin, Grb2, Protein-ligand interaction / **Supported by:** CAPES, FAPESP

E.30 - Dynamic Properties of the Dengue Virus Capsid Protein Bound to Nucleic Acid through Invisible States

Gabriela Rocha de Araújo [1,2], Glauce Moreno Barbosa[1], Andrea Thompson Da Poian[1], Fabio Ceneviva Lacerda Almeida[1,2]

[1]IBqM, Institute of Medical Biochemistry Leopoldo de Meis, Program of Structural Biology, Federal University of Rio de Janeiro, Brazil (Rio de Janeiro, Brasil), [2]CENABIO/CNRMN, National Center for Structural Biology and Bioimaging (CENABIO) / National Center for Nuclear Magnetic Resonance (CNRMN), Federal University of Rio de Janeiro, Brazil (Rio de Janeiro, Brasil)

INTRODUCTION: Dengue virus (DENV) is a mosquito-borne flavivirus considered a challenge to public health by WHO. The structure of DENV serotype 2 (DENV2) has been solved by cryo-electron microscopy, revealing an icosahedral symmetry on the viral envelope. There is no structural information for the nucleocapsid (NC. One hypothesis raised to explain this behavior was the presence of a dynamic equilibrium, with an intermediate state in the NC formation. The capsid protein of DENV2 (DENVC2) presents itself as a stable dimer, composed by four intertwined α-helices and a flexible intrinsically disordered N-terminal region. DENVC2 interacts with viral RNA, having a key role in the packaging of the viral genome. Our group has been studying the NC assembly *in vitro*, demonstrating the formation of an organized but very dynamic nucleocapsid-like particles by the addition of oligonucleotides such as 5 'GGG GG 3' (5G). OBJECTIVES: We aim to understand the dimer/oligomer balance in the capsid assembly. Since the saturation of DENVC2 with nucleic acid leads to precipitation, we used Nuclear Magnetic Resonance (NMR) to study the bound conformation in semi-saturated states. MATERIALS AND METHODS: Using NMR in solution and a 5G:DENVC2 molar ratio < 0.5, in which only soluble oligomers are formed, we measured the protein signal intensity decay, exposure of the amide groups to the solvent by sPRE and the relaxation dispersion data via CPMG. DISCUSSION AND RESULTS: We showed that the intensity of protein signal decreases with the addition of 5G, leading to the formation of invisible excited species. The characterization of these species by CPMG presented significant changes in the aromatic skeleton at the symmetry axis of DENVC2. CONCLUSION: These data present a fast dimer/oligomer balance, and this approach can reveal transient states of the nucleocapsid structure undetectable by other methodologies.

Keywords: Dengue virus, Capsid protein, Invisible species / **Supported by:** FAPERJ; CNPq; CAPES; FINEP

E.31 - Structural Characterization and DNA Binding Properties of the RRM Domain of the Plant Regulatory Protein AtGRP7

Gustavo DallOlio Cardoso [1]
[1]Departamento de Bioquímica, Instituto de Química, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: AtGRP7 (Arabidopsis thaliana glycine-rich protein 7) is a glycine-rich, RNA-binding protein that plays a central role in plant growth, development, and abiotic stress response. AtGRP7 consists of an N-terminal RNA recognition motif (RRM) followed by an intrinsically disordered, glycine-rich region. Despite the role played by AtGRP7 in cold adaptation and flowering time regulation in Arabidopsis thaliana, the biochemical mechanisms underlying its function are largely unknown. OBJECTIVES: Here, we used a plethora of biophysical techniques to characterize the structure, stability, and DNA binding affinity of AtGRP7-RRM. MATERIALS AND METHODS: The RMM domain of AtGRP7 (residues 1-90) was cloned into RP1B and expressed in Escherichia coli BL21 DE3 as a His6-fusion protein. AtGRP7-RRM was purified by a combination of nickel-affinity and size exclusion chromatography, and further analyzed by circular dichroism, NMR and fluorescence spectroscop DISCUSSION AND RESULTS: Circular dichroism revealed features of a typical RRM fold, containing a mixture of α-helices and β-sheets. AtGRP7-RRM displayed a melting temperature of 38 ° C, suggesting a lowly stable protein. Using multidimensional, triple resonance NMR, we unambiguously assigned 90% of the backbone resonances. The missing NH resonances belong to residues Met1, Ala2, Ser3, Gly4, Trp17, Arg43, Gln85, Ser86, Arg87, Gly88, Ser89, Gly90, most likely due to conformational dynamics and/or solvent exchange. At present, we are completing the side chain resonance assignments. The fluorescence emission spectrum of AtGRP7-RRM showed a maximum at ~349 nm, indicating that Trp17 is solvent exposed. Increasing concentrations of a 7-mer DNA oligonucleotide, consisting of a previously identified binding site, led to fluorescence quenching and AtGRP7-RRM interaction with DNA occurred with an apparent K_D of 17.9 ± 4.2 μM. CONCLUSION: This is an important first step toward the structure determination of AtGRP7-RRM, which will shed light into its DNA recognition mechanism.

Keywords: AtGRP7, Arabidopsis, structure / **Supported by:** CNPq, FAPERJ, CAPES

E.32 - Biophysical Analysis and Potential Applications of Porcine Circovirus Type 3 (PCV3) Virus Like Particles

Tamiris de Souza Rocha [1], Fernanda Angelica Sala[2], Richard Charles Garratt[1], Otavio Henrique Thiemann [1,3]
[1]Grupo de Biofísica e Biologia Estrutural, Instituto de Física de São Carlos (São Paulo, Brasil), [2]DRNPA-Ar, Univ. Federal de São Carlos (São Paulo, Brasil), [3]Departamento de Genética e Evolução, Univ. Federal de São Carlos (São Paulo, Brasil)

INTRODUCTION: Porcine circovirus is a single-stranded DNA virus known to infect swine. Four species are currently known: PCV1, PCV2, PCV3 and PCV4. It is, currently, the main etiological agent with severe economic impacts on swine production worldwide, therefore, the elucidation of the structure of the proteins involved in the composition of swine circoviruses can provide, based on structural information, the virulence factor in some members of this virus family and relevant targets to vaccine production or virus detection. OBJECTIVES: Purification of porcine circovirus type 3 virus-like particles (VLPs) followed by biophysical analysis of the VLPs by dynamic light scattering (DLS), differential scanning fluorimetry (DSF) and comparison with homologous PCVs from the literature. Furthermore, we will perform structural studies by negative staining, crystallography and/or cryo microscopy. MATERIALS AND METHODS: PCV3 genes were subcloned into pET28a and transformed into *Escherichia coli* BL21(DE3) cells and the protein production was induced by the addition of IPTG. The cultures were lysed, and the crude extract (CE) was clarified to collect the soluble fraction (FS) which was purified by sequential steps of ultracentrifugation in sucrose gradients and size exclusion chromatography. Based on SDS Page and western blotting analysis samples were chosen for DLS characterization. DISCUSSION AND RESULTS: The sucrose gradient followed by the size exclusion chromatography proved to be an appropriate method of purification. Purified samples revealed the formation of homogeneous VLPs of approximately 20nm, by DLS. CONCLUSION: Studies of coding genes and the results shown indicate that the proposed methodology is effective for the expression of soluble PCV3 proteins and formation of VLPs. Currently, the project is ongoing in order to refine the purification protocols for electron microscopy characterization for further structural analysis as well as the production of antiserum.

Keywords: negative staining, porcine circovirus, sucrose gradiente / **Supported by:** FAPESP, CNPq and CAPES

E.33 - Studies on mitochondrial chaperone system proteins TRAP-1 and CyP-D, characterization e interaction

Eduardo Feliciano de Lima Santos [1], Marina del Giudice[1], Sílvia Helena Libardi[2], Carlos Henrique Inácio Ramos[3], Júlio César Borges[2], Lisandra Marques Gava Borges[1]

[1]Departamento de Genética e Evolução (DGE), Universidade Federal de São Carlos (UFSCar), Centro de Ciências Biológicas e da Saúde (CCBS) (São Paulo, Brazil), [2]Departamento de Química e Física Molecular (DQFM), Universidade de São Paulo (USP), Instituto de Química de São Carlos (IQSC) (São Paulo, Brazil), [3]Departamento de Química Orgânica (DQO), Universidade Estadual de Campinas (UNICAMP), Instituto de Química de Campinas, IQM, (São Paulo, Brazil)

INTRODUCTION: Many of the biological functions are performed by proteins, including the maintenance of cellular homeostasis. Molecular chaperones are essential in this process, such as mitochondrial HSP90, TRAP-1, due to their ability to ensure correct protein folding and protect the cell from apoptosis. TRAP-1 is responsible for maintaining mitochondrial integrity and playing a protective role against reactive oxygen species. In this context, Cyclophilin-D (CyP-D) is also highlighted, a co-chaperone that regulates the activities of other interacting proteins. Both proteins are heavily studied as therapeutic targets, as imbalances in their functionality are related to diseases such as cancer. OBJECTIVES: The production of proteins was the initial objective, followed by the main focus of characterizing both recombinant proteins, as well as detecting and evaluating the *In vitro* interaction between them. MATERIALS AND METHODS: Both proteins were produced in E. coli BL21(DE3), and protein expression was induced by IPTG. Purification was achieved through immobilized metal affinity and size-exclusion chromatographies. The efficiency of the methods was verified by SDS-PAGE and protein concentration was determined by spectrophotometric measurements. A pulldown assay was performed to detect the interaction, and both proteins were submitted to analytical gel filtration for characterization. DISCUSSION AND RESULTS: Trap-1 and Cyp-d were efficiently expressed and purified, soluble, with a high degree of purity and homogeneity. Both were well characterized by analytical gel filtration and the pulldown assay was able to detect the interaction between Trap-1 and Cyp-d. CONCLUSION: Proteins were successfully produced, applying selected methodologies and protocols. TRAP-1 was detected as an asymmetric dimer in solution, while CyP-D was found as a globular monomer. The pulldown assay confirmed the physical interaction between the proteins under all conditions tested, which justifies and encourages a more in-depth and detailed investigation of this interaction, in the way of the search for the thermodynamic signature of the interaction and involvement in the mitochondrial chaperone system.

Keywords: Molecular chaperones, Trap-1, Cyp-D / **Supported by:** CNPq and FAPESP

E.34 - DNA-mediated Fabrication of Bradykinin Nanostructures

Thiago da Costa Lourenço [1], Lucas Rodrigues de Mello[1], Ian William Hamley[2], Clovis Ryuichi Nakaie[1], Marcelo Yudi Icimoto[1], Emerson Rodrigo da Silva[1]

[1]Biofísica, Univ. Federal de São Paulo (São Paulo, Brasil), [2]Chemistry Department, University of Reading (, United Kingdom)

INTRODUCTION: Introduction: Bradykinin (BK) is a short peptide-hormone belonging to the kinin-kallikrein system, having a vasodilator effect, and being related to the release of inflammatory mediators. For this reason, it has been explored as a blood pressure reducer, and its application is well established in the pharmaceutical industry as an active ingredient for antihypertensive drugs. On the other hand, no reports are available on nanostructured BK materials. An important BK analog is des-Arg9-BK (DBK), whose sequence is almost identical to that of BK, but lacking an arginine residue. DBK has a different mechanism of action, being an agonist of the B1 receptor. OBJECTIVES: Objective: We aimed at producing supramolecular assemblies based on BK and DBK. We also intended to provide detailed structural data on these assemblies and investigate if they exert cellular responses similar to the peptide in its monomeric form. MATERIALS AND METHODS: Methods: BK and DBK were complexed with DNA fragments. Steady-state fluorimetry was used to determine critical aggregation concentrations, whereas the secondary structure was analyzed through circular dichroism experiments. Atomic force microscopy and small-angle X-ray scattering were used to investigate the nanoscopic structure of the complexes. The cellular response was evaluated by monitoring calcium influx in HuVEC cells. DISCUSSION AND RESULTS: Results: We have found that DNA behaves as a template for BK strands, leading to the formation of nanoscopic fibrils stabilized by β-sheets. Interestingly, the same effect is not observed for DBK. We also identified that BK preferentially binds to the major grooves of DNA duplexes, whereas DBK intercalates in-between nucleotide bases. Importantly, BK-DNA fibrils were found to modulate calcium influx in HuVEC cells, thus preserving the bioactivity of the native peptide. CONCLUSION: Conclusion: We demonstrated a viable strategy to fabricate nanostructured matrices based on BK, which are potentially exploitable for the development of biomaterials endowed with hypotensive capabilities.

Keywords: peptide-hormone, DNA-peptide complexes, nanostructure / **Supported by:** FAPESP

E.35 - Importance of zinc ion in the stability of human DjC20 molecular chaperone

Amanda Laís de Souza Coto [1], Arthur Alexandre Pereira[1], Sabrina Dorta de Oliveira[1], Thiago Vargas Seraphim[1], Júlio César Borges[1]
[1]Instituto de Química de São Carlos, Universidade de São Paulo (SP, Brazil)

INTRODUCTION: The J-domain proteins (JDP) form a huge molecular chaperone family involved in protein homeostasis processes like protein folding, traffic through membranes and degradation/disaggregation. The JDPs are Hsp70 co-chaperones being capable of stimulating its ATPase activity as well as selecting and presenting client proteins to Hsp70. The JDP family members can be classified in 3 types or classes. Type III (or C) JDPs present only the J-domain conserved in some region of the protein, not necessarily at the N-terminal domain. In mitochondria DjC20 (a type III JDP) is involved in the biogenesis of Fe-S proteins and interacts with human mitochondrial Hsp70 (HSPA9). In this context, the zinc-finger domain present in the N-terminal of DjC20 appears to be essential for its functioning. OBJECTIVES: Understand the importance of Zn^{+2} for the hDjC20 structure stability. MATERIALS AND METHODS: The recombinant hDjC20 was expressed in E. coli BL21(DE3) strain by the pET28a expression vector and purified by Ni^{+2} affinity and size exclusion chromatographies. The structural stability of the protein in the absence and presence of EDTA was assessed by CD, DSC and intrinsic tryptophan fluorescence. Urea was used as chemical denaturant. DISCUSSION AND RESULTS: The removal of Zn^{+2} by dialysis with high concentrations of EDTA was not efficient and EDTA did not cause change in the spectroscopy properties of the protein. Thermal denaturation in the presence of EDTA led to reduction of ~4 ºC in the T_m. Urea-induced denaturation to hDjC20 provided a C_m of 4.0 M. The addition of 5 mM EDTA did not change the value of C_m, but caused a further red shift. CONCLUSION: The affinity of the Zn^{+2} with the protein is very high, evidencing its importance for hDjC20 structure. Urea and EDTA had a synergic action and caused an addition protein unfolding dependent of the removal of the metal.

Keywords: HscB, DjC20, molecular chaperones
Supported by: FAPESP, CNPq and CAPES

E.36 - Kinetic And Structural Characterization Of Dihydroorotate Dehydrogenase From Mycobacteria Tuberculosis

Olivia Teixeira[1,2], Ingrid Martins[3], Alexandre Suman de Araújo[4], Maria Cristina Nonato[1,2]
[1]Department of BioMolecular Sciences, School of Pharmaceutical Sciences of Ribeirão Preto (São Paulo , Brazil), [2]Department of Bio Molecular Sciences, Center for the Research and Advancement of Fragments and molecular Targets (São Paulo , Brazil), [3]Biophysics Institute Carlos Chagas Filho, Federal University of Rio de Janeiro (Rio de Janeiro , Brazil), [4]Department of Physics of the Institute of Biosciences, Letters and Exact Sciences (São Paulo , Brazil)

INTRODUCTION: Tuberculosis is a global challenge. Although treatable, resistant strains are becoming a serious threat to public health. The enzyme dihydroorotate dehydrogenase (DHODH) present in Mycobacterium tuberculosis fits into this scenario as a new and promising target. OBJECTIVES: Kinetic and structural characterization of the enzyme DHODH from Mycobacterium tuberculosis (MtDHODH) MATERIALS AND METHODS: Material and Methods: MtDHODH was expressed in E. coli CD43 and induced with IPTG and purified by metal affinity chromatography. The activity was measured by monitoring the orotate formation at 287 nm. Enzyme inhibition was measured by an indirect assay monitoring 2,6-dichloroindophenol (DCIP) reduction at 610 nm. A homology model was built using Modeller, and Molecular Dynamic experiments were performed using NAMD. DISCUSSION AND RESULTS: The protein obtained after purification was pure with a yield of 12 mg/L of cell culture. MtDHODH with the substrates (DHO and menadione (K3) performs the catalysis through a ping-pong mechanism, with KmDHO = 258±17 uM, KmK3= 145±10 uM, Kcat=2.69 ±0 .08 (s-1). In a second experiment, the quinone Qo, identified as the oxidant agent for all class 2 DHODH enzymes previously described, was also kinetically evaluated, with KmDHO= 250±60 uM, KmQ0= 334±55uM, Kcat=2.8±0.3(s-1) ki= 75±21uM. The catalysis with Q0 was identified to perform a ping-pong mechanism but surprisingly with the inhibition by the Qo substrate, Qo. Potent human DHODH (HsDHODH) inhibitors (teriflunomide, brequinar, atovaquone and ML390) were also evaluated against MtDHODH and negligible inhibition was observed. Molecular dynamics showed an N-terminal mobility already reported for other DHODHs, important for the druggability of inhibitor binding site. CONCLUSION: The kinetic characterization shows great potential for selectivity between HsDHODH and MtDHODH. Homology modeling together with the molecular dynamics simulation was crucial to map the structural bases that justify substrate and inhibitor selectivity between HsDHODH and MtDHODH. X-ray crystallography studies are under way.

Keywords: Kinetic , Mycobacterium tuberculosis DHODH, Molecular modeling and Dynamics / **Supported by:** Fapesp

E.37 - Title: Structural and interaction studies of RpiB from M. tuberculosis: screening of new compounds to a specific target

Karine Lima[1], Leonardo Bartkevihi[2], Danielle Oliveira[1], Fábio Almeida[2], Cristiane Anobom[1]
[1]Department of Biochemistry, Institute of Chemistry, Federal University of Rio de Janeiro (RJ, Brasil), [2]Institute of Medical Biochemistry, National Center of Nuclear Magnetic Resonance, Federal University of Rio de Janeiro (RJ, Brasil)

INTRODUCTION: Tuberculosis is one of the most lethal diseases widespread worldwide. It is considered an epidemic disease in several countries with an alarming number of cases. However, the investments in pharmaceutical industries are still low and some often used drugs have 50 years since their development turning conventional therapy ineffective. Thus, the research of more effective drugs for this disease is essential. Ribose 5-phosphate isomerase (Rpi) is an important enzyme involved in cellular anabolism, resulting in the synthesis of molecules such as nucleotides and cofactors. The RpiB is usually found in microorganisms and has no homologs in humans. Also, it is essential to cell growth, being considered an excellent target for the development of new inhibitors. OBJECTIVES: This work aims to study the structural, dynamics, and fragment screening studies of RpiB of Mycobacterium tuberculosis (MtRpiB) using Nuclear Magnetic Resonance. MATERIALS AND METHODS: MtRpiB was expressed in BL21(DE3), induced with 1 mM of IPTG, and purified with two steps of nickel-affinity and size-exclusion chromatography. All experiments required for the initial screening of a 560 fragments library was done using 50 µM of unlabeled MtRpiB using a Bruker Avance 500 MHz spectrometer at 298K. MtRpiB was isotopically labeled, the resonances were assigned, and the 15N relaxation experiments were acquired to analyze the protein dynamics. DISCUSSION AND RESULTS: Recently, MtRpiB was assigned, and interaction studies using different phosphorylated ligands showed the phosphate group importance to ligand binding. The NMR-dynamics studies of MtRpiB free and bound to ligands will be essential to monitored details of interaction. The pools containing fragments that interact with MtRpiB will be further isolated, and the interaction studied using chemical shift perturbation (CSP) and saturation transfer difference (STD) experiments. CONCLUSION: The structural studies and fragment screening could bring new information to the design of inhibitor compounds against tuberculosis.

Keywords: Ribose-5-phosphate-isomerase-B, dynamics, biomolecular interaction

E.38 - Sequence Diversity of *Trypanosoma cruzi* Ribose-5-phosphate Isomerase B: Mapping and Potential Impact on Drug Design

Daniel Cêdo Borges [1], Sophia Lincoln Cardoso de Azevedo[1], Rafael Ferreira[1], Mayla Abrahim[2], Marcos Catanho[2], Teca Calcagno Galvão[1], Ana Carolina Ramos Guimarães[1]
[1]Laboratório de Genômica Funcional e Bioinformática, Fundação Oswaldo Cruz (, Brasil), [2]Laboratório de Genética Molecular de Microrganismos, Fundação Oswaldo Cruz (, Brasil)

INTRODUCTION: Chagas disease's treatment is associated with many problems, such as drug resistance. So, therapeutic alternatives are being researched, and different authors suggest the *Trypanosoma cruzi* enzyme ribose-5-phosphate isomerase (TcRpiB) as a potentially new pharmacological target. In previous work, we virtually screened molecules that potentially bind to this protein, and that could be new drugs to treat the disease by selectively inhibiting the activity of TcRpiB. However, *T. cruzi* genetic diversity has been a significant obstacle to successful drug development, since variation in the sequence of pharmacological targets can affect the susceptibility of the parasite to new chemotherapeutic agents. OBJECTIVES: To map the diversity of TcRpiB protein sequences to investigate whether the variations found can impact drug design. MATERIALS AND METHODS: TcRpiB sequences were obtained from the GenBank, TriTrypDB and UniProt KB databases. Additionally, whole genome sequencing files of *T. cruzi* present in the SRA were processed to retrieve TcRpiB gene from 212 unique isolates from different countries. The three-dimensional models of the variants were obtained with the Modeller program, and evaluated according to the MOLPDF function and normalized DOPE score. DISCUSSION AND RESULTS: From 277 compiled TcRpiB sequences, 36 were obtained with substitutions at fourteen positions. Seven of these are close to residues of functional relevance. The Y46C (present in a single sequence) and M98I (found in ~50% of the sequences) substitutions can be considered relevant, since they are adjacent to the amino acids involved in the enzymatic function. The generated three-dimensional models confirmed that Y46C and M98I are located in regions with a critical role in catalysis. CONCLUSION: Our results demonstrate the essentiality of considering sequence variations during the development of drugs against the parasite and point to the need for additional analyzes that investigate in more detail any implications that the variations may have.

Keywords: *Trypanosoma cruzi*, Drug Design, Ribose-5-phosphate isomerase / **Supported by:** CNPq

E.39 - Structural Studies of the Leishmania spp PinX1 Orthologue

Stephany Cacete de Paiva [1], Hamine Cristina de Oliveira[2], Guilherme Henrique M. Salvador[2], Carlos Alexandre H. Fernandes[3], Marcos Roberto de Mattos Fontes[2], Carlos Henrique Inácio Ramos[4], Maria Isabel Nogueira Cano[1]
[1]Department of Chemical and Biological Sciences, Institute of Biosciences, São Paulo State University (São Paulo, Brazil), [2]Department of Biophysics and Pharmacology, Institute of Biosciences, São Paulo State University (São Paulo, Brazil), [3]IMPMC UMR 7590, Sorbonne Universite (, Ilê de France), [4]Institute of Chemistry, University of Campinas (São Paulo, Brazil)

INTRODUCTION: Eukaryote telomeres, including *Leishmania* spp., are nucleoprotein structures key for genome stability and cell proliferation. They are maintained by the telomerase ribonucleoprotein complex minimally composed of the TERT (telomerase reverse transcriptase) and the TER (Telomerase RNA). Regulation of telomerase activity is due to the coordinated action of proteins associated with the RNP complex and telomeric proteins. An ortholog of one of these regulators found in *Leishmania*, named PinX1, preserves the N-terminal G-patch domain, although it presents a less conserved TID (telomerase inhibitory domain). PinX1 is considered a natural inhibitor of telomerase activity since it can sequester the TERT component from the complex by competing with the TER. OBJECTIVES: Our goal is to elucidate the structure of Leishmania PinX1 (LPinX1) and its interactions with the telomerase components. MATERIALS AND METHODS: To predict the secondary structure of the protein and the telomerase-PinX1 relationships, we used AlphaFold. Recombinant LPinX1 was obtained using a bacterial system. The protein was isolated from inclusion bodies and purified by affinity chromatography. Western blot analysis with a specific polyclonal serum was used to confirm protein purification. Circular Dichroism (CD) was used to determine its secondary structure and thermostability. DISCUSSION AND RESULTS: AlphaFold secondary structure prediction revealed that LaPinX1 is composed of random coils and α-helices. The recombinant LPinX1 was expressed in its biologically active form, and its CD analysis confirmed the presence of mainly α-helices and random coils. Moreover, the protein also showed high thermal stability preserving its secondary structure up to 90°C. Western blot analysis helped identify the recombinant protein and its presence in parasite nuclear extracts. The next step will be to perform LPinX1-TERT interaction assays. CONCLUSION: Recombinant LPinX1 was obtained in purified form, is thermostable, and its structure is mainly composed of random coils and α-helices.

Keywords: PinX1, Leishmania, telomeres / **Supported by:** Capes

E.40 - Study of the interaction of substrates and inhibitors of the enzymatic activity of the Old Yellow Enzymes of Leishmania braziliensis

Thais Carvalho de Moura [1], Silvia Helena Libardi[1], Júlio César Borges[1]
[1]Instituto de Química de São Carlos, Universidade de São Paulo (São Paulo, Brazil)

INTRODUCTION: The disease caused by the protozoan*Leishmania braziliensis* affects thousands of Brazilians and still doesn't have an effective treatment. The search for new therapies involves the study of specific targets for the development of new active compounds and/or inhibitors. The Old Yellow Enzymes (OYE) are flavoenzymes that carry out oxidation reactions of NAD(P)H promoting the reduction of FMN prostetic group. Reduced FMN reacts with substrates containing an alkene group neighboring an electron-withdrawing group such as aldehydes and ketones. OYEs have an affinity for phenolic compounds that, when bound, form "π–π stacking" interactions. OBJECTIVES: This work seeks to evaluate potential substrates already studied for other OYEs and to determine the enzymatic activity of OYE from *L. braziliensis* (LbOYE) in the presence of phenolic and ketone compounds to observe their inhibitory effect on the enzymatic activity. MATERIALS AND METHODS: The recombinant protein was expressed in Escherichia coli and purified by nickel affinity and preparative size exclusion chromatographies. The enzyme activity was followed by the decay of the NADPH absorbance at 340 nm in the presence of 4-hydroxydobenzaldehyde, 4-Hydroxy-3-methoxybenzaldehyde (vanillin), 4-hydroxy-3-methoxybenzoic acid (vanillic acid) and menadione, using NEM (n-ethylmaleimide) as a substrate. The interaction between LbOYE and the aforementioned ligands was evaluated by absorbance and fluorescence quenching at different temperatures to obtain the thermodynamic signature by van't Hoff equation. DISCUSSION AND RESULTS: LbOYE showed higher Kcat values using NEM as a substrate than O_2. Some of the ligands showed significant inhibitory effects upon LbOYE activity upon NEM. Fluorescence quenching experiment pointed that all ligands interacted with LbOYE with dissociation constant at micromolar range. In addition, the absorbance spectra of LbOYE in the presence of the phenolic compounds indicated the existence of a charge transfer band derived from a "π–π stacking" interaction. CONCLUSION: All studied compounds interacted with LbOYE but showed a divergent thermodynamic signature.

Keywords: old yellow enzymes, L. Braziliensis, flavoenzymes

E.41 - Interaction Studies of Hsp90 and a Putative TPR-protein from Plant

Gabriel da Silva Possato Gonçalves [1], Annelize Zambon Barbosa Aragão[1], Carlos Henrique Inácio Ramos [1]
[1]institute of Chemistry , University of Campinas (São Paulo, Brazil)

INTRODUCTION: Hsp90 is an important chaperone that interacts with about 10% of the cellular proteome, acting in maintenance of the homeostasis and thus helping many clients to fold correctly. Several co-chaperones interact with Hsp90 modulating their function. This interaction is defined by the presence of TPR (tetratricopeptide repeat) domains that binds the C-terminal MEEVD motif of the Hsp90. OBJECTIVES: In this work, the objectives were to express, purify TPR-protein from plant and identify the interaction with a plant Hsp90 MATERIALS AND METHODS: Both recombinant proteins were expressed in E. coli, strain BL21 (DE3), using a pET28a vector system, purified on Nickel Affinity Chromatography followed by Size Exclusion Chromatography (SEC). Purifications were of about 95% and yields of about 0.5 mg/mL. Interaction studies were performed using analytical SEC (a-SEC), mixed the sample in different proportions of concentration monomers of each protein. After the a-SEC, we used SDS-PAGE analyzed by ImageJ software DISCUSSION AND RESULTS: The results indicated positive interaction with a stoichiometry of 2 Hsp90: 1 TPR-protein. CONCLUSION: In summary, this study identified a putative TPR-protein that interacts with Hsp90.

Keywords: Heat Shock Protein, Interaction, TPR-domain Protein

E.42 - Double Helix RNA Denaturation Activity and Specificity of Nucleocapsid Protein Interaction of SARS-COV-2 by Nucleic Acids.

Peter Reis Bezerra [1], Icaro Caruso[3], Gisele Amorim[2], Fabio Almeida[1]
[1]Instituto de Bioquímica Médica Leopoldo de Meis, Federal University of Rio de Janeiro (RJ, Brazil), [2]Campus Duque de Caxias, Federal University of Rio de Janeiro (RJ, Brazil), [3]Depto de Física, IBILCE, São Paulo State University (SP, Brazil)

INTRODUCTION: Betacoronaviruses possess an RNA genome with approximately 30,000 bases where TRS leader (TRS-L) and body (TRS-B) regions are present. They regulate the discontinuous transcription process of coronaviruses through the formation of subgenomes. The Nucleocapsid (N) protein is essential for the efficient replication of the viral genome and participates in the catalysis of the transcription of subgenomes. Despite performing functions with nonspecific interactions such as viral nucleocapsid assembly, the N protein has other specific intracellular activities, such as regulation of viral protein expression via binding to transcriptional regulatory sequences (TRSs). OBJECTIVES: To understand the molecular recognition, dynamics, and structural aspects of interactions of the N-terminal domain of the SARS CoV 2 N protein with regulatory RNAs, we used the Nuclear Magnetic Resonance technique among other biophysical methods. MATERIALS AND METHODS: N-protein samples were obtained by heterologous expression with His-Tag in BL-21 (DE3) from pET-28a plasmid vector, followed by His-tag nickel affinity purification. For the acquisition of NMR experiments, samples were labelled with 15N and 13C isotopes. Relaxation parameters were collected by relaxation dispersion experiments via CW-CPMG. DISCUSSION AND RESULTS: The CPMG data indicated the existence of a conformational change in the intermediate time scale, from μs to ms, in the regions where interactions with nucleic acids occur. The thermodynamic analysis in the transition to the excited state revealed a greater entropic contribution to the ΔG. Fluorescence Resonance Energy Transfer (FRET) experiments showed the denaturing activity of the DNA and RNA double helix. Anisotropy experiments displayed the affinity constants with RNA TRSs. CONCLUSION: Our data demonstrate that the N protein has DNA and RNA double-stranded opening activity in TRSs. Furthermore, residues in conformational exchange map the region of N protein interaction with nucleic acids and characterizes a conformational equilibrium that can select interactions promiscuous or specific to promote diverse functions.

Keywords: SARS-CoV-2, Nucleocapsid protein, RNA/DNA binding
Supported by: CAPES, CNPq, FAPERJ, FAPESP

E.43 - Structural Studies of Zika Virus Envelope Protein at Different pH Values that Mimic the Infectious Cycle

Antonio Leonardo Freitas Casalinho[1], Fabiana Pestana Albernaz[1], **Ana Carolina Duarte Bavier** [1], Suellen Souza Barros de Moura[1], Joana D'arc Ribeiro Argibay[1], Cristiane Dinis Ano-Bom[2], Ana Paula Canedo Valente[1], Jerson Lima da Silva[1], Andre Marco de Oliveira Gomes [1], Andrea Cheble de Oliveira[1]
[1]Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro (, Brazil), [2]Instituto de Química, Universidade Federal do Rio de Janeiro (, Brazil)

INTRODUCTION: Zika Virus was first isolated in Zika forest, Uganda. It belongs to the Flaviviridae family and has a single-stranded positive-sense RNA encoding a polyprotein which is cleaved in 10 proteins, including the envelope glycoprotein (E). The envelope protein plays a crucial role in the infection cycle as in the cell-entry and membrane fusion, and E is also the major target of neutralizing antibodies described in literature. Zika has been associated to newborn microcephaly and Guillain-Barré syndrome. Great efforts are being made to increase our understanding of Zika infection aiming the prevention, treatment or cure of Zika pathologies. OBJECTIVES: In this work we seek to obtain a recombinant E full length and the truncated Domain I and Domain II (DIDII) forms and develop a structural characterization of the E protein in different pH values as seen in infection and search for a structural stabilizer for future Nuclear Magnetic Resonance (NMR) characterization. MATERIALS AND METHODS: We express the recombinant E and DIDII proteins in an E.coli expression system and use affinity chromatography as purification strategy. Bruker 600 MHz spectrometer was used to obtain the NMR spectra and tertiary structural changes were studied by Intrinsic Tryptophan Fluorescence (ITF) spectroscopy. DISCUSSION AND RESULTS: Our ITF data suggest that the SDS 0,1%, a non-desaturating concentration, is capable of inducing tertiary structural gain at both pH 6 and pH 7 to both E and DIDII proteins. 1H NMR experiments at pH 6 showed a good dispersion of both proteins and confirmed the absence of urea and imidazole used to obtain both proteins. 15N HSQC spectra of E and DIDII were obtained to first observe the behavior of both proteins at pH 6. CONCLUSION: Our data are very promising in the structural studies of E protein dynamic and may contribute with important information about Zika pathogenesis.
Keywords: Envelope Protein, Flavivirus, Zika Virus

E.44 - Crystallographic and biophysical analysis of ANC1

Mariana Lino de Souza Gil [1], Carolina Gimenes Oliveira[1], Luis Felipe Santos Mendes[1], Olívia Teixeira[2], Maria Cristina Nonato[2], Antonio José da Costa Filho[1]
[1]1 Molecular Biophysics Laboratory; Physics Department - FFCLRP , University of São Paulo (São Paulo, Brasil), [2]Protein Crystallography Laboratory - FCFRP, University of São Paulo (São Paulo, Brasil)

INTRODUCTION: The family of proteins called Golgi Reassembly and Stacking Proteins (GRASP) is essential for the lateral connections of the Golgi complex, as well as for the unconventional secretion of protein cargos. The first reconstructed node in the evolution of GRASP was the connection between GRASP55 and GRASP65 within the Metazoa kingdom. This first ancestor (ANC1) would represent the GRASP of the first Metazoan to appear in evolution. The study of the GRASP ancestor is important for understanding, for example, the fundamentals of Golgi formation. Moreover, although we already know the structure and function of the GRASP domain, there is no structural information about the entire GRASP structure (including the SPR domain). In the ongoing project, crystallographic analyses and denaturation studies were performed with the ANC1 protein of the GRASP family. For the denaturation studies, circular dichroism, steady-state fluorescence, and differential scanning calorimetry were used to follow the thermal denaturation of ANC1. Preliminary crystallization studies were performed to optimize the obtention of good crystals for future X-ray diffraction experiments. The data will shed light on the similarities and differences between ANC1 and the modern GRASPs.
Keywords: ANC1, crystallography, protein
Supported by: FAPESP, CNPq and CAPES

E.45 - Characterization of an alpha neurotoxin from the venom of *Micrurus altirostris* species

Raquel Teixeira Lessa [1], Jessica Matos Kleiz Ferriera[1], Marcius da Silva Almeida[1], Jan Tytgat[2], Russolina Benedeta Zingali[1]

[1]IBqM, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil), [2]CENABIO, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brazil), [3]Toxicology and pharmacology, KU Leuven (, Belgium)

INTRODUCTION: Acetylcholine is the main neurotransmitter in the arasympathetic and somatic nervous systems, playing an essential role in the control of voluntary and involuntary movements through interactions with muscle-type nAChR. This channel is a pentameric ligand-gated ion-channel widely expressed in both central and peripheral nervous systems. Considering the involvement of nAChR in diverse physiological and pathological conditions, the characterization and understanding of these receptors are necessary. The Three-finger toxins (3FTx) are an abundant group of neurotoxins found in the venoms of elapids found worldwide, including in the Brazilian coral snakes. These toxins are used in the study of nAChR since they are potent modulators, therefore, becoming important tools for research on the harmacology/biotechnology fields. The *Micrurus altirostris* species is an elapid snake well distributed in the south of Brazil and has about 80% of its venom composed by 3FTxs. **OBJECTIVE:** In the present work, our goal is to characterize the structure-function of a 3FTx isolated from the *M. altirostris* venom with promising activity on nAChR. **MATERIAL E METHODS:** In order to screen the venom, we fractionated it by RP-HPLC and tested the fractions activities on nAChR expressed in Xenopus laevis oocytes by *Two-microelectrode Voltage Clamp technique.* **DISCUSSION AND RESULTS:** In a previous work, we found that M. altirostris venom fully inhibited the muscle-type nAChR. The second RP-HPLC venom fraction showed potent activity on the muscle-type and partial activity on α7 neuronal-type of nAChR, proving itself to be even more potent than the whole crude venom, in terms of reversibility. Intact mass analysis of this fraction revealed two proteins, with the major one called “TF1” by our group. Our next step is to express the TF1 in Pichia pastoris system. Structure studies by NMR and other biological experiments will be conducted to use TF1 as a tool to understand nAChR features.

Keywords: Micrurus altirostris, Nicotinic Acetylcholine Receptors, Three finger toxins / **Supported by:** CNPq

E.46 - Biophysical Characterization of a TPR-protein from Sorghum bicolor

Larissa Machado Antonio [1], Gustavo Henrique Martins[1], Annelize Zambon Barbosa Aragão[1], Carlos Henrique Inacio Ramos[1]

[1]Institute of Chemistry, University of Campinas (São Paulo , Brazil)

INTRODUCTION: All cells have specific mechanisms to defend themselves from aggression caused by a variety of external stresses that are imposed by the environment. Stresses can induce unproductive protein species, misfolding and aggregation. To verify these undesired events, proteostasis is maintained by the protein quality control system, constituted by molecular chaperones. The Heat shock protein 90 (HSP90) is one of the most abundant and conserved molecular chaperone that contributes to various cellular processes including signal transduction, intracellular transport, and protein degradation. A conserved MEEVD motif at the C terminus of HSP90 serves as the docking site for the interaction with tetratricopeptide repeat (TPR) proteins. OBJECTIVES: In this work, we aimed to investigate the structure-function relationship of a TPR-protein from S. bicolor. MATERIALS AND METHODS: DNA sequence was used to generate a clone inserted into a pET28a vector system, which were transformed into the Escherichia coli strain BL21 (DE3). The recombinant TPR-protein was found in the soluble fraction and was submitted to a Nickel Affinity Chromatography followed by Size Exclusion Chromatography (SEC) for purification. Protein conformation was done by Circular Dichroism (CD), Tryptophan fluorescence, Size Exclusion Chromatography coupled to Multi-angle and Quasi-Elastic Light Scattering (SEC-MALS-QELS) and Analytical SEC (a-SEC). DISCUSSION AND RESULTS: CD and fluorescence spectroscopy analyses showed that the protein was folded, had predominantly α-helical secondary structure and was stable up to 67°C. Moreover, SEC-MALS-QELS and a-SEC experiments determined that the putative TPR-protein was a monomer in solution with hydrodynamic radius of 4.3 (± 0.5) nm and diffusion coefficient of 6.3x10-7 (± 1.4x10-7) $cm^2.s^{-1}$. CONCLUSION: Thereby, these results confirm important contributions on the structure of a TPR-protein by biophysical techniques. Functional assays to determine activity are underway and the results might contribute to better understand protein interactions in plants.

Keywords: Chaperone, Heat Shock proteins, TPR-domain protein / **Supported by:** CNPq; FAPESP

E.47 - Myristoylation and its effects on the human Golgi ReAssembly and Stacking Proteins

Emanuel Kava[1], Antônio José da Costa Filho[1]
[1]Física, Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: GRASPs are myristoylated peripheral membrane proteins localized in the Golgi faces and involved in the Golgi structure maintenance and the regulation of unconventional secretion pathways. GRASPs achieves its main functionalities in the Golgi organization and, when bound to the lipid bilayer, its orientation relative to the membrane surface is restricted to determine its proper trans-oligomerization. The role of myristoylation in GRASP is involved with its oligomerization, and there is an impact of such protein organization on the membrane-anchoring properties. OBJECTIVES: This work aims to elucidate the influence of the lipidation in GRASPs protein-protein and protein-lipid interactions. MATERIALS AND METHODS: Circular Dichroism (CD), Fluorescence Spectroscopy and Liposome Electrophoretic Mobility Assay (LEMSA). DISCUSSION AND RESULTS: There is an influence of this co-translational modification in GRASPs oligomerization , which leds to a clear effect in lipid interaction. CONCLUSION:
Keywords: Myristoylation, Oligomerization, Membrane protein
Supported by: FAPESP

E.48 - L-asparaginase from Escherichia coli: novel insights about the behavior of flexible part of the active site

Jhenifer Yonara de Lima [1], Stephanie Bath de Morais[1], Tatiana De Arruda Campos Brasil de Souza[1]
[1]Laboratory for structural and computational proteomics, Fiocruz/PR - Instituto Carlos Chagas (Paraná, Brasil)

INTRODUCTION: Acute Lymphoblastic Leukemia (ALL) is primary cancer in childhood and has a significant occurrence after the age of 50. L-asparaginase II of *Escherichia coli* (EcA) has been used as a powerful tool against ALL, the leading childhood cancer, since 1978. L-asparaginase is essential for high survival rates for patients fighting ALL in which abnormal lymphocytes are unable to differentiate and proliferate unchecked, which leads to impaired functions of various organs. L-asparaginase works by depleting circulating asparagine, essential for tumor cell survival. EcA preparations are the most efficient among the available asparaginases for therapy, promoting a longer event-free time rate and the highest half-life in patient blood. However, their use might cause hyper sensibility reactions in 60% of patients and toxic effects. EcA variants could result in less harmful/immunogenic alternatives to ALL treatment. OBJECTIVES: The understating of EcA activity determinants towards asparagine is crucial to generating optimized molecules. MATERIALS AND METHODS: Herein, crystals were obtained by the sitting-drop vapor-diffusion method, and X-ray diffraction data were collected at the Brazilian Synchrotron Light Laboratory, Campinas, Brazil. Three EcA structures with different ligand in active site were solved by molecular replacement method using the program Phaser and the atomic coordinates of *E. coli* L-asparaginase II (PDB ID: 3ECA). The model was examined and manually fitted using the program COOT; refinement cycles included secondary structure, reference-model restraints, and translation/libration/screw parameters and were made onto Phenix and with a visual inspection of the electron density maps and manual rebuilding with COOT, its quality was assessed using Molprobity. DISCUSSION AND RESULTS: The structures presented here indicated a pattern of flexibility/rigidity within EcA homotetramer. CONCLUSION: Our analysis brings novelty to understanding the dynamic behavior of EcA based on a set of structures that, different from the others, presents a mixed profile of flexibility among the subunits comprising the homotetramer.
Keywords: L-asparaginase, Catalytic mechanism, Protein dynamics

E.49 - Structure-Activity Analysis Of The TrkA - PLCγ Interaction And The Development Of Inhibitors That Play An Analgesic Role Based On Such Interaction

Allan Pradelli Roldão [1], Noemi Jacó de Souza[1], Helder Veras Ribeiro Filho[2], Fernanda Aparecida Heleno Batista[2], Gustavo Penteado Battesini Carretero[1], German Gustavo Sgro[3], Ana Carolina Migliorini Figueira[2], Paulo Sérgio Lopes de Oliveira[2], Deborah Schechtman[1]
[1]Dep. of Biochemistry, Institute of Chemistry (, Brazil), [2]Brazilian Biosciences National Laboratory, Brazilian Center for Research in Energy and Materials (, Brazil), [3]School of Pharmaceutical Sciences of Ribeirão Preto, University of Sao Paulo (, Brazil)

INTRODUCTION: Our group is investigating specific signaling pathways activated in pain, based on the TrkA/NGF system to identify new targets. TrkA, a tyrosine kinase coupled receptor, is the high affinity receptor for NGF. Upon binding, several well defined signaling pathways are activated. Amongst them we have shown that activation of phospholipase PLCγ is critical for mechanical nociception in an inflammatory pain model. PLCγ regulatory core presents two tyrosine kinase binding domains, n and cSH2, and an intramolecular interaction inhibiting cSH2 has been proposed to keep the lipase inactive. It is important to track which domain is best suitable for receptor anchoring and the specificity of this intramolecular interaction. Importantly, we developed a peptide inhibitor of the interaction between TrkA and PLCγ that led to analgesia. OBJECTIVES: To rank the specificity of PLCγ SH2 domains and to characterize the TrkA/PLCγ interaction site for the development of molecules that target this interaction. MATERIALS AND METHODS: Molecular dynamics simulations using Amber, and MMPBSA for *in silico* affinity determination were used to predict binding of the domains for peptides derived from receptor tyrosine kinases that bind to PLCγ and a proposed auto-inhibitory peptide in the phospholipase. Fluorescence anisotropy and 8-anilino-1-naphtalenesulfonic acid fluorometry assays of the purified domains and synthetic peptides for determination of binding and affinity constant. DISCUSSION AND RESULTS: The *in silico* affinity was determined for a set of peptides derived from 11 receptors and a peptide derived from PLCγ, ranking the most favorable interactions between the two SH2 domains. The binding of nSH2 to TrkA peptide was confirmed by fluorometry and its affinity constant determined by anisotropy. CONCLUSION: We are developing a platform for new analgesic drugs discovery based on the TrkA/PLCγ interface structure. The binding of each domain to several receptors may give evidences for specificity and help us develop more specific modulators along with understanding basic signaling of PLCγ.

Keywords: TrkA, PLCg, Structural characterization / **Supported by:** FAPESP and CAPES

E.50 - Septin engineering: chimeras involving the coiled-coil region

Eloy Condori Mamani [1], Ana Paula Ulian de Araujo[1], Richard Charles Garratt[1]
[1]Instituto de Física de São Carlos, Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: Septins are considered the fourth component of the cytoskeleton, as they are found in various eukaryotes and participate in different cellular processes. An important property of septin subunits is their ability to assemble into a specific linear sequence forming hexameric and octameric heterocomplexes, which pol-ymerize into filaments. OBJECTIVES: Seeking to assess the importance of the C-terminal region of septins in the se-lection of partners for septin-septin interactions, we produced a chimeric human septin. MATERIALS AND METHODS: The C-terminal domain, including a coiled coil region of SEPT7, was fused to the G domain of SEPT9, generating a SEPT9G/7C chimera. Using this con-struct, we tested if the chimera, when co-expressed with the partner septins SEPT2G and SEPT6 (in the absence of SEPT7), would assemble into a hex-amer, indicating the replacement of SEPT7 by SEPT9G/7C. DISCUSSION AND RESULTS: Septins 2-6-9/7 were co-eluted as a complex after affinity and size-exclusion chromatographies (Superdex 200). SEC-MALS measurements showed that the 2-6-9/7 heterocomplex exhibited some oligomeric states, one of which corre-sponds to a hexamer. CONCLUSION: The detection of a hexameric assembly, with the SEPT9G/7C chimera in place of SEPT7, reinforces the importance of the coiled coil region for correct partner selectivity. Taken together, the results show that it is possible to replace SEPT7 by SEPT9 in the hexamer, just by grafting the C-terminal domain of SEPT7 into the G-domain of SEPT9.

Keywords: Septin, chimera, coiled-coils.
Supported by: FAPESP grant # 2020/02897-1

E.51 - Cryo-EM of Leishmania braziliensis Hsp100 Disaggregation Machine Reveals a Split Spiral Structure and Mg2+/Nucleotide-Dependent Dynamics.

Thiago Vargas Seraphim[1], Sérgio Luís Ramos Junior[1], Fernanda Aparecida Heleno Batista[1], Vitor Hugo Balasco Serrão[2], **Júlio César Borges[1]**

[1]Dept of Molecular Physics and Chemistry, São Carlos Chemistry Institute, University of São Paulo (São Paulo, Brazil), [2]Biomolecular Cryo-Electron Microscopy Facility, University of California Santa Cruz (California, USA)

INTRODUCTION: ClpB/Hsp104 is a ring-like hexameric AAA+ ATPase, typically involved in protein remodeling by threading polypeptides through its central pore using chemical energy from ATP. ClpB/Hsp104 bears a N-terminal regulatory region (ND) and two stacked AAA+ ATPase domains (NBD1 and NBD2); a coiled-coil middle domain (MD) lies within NBD1. As part of a bichaperone system involved in thermotolerance, ClpB/Hsp104 cooperates with Hsp70/Hsp40 chaperones to recover proteins from aggregates. In trypanosomatids of the genus *Leishmania*, a ClpB/Hsp104 orthologue, named Hsp100, has been found to be critical for parasite development and profusely expressed during heat stress. Additionally, it has been shown that Hsp100 plays a role in the biogenesis of exosomes and secretion during mammalian host invasion by the parasite. Despite the central role of Hsp100 during *Leishmania* life cycle, a deep understanding of its molecular basis of function is still missing. OBJECTIVES: This work aimed to unravel the solution behavior and structure of a Hsp100 from *Leishmania braziliensis* (LbHsp100). MATERIALS AND METHODS: The oligomeric state of LbHsp100 and effect of adenosine nucleotides were investigated by size exclusion chromatography (SEC) and analytical ultracentrifugation (AUC). High-resolution structures of LbHsp100 in its apo, Mg^{2+} and nucleotide-bound states were solved by cryogenic electron microscopy (cryo-EM). DISCUSSION AND RESULTS: We found that, in solution, LbHsp100 protomers assemble into hexamers in a concentration-dependent manner through at least three intermediate species; adenosine nucleotides shifted the equilibrium towards oligomerization. High-resolution electronic maps of LbHsp100 revealed dodecameric, split lock-washer structures with central pore loops organized in a spiral manner. Interestingly, we observed that apo LbHsp100 is highly dynamic, whereas Mg^{2+} and ATP-bound LbHsp100 adopts more rigidified structures, suggesting the conformational control exerted by Mg^{2+} /adenosine nucleotides and the reinforcement of protomer-protomer contacts. CONCLUSION: LbHsp100 is a hexameric spiral-like disaggregation machine whose conformations are in part controlled by Mg^{2+} and adenosine nucleotides.

Keywords: Molecular chaperones, AAA+ ATPase, Cryo-EM / **Supported by:** FAPESP, CNPq

E.52 - Cloning, Expression, and Purification of Uga1p Protein of *Saccharomyces cerevisiae*

Bianca Lujan Pereira [1], José Ribamar dos Santos Ferreira-Junior[1]

[1]Biomedical Sciences Institute, University of Sao Paulo (Sao Paulo, Brazil), [2]School of Arts, Sciences and Humanities, University of Sao Paulo (SP, Brazil)

INTRODUCTION: GABA (gamma-aminobutyric acid) is a non-protein amino acid present in several organisms with different functions. In mammals, especially humans, it is an inhibitory neurotransmitter in the brain that is, directly and indirectly, involved in several neurological disorders, such as schizophrenia, seizures that may be related to autism, mood and anxiety disorders. In yeast, it regulates the excess of glutamate, and this regulation is made by redox reactions. Therefore, GABA also modulates the oxidative stress status of the cell, limiting the production of reactive oxygen species. Uga1p protein (ABAT in humans) is a transaminase responsible for converting GABA and 2-oxoglutarate to glutamate and succinate semialdehyde, respectively. OBJECTIVES: The crystallographic structure of Uga1p has not yet been resolved, and this project aims to purify this protein to perform crystallization experiments. MATERIALS AND METHODS: The gene UGA1 was cloned into pET28a(+) vector, and the protein expressed in BL21(DE3) cells. Protein extraction was performed by osmotic shock procedure. DISCUSSION AND RESULTS: The pET28a(+)-UGA1 expression system was successfully transformed into the competent cells, and the identity of the clone confirmed by DNA sequencing. After extraction of the protein, the lowest abundance suggested that codon optimization is necessary to improve its expression. A yeast expression system is also being considered. CONCLUSION: The project is progressing towards structural resolution of Uga1p, It is expected that the crystallographic data to be obtained can be used to elucidate the mechanism of the Uga1p catalyzed reaction.

Keywords: Uga1p, GABA, *Saccharomyces cerevisiae* / **Supported by:** CAPES

E.53 - Characterization of a novel dimeric variant of the amyloidogenic protein transthyretin involved in familial amyloidotic cardiomyopathy

Lucas do Amaral Martins [1], Priscila Ferreira[1], Cinthia Lima[1], João Vasques[1], Marcus Jager[2], Cecilia Monteiro[2], Humberto Pereira[3], Fernando Palhano[1], Jeffery Kelly[2], Márcia Waddington-Cruz[4], Debora Foguel[1]
[1]Instituto de Bioquímica Médica Leopoldo De Meis, Univ. Federal do Rio de Janeiro (Rio de Janeiro, Brasil), [2]Department of Chemistry, The Scripps Research Institute (, USA), [3]Instituto de Física de São Carlos, Univ. Federal de São Carlos (São Paulo, Brasil), [4]Centro de Estudos de Paramiloidose Antônio Rodrigues de Mello, Univ. Federal do Rio de Janeiro (, Brasil)

INTRODUCTION: The Transthyretin (TTR) is an homotetrameric serum protein enrolled in the transport of thyroid hormones and retinol binding protein. Over 100 different mutations have been described in its gene and one of the most prominent types of TTR related amyloidosis is the familial amyloid cardiomyopathy (FAC), a slow, progressive, and fatal disease. Recently, our group described a new variant in a Brazilian family that leads to FAC: A39D-TTR. Position 39 is located within the AB-loop, a critical region for tetramer formation and which is very important to the context of aggregation. OBJECTIVES: Characterize the structure of A39D-TTR and understand its implications to FAC. MATERIALS AND METHODS: We expressed A39D-TTR in E. coli BL21 DE3, analyzed it using Size Exclusion Chromatography (SEC) and solved its structure using X-Ray Crystallography. We accessed A39D-TTR stability with High Hydrostatic Pressure (HHP), Urea Denaturation and Bis-ANS binding. Also, we evaluated its aggregation kinetics using 330nm absorbance and ThioflavinT binding, observing the aggregates with Transmission Electron Microscopy (TEM) together with dot blotting to identify oligomers used to test its toxicity in a cardiac cell line, H9C2. DISCUSSION AND RESULTS: SEC indicate that this variant is a dimer in solution due to side chain rearrangement in the AB-loop region visualized by X-Ray Crystallography. HHP, urea denaturation and Bis-ANS binding experiments revealed the low stability of A39D-TTR and studying the aggregation kinetics together with TEM images showed us that this mutation is more aggregation prone than WT-TTR. Finally, dot blotting A39D-TTR showed us that this mutation also leads to the formation of oligomers that proved to be toxic to H9C2 cell lines. CONCLUSION: A39D-TTR is a dimer in solution due to the instability in its structure, that leads to a faster aggregation kinetics than WT-TTR and to toxicity to cardiac cells, mimicking the severe FAC observed in the patient.

Keywords: Amyloidosis, Familial Amyloidotic Cardiomyopathy, Transthyretin / **Supported by:** CNPq

E.54 - Title: Structure, Dynamics and interaction of FKBP12 Enzyme from Different Microorganisms: a New Biological Target for Inhibitors Against Tuberculosis and Neglected Diseases

Victor Cantanhede de Souza [1], Karine Belo Rocha de Lima [1], Isabela Cardoso de Azevedo e Sousa[1], Guilherme Caldas Andrade [2], Francisco Gomes Neto[3], Danielle Maria Perpetua de Oliveira Santos[1], Fábio Ceneviva Lacerda de Almeida[2], Cristiane Dinis Anobom[1]
[1]Department of Biochemistry, Institute of Chemistry , Federal University of Rio de Janeiro (Rio de Janeiro, Brasil), [2]Institute of Medical Biochemistry, National Center of Nuclear Magnetic Resonance, Federal University of Rio de Janeiro (Rio de Janeiro, Brasil), [3]Oswaldo Cruz Institute, Oswaldo Cruz Foundation (Rio de Janeiro, Brasil)

INTRODUCTION: Neglected Tropical Diseases (NTDs) are mainly found in tropical and subtropical regions and constitutes a serious public health problem with significant economic impact. Tuberculosis epidemic annually affects about 10 million people worldwide.The rising resistance and lack of more effective therapy have become a considerable concern. Thus, the investigation of new targets and the development of more effective drugs against these diseases is crucial. FKBP12 proteins, an essential peptidyl-prolil-cis-trans-isomerase to numerous disease-causing microorganisms, are reported as potential biological targets. They differ about 40% in their primary sequence when compared to their human ortholog. OBJECTIVES: This work aims to study the structure, dynamics, and interaction of FKBP12 from different microorganisms and compare it to Homo sapiens ortholog. MATERIALS AND METHODS: Prospection studies using the primary sequence of human FKBP12 was done using bioinformatics tools. FKBP12 from Mycobacterium tuberculosis (MtFKBP12), *Trypanosoma cruzi* (TcFKBP12), and Leishmania infantum (LiFKBP12) were selected. Previously, the group performed structural, dynamics, and interaction studies with MtFKBP12. TcFKBP12 and LiFKBP12 were cloned into pET28a and transformed in E. coli strains. Expression tests were performed using different parameters and were monitored by 15% SDS-PAGE. DISCUSSION AND RESULTS: TcFKBP12 best expression condition was achieved using BL21(DE3) strain and induction with 0.5 mM of IPTG (OD600:0.8) at 18oC for 16 hours. LiFKBP12 best expression condition was achieved using the same strain and induction with 0.2 mM of IPTG (OD600>1.0), at 37oC for 4 hours. Purification methods were applied using two steps: affinity and size-exclusion chromatography. Afterward, TcFKBP12 and LiFKBP12 proteins will be isotopically labeled with 15N and 13C to perform all experiments required for NMR assignment and dynamics studies. CONCLUSION: These studies will bring important information about specific regions of FKBPs that will be important to the studies of fragment screening for the search for new compounds against Tuberculosis and NTDs.

Keywords: Enzymes, FKBP12, Structure

E.55 - Modulation of Alpha-synuclein Aggregation by Organic compounds

Adriana Pereira Ramos [1], Gabriel Dos Santos Ribeiro[1], Lucas Caruso Araujo Bezerra[2], Arthur Eugen Kümmerle[2], Maria Leticia C. Barbosa[1], Ingrid Del Castilo[1], Julia R. Clarke[3], Yraima Moura Lopes Cordeiro[1]
[1]Faculdade de Farmácia, Universidade Federal do Rio de Janeiro (Rio de janeiro, Brasil), [2]Programa de Pós-Graduação em Química (PPGQ), Universidade Federal Ruraldo Rio de Janeiro (Rio de Janeiro, Brasil), [3]Instituto de Ciências Biomédicas, Universidade Federal Rural do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: Parkinson's disease (PD) is the second most prevalent neurological disorder and is characterized by the degeneration of dopaminergic neurons in the substantia nigra and the subsequent loss of dopamine innervation on striatum. Affected neurons accumulate intracytoplasmic inclusions called Lewy bodies, which are composed mainly of α-synuclein (α-syn) protein. α-syn aggregates are also associated to other neurodegenerative disorders known as synucleinopathies. Several lines of evidence suggest that small aggregates such as oligomers are the most toxic forms of α-syn. Thus, compounds that reduce the formation of toxic α-syn species may be of interest to PD therapy. OBJECTIVES: Considering that α-syn has prion-like properties, we aimed to investigate the efficacy of trimethoxy-chalcones (J8, LC90 and LC91) previously shown to have anti-prion activity, in modulating the aggregation of wild-type and mutant forms of α-syn. LC90 and LC91 were previously shown to remodel α-syn fibrils. MATERIALS AND METHODS: We expressed and purified recombinant α-syn (WT and the E46K mutant) to produce oligomeric species of α-syn. Aggregation was monitored by binding of thioflavin-T. Aggregated α-syn were characterized by Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM). DISCUSSION AND RESULTS: Our preliminary TEM results showed that we obtained both fibrils and oligomeric α-syn species after the aggregation protocol, with a wide hydrodynamic radious range, as evidenced by DLS. Aggregation in the presence of J8 resulted in more organized α-syn fibrils, indicating fibril eemodeling by this trimethoxy-chalcone. CONCLUSION: We will next evaluate the *In vitro* and *In vivo* efficacy of the selected organic compounds in mammalian cell culture and in animal models of PD.
Keywords: Aggregation, Alpha-synuclein, Oligomers / **Supported by:** CNPq

E.56 - Investigating the molecular interaction of human Bip (Heat Shock Protein A5) with Calcium

Pâmela Thays da Silva Baima [1], Noeli Soares Melo da Silva[1], Júlio César Borges[1]
[1]Dept. Chemistry and Molecular Physics, São Carlos Institut of Chemistry, University of São Paulo (SP, Brazil).

INTRODUCTION: 70 kDa Heat shock proteins (Hsp70) are ubiquitous molecular chaperones acting in protein homeostasis, including folding of nascent proteins, translocation of polypeptides through membranes, assembly and disassembly of protein complexes, targeting proteins for clearance, among other functions. HSPA5, also called immunoglobulin binding protein (BiP), is the human Hsp70 abundantly present in the endoplasmic reticulum (ER). Under stressful conditions, HSPA5 migrates to the nucleus, cytoplasm, mitochondria and cell surface, in which it performs functions related to cell proliferation, apoptosis, regulation of innate and adaptive immunity and modulation of the response to unfolded protein response. Recently, it has been seen that the Ca^{2+} strengthening the Hsp70 binding to the adenosine nucleotides, mainly to the ADP. Such interaction would lead Hsp70 to a slower releasing of client proteins, letting a longer time of chaperone-client interaction, which should allow to reach an adequate client-protein fate. OBJECTIVES: To study the molecular interaction of the recombinant HSPA5 (rHSPA5) with calcium ions. MATERIALS AND METHODS: rHSPA5 was produced in bacterial system and purified by 2 chromatographic steps: Ni $^{2+}$ affinity and size exclusion. The interaction with Ca^{2+} was monitored by circular dichroism (CD), intrinsic tryptophan fluorescence emission, isothermal titration calorimetry (ITC), differential scanning calorimetry (DSC) and ATPase activity. These experiments were done in comparison to Mg^{2+}. DISCUSSION AND RESULTS: Our results have shown that rHSPA5 was obtained in the folded and functional states. The presence of Ca^{2+} led to a reduction of the ATP hydrolysis rate in comparison to the presence of Mg^{2+}. The intrinsic fluorescence of tryptophan showed that calcium causes a suppression in the protein fluorescence, while ITC and DSC have shown some differences regarding its interaction with nucleotides in comparison with assays in the presence of Mg^{2+} CONCLUSION: These results help in a better understanding and guiding future studies on the Hsp70 chaperone family.
Keywords: HSPA5, Calcium, Molecular chaperone
Supported by: FAPESP, CNPq and FAPEMA.

E.57 - Interdomain Dynamics and Regulation of Grb2 Activity in the Presence of Effectors

Aléxia Santos Silva Valadares [1,2], Larissa Saraiva da Silva Santos[1,2], Ramon Pinheiro Aguiar[1,1], Fernando Alves de Melo[3], Fábio C. L. Almeida[1,1]

[1]Institute of Medical Biochemistry Leopoldo de Meis (IBqM), Federal University of Rio de Janeiro (Rio de Janeiro, Brazil), [2]National Center of Structural Biology and Bioimage (CENABIO), Federal University of Rio de Janeiro (Rio de Janeiro, Brazil), [3]Department of Physics, Multiuser Center for Biomolecular Inovation (CMIB), São Paulo State University (São Paulo, Brazil)

INTRODUCTION: Growth factor receptor-bound protein 2 (Grb2) is an adapter protein that participates in the activation chain of some receptor tyrosine kinases, such as EGFR and FGFR. Grb2 is of paramount importance in preventing these receptors from being activated without the ligands. Grb2 forms complexes that activate the mitogen-activated protein kinases (MAPK) signaling pathway, leading to cell proliferation. OBJECTIVES: Our goal is to evaluate the hypothesis that the microenvironment modulates the interdomain dynamics and consequently can regulate Grb2 activity. We used effectors that changes the protein solvation and measured the protein dynamics by Nuclear Magnetic Resonance (NMR). MATERIALS AND METHODS: The protein expression with isotopic labeling of the atoms ^{15} N, ^{19} F, and ^{2} H was used *E. coli* BL21 DE3 strain. We purified Grb2 by nickel affinity followed by a molecular exclusion. The NMR experiments consist of measuring the interaction with peptides derived from the SOS1 recognition region (VPPPVPPRRR) to the SH3 domain of Grb2 and a phosphopeptide from the EGF recognition region (EpYINSQV) by the SH2 domain. DISCUSSION AND RESULTS: We collected the relaxation parameters (R1, R2) of ^{19} F-Grb2, and all experiments were performed with free protein and in the presence of peptides and effectors. Our results indicate that ^{19} F-tryptophan is a good probe for the domain dynamics. The ^{19} F measurements provide information on the dynamics of each of the ^{19} F-tryptophans present in the protein. Two tryptophan's are in the N-terminal SH3 domain, two in the SH2 domain, and one in the C-terminal SH3 domain. CONCLUSION: The presence of SOS1 and EGF peptides increased the ^{19} F-Trp freedom, as expected from the Grb2 monomerization. Beyond that, some of them are in conformation exchange and have relevant interdomain motion.

Keywords: fluorine NMR, Grb2, interdomain dynamics / **Supported by:** FAPESP, FAPERJ, CNPq, CAPES

E.58 - There and back again. Reincorporation of the Fe (II) cofator in 1,2-dihydroxynaphthalene dioxygenase (DoxG)

Diego Magno Martins [1], Mozart Silvio Pereira[1], Tiago Antônio da Silva Brandão[1]

[1]Department of Chemistry, Federal University of Minas Gerais (Minas Gerais, Brazil)

INTRODUCTION: The 1,2-dihydroxynaphthalene dioxygenase from ⟨i⟩Pseudomonas⟨/i⟩ sp. C18 (DoxG) is a Fe(II)-dependent extradiol dioxygenase that catalyzes the conversion of 1,2-dihydroxynaphthalene into 2-hydroxy-2H-chromene-2-carboxylate. This enzyme exhibits a large active site endorsing promiscuity able to cleave, in addition to catechol substrates, polychlorinated biphenyls. This unusual capacity contrasts with the facile oxidation of the Fe(II) cofactor by dioxygen to yield Fe(III), a process that occurs throughout the convenient enzyme purification under aerobic conditions. OBJECTIVES: Herein, we determine what factors affect cofactor reconstitution of the enzyme activity. MATERIALS AND METHODS: Steady-state kinetics using UV-vis spectroscopy were carried out for the reaction of the substrate 3-methylcatechol to determine the effect of pH, buffer nature, ionic strength, and cofactor concentration for enzyme activation. DISCUSSION AND RESULTS: The rate of holoenzyme reconstitution depends on several factors: (1) activation is rate-dependent up to about 0.4 mM Fe(II) and was more efficient than considering common reducing agents; (2) the reactivation follows a bell-shaped pH-rate profile with maximum at pH 5.7, in which rates were impaired due to the limited availability of the cofactor at higher pHs and to protonation of residues involved in cofactor binding at lower pHs; (3) salts like NaCl and KCl did not affect the reactivation, but sulfate ion, a stronger ligand to Fe(II) affected the cofactor availability; (4) reactivation of 90% of enzyme is achieved after 2.5 hours of incubation; (5) enzyme co-purified containing Mn(II) were more stable and still prone of reactivation by replacement with Fe(II). CONCLUSION: We have established a protocol for DoxG reactivation that provided enzyme activities similar to those reported under anaerobic conditions. Our studies provided ground to understand how the interaction between Fe(II) and DoxG is affected by experimental conditions and to pave the way of further aerobic studies with DoxG and other Fe(II)-dependent extradiol dioxygenases.

Keywords: Enzyme, Extradiol Dioxygenase, Reactivation

E.59 - Characterization of the thermodynamic profile of the interaction between Grb2 - SH2 domain and coumarin

Gabriela Tognoni Moreira [1,2], Renan Pereira Pedro[1,2], Icaro Putinhon Caruso[1,2,3], Fernando Alves de Melo[1,2]
[1]Departament of Physics, Institute of Biosciences, Humanities and Exact Sciences, São Paulo State University "Júlio Mesquita Filho " (SP, Brazil), [2]Multiuser Center of Biomolecular Innovation, São Paulo State University "Júlio Mesquita Filho" (SP, Brazil), [3]Institute of Medical Biochemistry, Federal University of Rio de Janeiro (Rio de Janeiro , Brazil)

INTRODUCTION: Grb2 is an adaptor protein with significant relevance in cell signaling transduction, specially on kinase-mediated signaling by linking receptor tyrosine kinases to Ras-MAPK pathway, which can lead to oncogenic outcome. Grb2 is composed of three domains, including the SH2-domain, which is responsible for binding to specific phosphotyrosine containing motifs on Receptor-Tyrosine-Kinases, transferring information for signaling in the cytosol. Due to its importance, it is interesting to analyze the interaction between SH2-domain and a small molecule, such as coumarin, a phenolic molecule with many important properties such as anticarcinogenic and anti-inflammatory response. OBJECTIVES: Therefore, this study aimed to characterize the interaction between these molecules by fluorescence spectroscopy in order to investigate the micro-environment changes induced by different physico-chemistry conditions. MATERIALS AND METHODS: SH2-domain was expressed in E.coli:BL21(DE3) and purified by cobalt-affinity column IMAC-HiTrap-HP equilibrated in 50mM-Tris-HCl buffer containing 100mm-NaCl and 500mM-imidazole at the pH 8.0. Afterwards, the sample was loaded into a Sephacryl-100 column equilibrated with 20mM Na_2HPO_4/NaH_2PO_4 buffer containing 100mM-NaCl at the pH 7.0 for size-exclusion chromatography. Fluorescence spectroscopy was used to characterize the interaction between SH2-domain and coumarin at the temperatures of 288K, 298K and 308K. DISCUSSION AND RESULTS: Our results have shown that the SH2-domain interacts with coumarin, in a 1:1 protein-ligand molar ratio, with an affinity constant of $K_b \sim 10^5\ M^{-1}$. Throughout the thermodynamic profile analyzed, it was seen that ΔH, $\Delta S > 0$, meaning that the interaction was hydrophobically guided; furthermore, Gibbs free energy (ΔG) was negative, indicating a spontaneous reaction for the interaction. The aforementioned analysis was made using a 100mM-NaCl concentration. CONCLUSION: This study allows us to characterize the interaction of Grb2-SH2-domain with coumarin at a molecular level and understand how the changes in the environment influences the physical and structure properties of the interaction.

Keywords: Coumarin, Fluorescence, Grb2

E.60 - Comparative genomics of R-body determinants

Gabriel Sánchez Hueck [1], Robson Francisco De Souza[1]
[1]Microbiologia, Instituto de Ciências Biomédicas (São Paulo, Brasil)

INTRODUCTION: R-bodies are proteic ribbon structures which, in appropiate plasmic conditions, can unroll and puncture the cell that produces them, causing death and release of cellular contents. However, the role and genomic context of reb genes responsible R-body polymerization is not yet understood. Reb genes appear on genomes of proteobacteria, bacteroidetes, acidobacteria and cyanobacteria, generally displaying several contiguous duplications, but operon configuration exhibits diversified order and distribution. R-bodies have been shown to require the presence of distinct reb family members to ensure polymerization. Some regulatory genes have been described, but their taxonomic distribution and evolutionary history have not been fully determined. The order of events of speciation and duplication of reb homologs also remains undefined. OBJECTIVES: In this work, we describe the evolutionary history of reb homologs and of reb loci in several bacterial phyla, identifying conserved components and neighboring genes which may be associated with R-body regulation. MATERIALS AND METHODS: For collection of homologs, we used iterative profile-based alignment tools (jackhmmer, PSI-BLAST). We annotated domains using hmmscan and hhsearch. Mmseqs2 was employed for clustering sequences based on e-value and identity of pairwise alignments. Multiple sequence alignments were built with MAFFT, while FastTree and figtree were used for phylogeny inference and visualization. The main databases used were the non-redundant RefSeq database for sequence data and Pfam for domain annotation. DISCUSSION AND RESULTS : We have found that reb homologs have a conserved common core and variable regions on the N and C-terminal portions, which might account for their distinct roles during R-body polymerization. Furthermore, reb loci are largely organized in triads of distinct reb components (rebBCD), while other loci represent more ancestral populations of reb genes. We have additionally identified conserved regulatory genes which may be associated with R-body function. CONCLUSION: Genomic context suggests that R-body assembly depends mainly on the polymerization of reb gene products and is subject to stringent regulation.

Keywords: R-bodies, reb genes, Phylogenetics / **Supported by:** FAPESP

E.61 - Validation of an Alternative Protocol for Crotalic Toxin Isolation and Comparative Analysis with the Classic Protocol.

Ana Júlia Loureiro Levada [1], Isabella Scarpa[1], Beatriz Silveira Augusto[1], Fábio Florença Cardoso[1], Marcos Roberto de Mattos Fontes[1]
[1]Departamento de Biofísica e Farmacologia, Instituto de Biociências de Botucatu - UNESP (São Paulo, Brasil)

INTRODUCTION: Crotoxin (CTX) is a potent heterodimeric neurotoxin, and the major fraction of *Crotalus durissus terrificus* (Cdt) venom. This toxin is composed by an acidic and non-toxic subunit (CA) non-covalently linked to a basic and toxic phospholipase A_2 (CB). CTX has systemic neurotoxic and myotoxic activities, resulting in the higher lethality rate by Brazilian snakebites, besides it has potential biotechnological applications. OBJECTIVES: Intends to compare possible structural differences in protein, with different types of treatment, which may indicate a better understanding of the CTX structure. MATERIALS AND METHODS: We initially propose a structural investigation about the subunit CB. Then, we define an alternative purification protocol, isolating CTX by size exclusion chromatography (SEC), and unbounding its subunits by reverse phase chromatography (RPC). This CB isoforms pool fraction was structurally compared to the CB pool obtained by classical protocol (SEC followed by ion exchange chromatography – IEC). Thus, the secondary structure of RPC-CB and IEC-CB were evaluated by circular dichroism (CD), and their tertiary/quaternary structure by photon correlation (DLS) spectroscopy. Moreover, the phospholipase activity of these fractions was evaluated by indirect hemolytic assays (IHA). DISCUSSION AND RESULTS: RPC-CB and IEC-CB demonstrated a majority α-helical content with some differences in their ellipticity pattern. However, RPC-CB presented a dimer assembly, while IEC-CB presented a combination of dimers and tetramers conformations. Interestingly, RPC-CB demonstrated low solubility in neutral and basic buffers, and the ammonium formate (an acidic buffer) as the best option for its proper solubilization. Thus, RPC-CB was submitted to the crystallization trials, using classical precipitant agents. Concurrently, IHA corroborated the enzymatic viability of toxins obtained by the isolation protocols. Although both protocols allow us to obtain two viable CB pools. CONCLUSION: The differences observed in the structural assays suggested the isolation of different isoforms in each protocol or, at least, the induction of different behavior in solution, by changes in the fractionation conditions

Keywords: Crotoxin B, High-performance liquid chromatography, Crystallizatio

E.62 - Structural Analysis of the Interaction Between SH2-Domain of the GRB2-Protein with the MRE11-Peptide of the MRE11-Complex

Renan Pereira Pedro[1,2], Zamal Ahmed[3], Icaro Putinhon Caruso[1,2,4], Fernando Alves de Melo[1,2]
[1]Física, Instituto de Biociências, Letras e Ciências Exatas - Universidade Estadual Paulista "Júlio de Mesquita Filho" (São Paulo, Brasil), [2]Física, Centro Multiusuário de Inovação Biomolecular (São Paulo, Brasil), [3]Molecular and Cellular Oncology and Cancer Biology, The University of Texas MD Anderson Cancer Center (Texas, United States of America), [4]Bioquímica Médica, Instituto de Bioquímica Médica - Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: DNA double-strand breaks (DSBs) can either occur by internal or external factors and a fast repair is essential for the cell viability. The repair is initiated by MRE11 nuclease for both homology-directed repair (HDR) and alternative end-joining repair (Alt-EJ) pathways. Recent studies showed that GRB2, the key factor protein in the RAS/MAPK pathway activation, to do a GRB2-MRE11 complex for effective HDR initiation. Sh2 domain of the GRB2, known for its interaction with phosphotyrosines regions, intermediates the interaction between GRB2 and MRE11. OBJECTIVES: Therefore, this work's goal is to structurallyl describe the interaction of MRE11 phosphopeptide and GRB2-SH2 by NMR. MATERIALS AND METHODS: GRB2-SH2 domain was expressed in E.coli:BL21(DE3). The cell culture was grown M9-minimal-medium broth enriched with $^{15}NH_4Cl$. Firstly, an affinity purification with cobalt-affinity-column IMAC-HiTrap-HP in buffer 500mM-imidazole, 50mM-Tris-HCl (pH:8.0) and 100mM-NaCl was realized. Then, applied in Sephacryl-100 resin in buffer 20mM-Na_2HPO_4/NaH_2PO_4 (pH:7.0), 100mM-NaCl and 0.5mM-PMSF for size-exclusion chromatography. The binding of the phosphopeptide pMRE111 with to GRB2-SH2 was analyzed by chemical shift perturbation (CSP) using the ^{15}N-HSQC spectra in the presence and absence of pMRE111. DISCUSSION AND RESULTS: The perturbation in GRB2-SH2 chemical shifts with peptide addition in the system, analyzed through NMR showed that the interaction occurs in the site I. Bigger CSP in A68, E89, S90, S96, F108 and K109 suggest that a conformational exchange occurs so that the peptide can access the site. CONCLUSION: This study provides an understanding of the molecular level of the microenvironment in which the peptide interacts and how the protein can be structurally affected. Mapping the interaction between GRB2-SH2/pMRE111 by chemical shifts perturbation suggests a motif involving α_2-helix, β_5-sheet, loop between β_5-sheet and β_6-sheet, β_6-sheet and β_7-sheet. This interaction motif is found in the site I, which is responsible for the phosphotyrosine's recognition.

Keywords: Interaction, GRB2, MRE11 / **Supported by:** CNPq

E.63 - Structural Dynamics Study of Thermophilic Proteins by NMR - TTHA0849 of Thermus Termophilus
Karen Stephanie Santos [1], Orlando Rodrigues Ribeiro[1], Adolfo H. Moraes[2], Ana Paula Valente[1]
[1]IBqMLdM, Univ. Federal do Rio de Janeiro (RJ, Brasil), [2]Depto de Química, Univ Federal de Minas gerais (MG, Brasil)

INTRODUCTION: TTHA0849 is a conserved hypothetical protein from ⟨i⟩Thermus thermophilus⟨/i⟩ that shares structural features with the StAR-related lipid-Transfer (START) domain superfamily. Our group has previously studied some members of these superfamily, the allergens Bet v 1 and Fag s 1, and the enzyme Norcoclaurine Synthase (NCS) that shares the presence of an cavity able to bind hydrophobic compounds. OBJECTIVES: Characterization of the structure and dynamics of the thermophilic TTHA0849 protein, in its apo and holo form and compare with mesophilic counterparts. MATERIALS AND METHODS: Materials and methods: TTHA0849 was obtained using heterologous expression and purified by ion exchange and gel filtration chromatography. Intrinsic fluorescence, circular dichroism and nuclear magnetic resonance was used to analyze the structural characteristics of apo e holo states. DISCUSSION AND RESULTS: Initially, the protocol for protein expression and purification was established. Thermal and chemical denaturation showed high stability as expected. Intrinsic fluorescence emission and STD-NMR showed evidence of complex formation with quercetin, ANS and retinoic acid. CONCLUSION: The TTHA0849 protein, in addition to presenting structural similarity, also interacts with the molecules that bind to proteins studied by the group. Triple resonance experiments have been collected and resonance assignment is in progress, in addition, relaxation parameters are being collected to assess the dynamics of TTHA0849 and compare with mesophilic protein data.
Keywords: nuclear magnetic resonance, thermophilic protein, dynamic
Supported by: FAPESP, FAPERJ, CNPq and CAPES.

E.64 - Structural and Biochemical Studies of 10 Enzymes with possible PET Hydrolase activity from an Antarctic Metagenome
Adriano Alves Furtado[1], Jhon Antoni Vargas Santillan[1], Humberto D´Muniz Pereira[1], Diego Antonio Leonardo Cabrejos[1], Susana Andréa Sculaccio[1], Richard Charles Garratt[1], Cesar A. Ramirez Sarmiento[2]
[1]Grupo de Biofísica e Biologia Estrutural, Instituto de Física de São Carlos, Universidade de São Paulo (São Paulo, Brasil), [2]Protein Biophysics, Biochemistry and Bioinformatics Lab, Institute for Biological and Medical Engineering, Pontificia Universidad Católica de Chile (Santiago, Chile)

INTRODUCTION: Amongst all industrial products, plastics have come to represent that with the highest level of accumulation over recent years and their fragmentation is one of the most critical pollutants. Polyethylene terephthalate (PET) is one of the most commonly used materials for the manufacture of disposable bottles and has become a strategic focus for pollution mitigation, albeit with only limited success. With the discovery of enzymes that degrade PET, the opportunity arises for a biotechnological approach to minimize these problems OBJECTIVES: In this work, we studied 10 recombinant enzymes from Antarctic bacteria with potential catalytic activity against PET, identified by metagenomics. MATERIALS AND METHODS: Protein expression parameters were optimized in 5 Escherichia coli strains and purifications were performed in all cases using either two or three steps (combinations of Affinity Chromatography, Ion Exchange and Molecular Exclusion). DISCUSSION AND RESULTS: So far six enzymes have been shown to be stable, expressed in soluble form and their SEC profile consistent with being monomeric. Crystallization assays were successful for 3 enzymes, but only one so far yielded a crystal. structure. This was solved at 1.2 Å, which has allowed for the understanding of intricate details of the strure and its comparison with other PET metabolizing enzymes. CONCLUSION: In the following stages of this work we intend to accumulate estructural and kinetic data on all 10 enzymes in order to draw structure-function relationships to aid in the development of PET-degrading molecular too.
Keywords: PET, crystallographic structure, Antartic / **Supported by:** CAPES

E.65 - Small Angle X-Ray Scattering (SAXS) As Tool For Obtaining Insights And Structural Models Of Molecular Chaperones: Recent Results And Perspectives

Luiz Fernando de Camargo Rodrigues[1,2], Julio Cesar Borges[3], Carlos Henrique Inacio Ramos[4], Leandro Ramos Souza Barbosa[1,2]

[1]Departamento de Física Geral, Instituto de Física - Universidade de São Paulo (São Paulo, Brazil), [2]Divisão de Matéria Mole e Biológica, Laboratório Nacional de Luz Síncrotron - Centro Nacional de Pesquisa em Energia e Materiais (São Paulo, Brazil), [3]Departamento de Química e Física Molecular, Instituto de Química de São Carlos - Universidade de São Paulo (São Paulo, Brazil), [4]Depto de Química Orgânica, Instituto de Química - Universidade Estadual de Campinas (São Paulo, São Paulo)

INTRODUCTION: Molecular chaperones are a diverse class of proteins with functions related to preserving cell proteostasis. Issues related to these phenomena are related to neurodegenerative diseases, such as Alzheimer and Parkinson, and some types of cancer. A well established technique for structural studies of biological systems is Small Angle X-Ray Scattering (SAXS), from which a user can obtain information regarding a scattering particle's structure in solution, but with the tradeoff of working at lower resolution than crystallography. Nevertheless, SAXS allows the researcher to obtain parameters regarding a particle's size and shape, information on protein folding state and also to calculate molecular envelopes at nm resolution. OBJECTIVES: This work aims to illustrate how to use SAXS for protein samples by showing recent studies on molecular chaperones that have employed this technique, how it benefits from results obtained by other experimental approaches and what experiments that will be possible in the future SAXS beamline, called SAPUCAIA, at SIRIUS. MATERIALS AND METHODS: Measurements were performed at the SAXS1 beamline, in LNLS-CNPEM. DISCUSSION AND RESULTS: For the presented chaperones we have been able to extract structural parameters, inferring about protein folding state and elaborate models of different types, from molecular envelopes to ensemble modeling using partial structures. It was also shown potential uses for techniques that will be available at the new SAXS beamline at SIRIUS. CONCLUSION: These results illustrate what information SAXS can bring users about their protein samples, and we hope it encourages participants on applying for beamtime when SAPUCAIA is open for users. These results illustrate what information SAXS can bring users about their protein samples, and we hope it encourages participants on applying for beamtime when SAPUCAIA is open for users.

Keywords: Chaperones, Protein Folding, SAXS / **Supported by:** CAPES, CNPq, FAPESP

E.66 - Biochemical and Structural Characterization of Shs1: a Unique *Saccharomyces cerevisiae* Septin

Giovanna Christe dos Reis Saladino [1], Italo Augusto Cavini[1], Ana Paula Ulian de Araujo[1]

[1]Instituto de Física de São Carlos, Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: *S. cerevisiae* septins were the first septins described in literature, but information regarding their structure and biochemistry remains incomplete. Shs1 is the only non-essential yeast septin for cellular division, and it is interesting due to its unique characteristics: Shs1 substitutes Cdc11 on the terminal subunit of the septin filament, binding to Cdc12 and blocking end-to-end polymerization but promoting instead ring and gauze formation. Nevertheless, studying integral septins has many drawbacks, but Shs1 and Cdc12 interact through their G-domains, thus being the G-domains the object of this study. OBJECTIVES: Characterization of the G-domain of Shs1 to understand its role in filament formation and its interaction with the G-domain of Cdc12. MATERIALS AND METHODS: DNA sequences encoding G domains were cloned and expressed on *E. coli* BL21(DE3). Shs1 was expressed as a single protein and co-expressed with Cdc12. Proteins were purified by affinity and size-exclusion chromatography and used in GTPase activity and crystallization assays. DISCUSSION AND RESULTS: Cdc12 was able to hydrolyze GTP and was co-purified bound to GDP. However, Shs1 was purified without any nucleotide. The dimer Shs1-Cdc12 shows the presence only of GDP bound, which is unexpected to septin heterodimers, since one subunit is usually catalytically inactive. The GTPase activity assay for Shs1 also did not indicate any catalytic activity when GTP was added in excess. Crystals of Shs1 were grown on sitting-drop plates and are waiting for beam-time to submission to X-Ray diffraction.CONCLUSION: Shs1 is a unique septin and clarifying its structure and function will help understanding the blockage of polymerization in septin filaments.

Keywords: septin, Saccharomyces, polymerization

Supported by: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)

E.67 - Steered molecular dynamics simulations reveal critical residues in human Dopamine transporter for binding of Dopamine with different patterns of phosphorylation

Roberto Carlos Navarro Quiroz [1], Eric Allison Philot[1], Ana ligia Scott[1]
[1]Centro de Matemática, Computação e Cognição, Universidade Federal do ABC (SP, Brazil)

INTRODUCTION: The Human Dopamine Transporter (hDAT) plays an essential role in modulating the Influx/Efflux of dopamine, and it is involved in the mechanism of certain neurodegenerative diseases such as Parkinson's disease. We have studied the conformational dynamics and metastable states of hDAT named as follows: the outer open, the outward closed, holo-occluded closed and the inward open under different phosphorylation states, using hybrid methods described by (Quiroz et al, 2022). OBJECTIVES: This work investigates details of the dopamine transport molecular mechanism and the interactions of this ligand with critical residues of several hDAT metastable states under different patterns of phosphorylation. MATERIALS AND METHODS: Steered molecular dynamics (SMD) were performed for the different systems representing the metastable states under different phosphorylation conditions: i) with 22 sites phosphorylated; ii) SER12 is not phosphorylated; iii) SER333 is not phosphorylated; and no residues are phosphorylated as described by (Lin et al.). The ligand-residue binding network was analyzed and the most relevant residues to control the ligand migration within the hDAT channel were identified for each system studied. DISCUSSION AND RESULTS: We identify that the different phosphorylation patterns affect the interactions of the functional residues responsible by controlling the ligand migration within the hDAT channel, indicating that distinct phosphorylation patterns are important in the DA transport regulation by hDAT. CONCLUSION: This results corroborated with the phosphorylation effect over the stability of metastable states and the influx rate as discussed by (Quiroz et al., 2022)(Lin et al, 2003).

Keywords: Roberto Carlos Navarro Quiroz, Eric Allison Philot, Ana Ligia Scott
Supported by: CAPES

E.68 - Structural characterization by Circular Dichroism (CD) and intrinsic tryptophan fluorescence emission (ITFE) of SARS-CoV-2 non-structural protein 4(NSP4)

Deborah Kimie Yonamine [1], Fávia Maia[1], Daniel Dorta[1], Taisa Magnani[1]
[1]Chemistry Department, University of São Paulo (SP, Brazil)

INTRODUCTION: Since 2019, the world has faced the COVID-19 pandemic, caused by SARS-CoV-2. Information about the structure SARS-CoV-2 NSP4 protein is particularly interesting to understand the pathogenicity of this virus, because it is involved in host cell metabolites and escape from immune responses by formation double-membrane vesicles (DMVs). OBJECTIVES: In this study we have focused on SARS-CoV-2 NSP4 protein structure. MATERIALS AND METHODS: NSP4 encoding gene was cloned into the expression vector pET28a(+) and expressed in *E. coli* strain Rosetta™ (DE3) pLysS. After purification, the recombinant protein was analyzed by SDS-PAGE. After, we excised the purified NSP4 from the gel and analyzed it on the LC-MS/MS Xevo TQS (Waters) to confirm that the protein was NSP4. The NSP4 structure prediction were performed by circular dichroism (CD) and intrinsic tryptophan fluorescence emission (ITFE) under different temperature conditions (20, 30, 40, 50, 60, 70, or 80 ºC) for 30 minDISCUSSION AND RESULTS: After expression with 1 mM IPTG at 14 ºC for 24 h and purification, we obtained the protein with molecular weight of approximately 44 kDa. The CD spectra displayed double minima, at 208 and 222 nm, indicating that the helical structure predominated. As the temperature rose during pre-incubation elicited changes in the CD spectrum, such as decreased peaks at 208 and 222 nm and spectral shift toward 200 nm, which is typical of an unordered form and indicates tendency toward denaturation. Moreover, the result obtained from the melting temperature (Tm) value was 45.9 ºC and 51.5 ºC obtained by CD and ITFE, respectively. CONCLUSION: These results are valuable and contribute to the fight against COVID-19 and other diseases caused by viruses belonging to the family Coronaviridae.

Keywords: COVID-19, non-structural protein 4, protein structure / **Supported by:** CAPES

E.69 - Crystal structure of the septin heterodimer Sep1-Sep2 from Drosophila melanogaster

Diego Antonio Leonardo Cabrejos [1], Adriano de Freitas Fernandes[1], Italo Augusto Cavini[1], Humberto D'Muniz Pereira[1], Richard Charles Garratt[1]
[1]Biofísica e Biologia estrutural, Instituto de Física de São Carlos (São Paulo, Brazil)

INTRODUCTION: Septins are GTPases involved in several fundamental cellular processes and are present in a wide range of organisms. In humans, to perform their functions, a septin from each of four different groups must associate to form linear heterofilaments stabilized by two types of interfaces, called G and NC. Studies on heterodimers of human septins, have allowed us to identify specific interactions that guarantee the correct pairing of septins at the G-interfaces along the heterofilament. However, we do not currently know if such interactions are conserved in the heterofilaments formed by septins from other organisms. OBJECTIVES: Here, we describe the first crystal structure of a septin heterodimer (Sep1-Sep2) from *Drosophila melanogaster* aimed at uncovering the structural determinants of interaction specificity in fly septins. MATERIALS AND METHODS: Sep1-Sep2 heterodimer was co-expressed and co-purified by affinity chromatography and SEC. The heterodimer was crystallized by the sitting drop vapor diffusion method using the Morpheus screening kit. Collected data were processed using Xia2 and the structure was solved by molecular replacement (Phaser) using 6UPQ as template. Refinement was done with phenix.refine. DISCUSSION AND RESULTS: Sep1-Sep2 structure presents a heterodimer in the asymmetric unit, with Sep1 bound to GDP and Sep2 bound to GTP. Both subunits have the classic αβα sandwich fold observed in septins in general. By comparing Sep1-Sep2 with the homologous human septin heterodimer SEPT2-SEPT11, similar inter-subunit interactions are observed, indicating that the origins of interface specificity have been conserved during evolution from flies to humans. CONCLUSION: In both cases these interactions involve switches I and II of both subunits of the heterodimer implying that they have been repurposed from conformational signalling elements in small GTPases to become interface components of the septin filament.

Keywords: Septin, Protein-protein interaction, crystal structure
Supported by: Fundação de Amparo à Pesquisa do Estado de São Paulo

E.70 - Structural Analysis Of Linear Epitopes Peptides Derived From Sars-cov-2 By NMR Spectroscopy

Victor Mendonça de Rezende Fabri [1], Bruno de Paula Oliveira Santos[2,3], Sergio Costa Oliveira[2], Mariana Torquato Quezado de Magalhães[1,3]
[1]Programa Interunidades de Pós-Graduação em Bioinformática, [2]Instituto de Ciências Biológicas, [3]Laboratório de Biofísica de Macromoléculas, Universidade Federal de Minas Gerais (Minas Gerais, Brasil)

INTRODUCTION: With the goal of developing an immunogenic vaccine against SARS-CoV-2, which caused 6 million deaths since december 2019, our research group described several B and T cells linear epitopes derived from SARS-CoV-2 structural proteins using immunoinformatics. Here, we selected three peptides. Two N-derived: one in the NTD region with high potential for T-cell cross-reactivity within the nucleocapsid proteins of several human coronaviruses, and another from an unstructured serine-arginine-rich domain that represents a strong B-immunodominant B cell epitope. The third is an S-derived peptide identified in the Receptor Binding Domain (RBD) in a high binding affinity region for multiple human MHC I and II alleles. OBJECTIVES: The main goal of this study is to describe the tertiary structure of these peptides and to use these features to aid the prediction of their antigenic potential for use in a multi-epitope vaccine, improving its rationale design. MATERIALS AND METHODS: The peptides were synthesized by GenScript® (USA), purified through HPLC and submitted to 3 NMR experiments: 1H-1H TOCSY, 1H-1H NOESY and 1H-13C HSQC. The spectra were recorded in DMSO medium in a spectrometer operating at a hydrogen frequency of 800 MHz at the Jiri Jonas NMR National Center (UFRJ). DISCUSSION AND RESULTS: Structure was calculated in silico using a simulated annealing algorithm with a modified protocol, generating 50,000 structures to improve energy minimization. The observation of large sequential dαN(i, i+1) NOEs with the absence of middle range distance correlation emphasizes the absence of stable helix and hairpin conformations in DMSO. This typical random shape of spectra was previously observed for longer β-sheet-forming peptides. Clusters of the most favorable conformations are shown, highlighting the conformational preferences of the peptide. CONCLUSION: These preferred conformations correlate with the immunogenic character of T cell epitopes, generally found as disordered structures with specific regions adopting preferred conformations.

Keywords: immunoinformatics, conformation, random-coil / **Supported by:** FAPEMIG, CAPES

E.71 - Studies on mitochondrial TRAP-1 and PINK-1 proteins: recombinant production, characterization and interaction

Marina Del Giudice [1], Eduardo Feliciano de Lima Santos[1], Silvia Helena Libardi [2], Carlos Sabino Oliveira[2], Júlio César Borges[2], Carlos Henrique Inácio Ramos[3], Lisandra Marques Gava Borges[1]
[1]Depto de Genética e Evolução, Universidade Federal de São Carlos (SP, Brasil), [2]Departamento de Química e Física Molecular, Universidade de São Paulo (SP, Brasil), [3]Depto de Química Orgânica , Univ. Estadual de Campinas (SP, Brasil)

INTRODUCTION: Eukaryotic cells are characterized by the presence of a nucleus and a series of membrane-enclosed organelles. Among these, there's mitochondria, a semi-autonomous, complex and dynamic organelle, essential for cell viability. Several protein machineries are involved in mitochondrial homeostasis, including molecular chaperones, their co-chaperones and interactors. Among them, TRAP-1 and PINK-1 are of particular interest in this work. TRAP-1 also ensures the correct protein folding and it is essential for the mitochondrial integrity, protecting cells against apoptosis, besides avoiding toxic effects of oxidants and anti-cancer drugs. It exerts its functions through interaction with other proteins, such as PINK-1, a mitochondrial serine-threonine kinase: a critical regulator of the cytoprotective activity of TRAP-1 through the chaperone phosphorylation. OBJECTIVES: The objective was the production of recombinant proteins in *E. coli*, followed by structural and stability characterization of both recombinant proteins, besides detecting and evaluating their *In vitro* interaction. MATERIALS AND METHODS: The proteins were produced in *E. coli* BL21(DE3) strain and protein expression were induced with isopropyl-β-D-thiogalactoside. The pellets were lysed and purified using immobilized metal affinity (IMAC) and size-exclusion (gel filtration) chromatographies. Polyacrylamide gel electrophoresis (SDS-PAGE) was used to verify the efficiency of the methods and the protein concentration determinated by spectrophotometric measurements. To characterize TRAP-1 and PINK-1 we used circular dichroism (including thermal unfolding assays), and intrinsic tryptophan fluorescence assays. Also, a pull-down test was performed to detect the interaction between the recombinant proteins. DISCUSSION AND RESULTS: Both proteins were successfully expressed and purified, with a high purity. They're are folded as characterized by CD spectropolarimetry and intrinsic tryptophan fluorescence spectroscopy. The interaction was detected in the pull-down assay, but control optimizations are still needed. CONCLUSION: Both recombinant proteins were obtained with a high degree of purity and well folded. As perspectives, we intend to repeat the assays with optimizations and carry out other tests focusing on the protein interaction.
Keywords: structural characterization, TRAP-1, PINK-1
Supported by: CNPq e FAPESP

E.72 - Evaluation of the Structure and Stability of Truncated Mutants of the Hsp70 Escort Protein 1

Maria Júlia Mattos Gomes [2,1], Sabrina Dorta de Oliveira[2], Milene Nóbrega Oliveira Moritz[2], Amanda Lais de Souza Coto[2], Júlio César Borges[2]
[1]Departamento de Genética e Evolução, Centro de Ciências Biológicas e da Saúde (São Carlos - SP , Brasil),
[2]Departamento de Química e Biofísica Molecular, Instituto de Química de São Carlos (São Carlos - SP , Brasil)

INTRODUCTION: Hsp70 is a molecular chaperone protein family needed for protein homeostasis. These proteins are ubiquitous, exceptionally conserved, found in various cellular compartments and are highly expressed in several stressful conditions and malignancies. HSPA1A is the main inducible human Hsp70 found in the nucleus and cytoplasm acting as a central component of the cellular chaperone network. Mitochondria contain the HSPA9 (also named mortalin), which is also involved in the importing process of nuclear encoded proteins. Both HSPA1A and HSPA9 require the assistance of the human Hsp70-escort protein 1 (hHep1) to remain functional. This co-chaperone is capable of remodeling Hsp70 supramolecular assemblies into smaller particles and stimulating their ATPase activity, as well as present intrinsic chaperone activity. hHep1 has a conserved "Zn2+-finger" domain and poor conserved N- and C-terminal regions of unknown functions. OBJECTIVES: This work intends to perform the structural characterization of hHep1's truncated mutants without N- or/and C-terminals regions. MATERIALS AND METHODS: The recombinant proteins were produced in Escherichia coli cells by pET28a expression vectors and purified by Ni2+-affinity and size exclusion chromatographies. The His-tag was removed by thrombin and the purity was evaluated by SDS-PAGE. The hydrodynamic properties were studied by analytical size exclusion chromatography. Circular dichroism and tryptophan fluorescence emission were applied to evaluate the secondary and local tertiary structure contents, respectively. DISCUSSION AND RESULTS: The recombinant proteins (hHep1, hHep1-core, hHep1-Ndel and hHep1-Cdel) were obtained with >95% purity, folded and in its monomeric states. In addition, the truncations caused subtle alterations in the spectral signatures acquired through the biophysical tools. CONCLUSION: In summary, the removal of the terminal regions didn't cause significant structural changes in the structure of hHep1, but indicates that both N- and C-terminal regions behaved, in comparison to the full-length protein, as natively unfolded regions. PERSPECTIVES: Functional characterizations of hHep1's mutants will be evaluated.
Keywords: Molecular chaperones , HSPAs, hHep1 / **Supported by:** CNPq, FAPESP, CAPES

E.73 - Interaction Studies of the Human Hep1 Truncated Mutants and Negatively Charged Liposomes

Arthur Moraes Franco da Rocha [1], Amanda Lais de Souza Coto[1], María Júlia Mattos Gomes[2,1], Milene Nóbrega Ortega Moritz[1], Julio Cesar Borges[1]
[1]DQFM, Instituto de Química de São Carlos, Univ. de São Paulo (SP, Brasil), [2]Departamento de Quimica, Univ. Federal de São Carlos (, Brasil)

INTRODUCTION: The Hsp70 family of molecular chaperones are involved in several cellular proteostatis processes including protein folding/refolding and disaggregation, protein targeting for membrane traffic and degradation. Hsp70 have isoforms addressed to different cellular compartments. The human mitochondrial Hsp70 (HSPA9 or mortalin) has several functions, including its participation in the import of proteins from the cytosol into the mitochondrial matrix. To correctly perform their function, Hsp70 are regulated by its dynamic association with adenosine nucleotides and are assisted by co-chaperones. Hep1 (Hsp70-escort protein 1) is a co-chaperone responsible for maintaining HSPA9 in its soluble and functional state. Hsp70 also have the ability to interact with lipid membranes and the same was reported to hHep1. This co-chaperone is formed by a conserved zinc-finger domain core and poor conserved N- and C-terminals of unknown structure and function. OBJECTIVES: To elucidate the mechanism of interaction of hHep1 and N- and/or C-terminals truncated mutants with liposomes formed by cardiolipin and POPS. MATERIALS AND METHODS: The recombinant hHep1 truncated mutants were expressed in E. coli BL21DE3 strain by the pET28a expression vector and purified by Ni+2 affinity and size exclusion chromatographies. The stability of the mutants in solution and their interaction with POPS and Cardiolipin were investigated using techniques such as circular dichroism (CD) and Intrinsic Tryptophan Fluorescence. DISCUSSION AND RESULTS: The hHep1 mutants were obtained under suitable conditions to interact with POPS and Cardiolipin. In structural terms, the interaction of mutants with liposomes does not differ significantly from the interaction between liposomes and full-length hHep1. CONCLUSION: Deletion of the terminal regions of hHep1 does not significantly modify the interaction with POPS and Cardiolipin. The results obtained showed the importance of the hHep1 zinc finger domain present in the core region of the protein for the interaction with liposomes.

Keywords: Hep1, Liposomes, Molecular Chaperones

E.74 - Norclaurine synthase structural characterization using NMR

Ana Julia Rezende Santos [1], Karen Sthephanie Santos[1], Yan Ferraz Ximenes[3], Adolfo Henrique Moraes[3], Ana Paula Valente[1]
[1]Institute of Medical Biochemistry , Federal University of Rio de Janeiro (Rio de Janeiro , Brazil), [2]National Center of Structural Biology and Bioimage, Federal University of Rio de Janeiro (Rio de Janeiro, Brasil), [3]Department of Chemistry , Federal University of Minas Gerais (Minas Gerais , Brasil)

INTRODUCTION: Norcoclaurine synthase (NCS) catalyzes the first step in the biosynthesis of benzylisoquinoline alkaloids (BIAs), a large and diverse group of natural products. Several BIAs are pharmacological active such as codeine, morphine and papaverine. Therefore, information about the mechanism of action of NCS is of great importance. NMR and crystallographic studies shows that NCS structure is similar to the Pathogenesis related-10 proteins. OBJECTIVES: Characterization of the protein dynamics, in its apo and holo form with both dopamine and (4-HPAA) for nuclear resonance magnetic (NMR) to better understand mechanism of catalysis. Also, we intend to compare the dynamics with proteins with similar fold such as: Bet v 1 and TTHA0849. MATERIALS AND METHODS: NCS was obtained by heterologous expression, and purification using ion exchange chromatography and gel filtration. Intrinsic fluorescence, circular dichroism and nuclear magnetic resonance were used for structure and dynamics characterization. DISCUSSION AND RESULTS: Initially, the protocol for protein expression and purification protocol was established. CONCLUSION: Once the protocol was established, at the current moment, dopamine titration experiments with the protein of NMR are being collected, so that can lead us to better information about the mechanism of action of NCS in its holo form, and also to compare the dynamics with proteins with similar fold.

Keywords: Nuclear Magnetic Resonance, Enzyme, Protein
Supported by: FAPESP, FAPERJ, CNPq, PIBITI and CAPES.

E.75 - Structural and Functional Characterization of the Phosphorylation Effects on the Globular Tail Domain of Human Myosin Va

Silas Pontes de Almeida [1], Mario Murakami[1], Andrey Nascimento[1]
[1]MANACA/Sirius, Brazilian Synchrotron Light Laboratory (LNLS) (São Paulo, Brasil), [2]LNBR, Brazilian Biorenewables National Laboratory (LNBR) (São Paulo, Brasil)

INTRODUCTION: The myosins V (MyoV) constitute a class of protein homodimers capable of converting chemical energy into movement through actin filaments. They play a key role in many essential cellular processes and, together with their molecular partners, are responsible for the intracellular transport of vesicles, organelles, proteins and mRNA. Some studies point that Ser ^{1652} residue phosphorylation, located at a long flexible loop (phospho-loop) of globular tail domain of the human MyoVa (GTD-MyoVa), have an important regulatory role on cell cycle control and nuclear localization. OBJECTIVES: However, since the structure of phosphorylated GTD has not been solved yet, both structural and functional implications of this covalent modification remain unknown. We believe phospho-loop might interact with positive charged nearby regions, potentially interfere with MyoVa auto-inhibition conformation and its capacity to recognize molecular partners. MATERIALS AND METHODS: In order to shed light on Ser ^{1652} phosphorylation effects for this protein class, we intend to solve the structure of the phosphomimetic GTD-MyoVa mutant using X-ray crystallography and analyze phosphorylation impacts on a chosen partner, the Rab11a GTPase, through a pulldown assay. DISCUSSION AND RESULTS: So far, preliminary results have shown that the mimetic phosphorylation mutation of S ^{1652} does not inhibits the interaction of GTD-MyoVa:Rab11a. We have also obtained plate-like crystals from phosphomimetic mutant of GTD-MyoVa which diffract up to 5 Å resolution. CONCLUSION: Additional experiments are in progress to confirm whether phosphorylation affects MyoVa ability to recognize Rab11a. Crystallization conditions for phosphomimetic GTD-MyoVa mutant have been identified and are currently being refined. These crystals will also be used to validate new crystallographic methods at MANACA beamline.

Keywords: Myosin Va, Rab 11, X-ray Crystallography / **Supported by:** FAPESP

E.76 - Characterization of the N-terminal of Tetracenomycin Aromatase/Cyclase Interactions with Substrates and Product Analogs

Veronica Silva Valadares [1], Luan Carvalho Martins[1], Adolfo Henrique de Moraes Silva[2]
[1]Department of Biochemistry and Immunology, Federal University of Minas Gerais (Minas Gerais, Brasil),
[2]Departamento de Química, Universidade Federal de Minas Gerais (Minas Gerais, Brasil)

INTRODUCTION: The N-terminal of Tetracenomycin aromatase/cyclase (TcmN) is an enzyme derived from *Streptomyces glaucescens* involved in polyketide cyclization and aromatization. Aromatic polyketides are an important class of secondary metabolites produced by certain bacteria, fungi, and plants with various biological activities. Examples include antibiotics, tetracycline, and anticancer drugs, such as doxorubicin. A mechanism was proposed in which the thermodynamics and kinetics of the TcmN conformational equilibrium modulate enzyme function by favoring ligand binding and avoiding aggregation. Because TcmN is a promising enzyme for *In vitro* production of polyketides, it is essential to characterize the TcmN enzymatic mechanism. OBJECTIVES: Investigate the mechanism of TcmN molecular recognition of substrates, intermediates, and products, and understand the role of TcmN conformational dynamics on its enzymatic function. MATERIALS AND METHODS: TcmN unfolding and stability were evaluated by circular dichroism and differential scanning calorimetry (DSC). Interaction with ligands was assessed by nuclear magnetic resonance (NMR) ^{15} N relaxation experiments, NMR saturation-transfer difference (STD), ^{1} H- ^{15} N chemical shift perturbation, and microsecond molecular dynamics (MD) simulations. DISCUSSION AND RESULTS: The naringenin-bound TcmN melting temperature, Tm, measured from DSC experiments, was higher than the free enzyme one. The naringenin binding site on TcmN, mapped by NMR experiments, was located in the core TcmN cavity; Conformational dynamics on the µs-ms timescale were detected for residues in the substrate-binding cavity for the naringenin-bound TcmN. CONCLUSION: The results provide insights into the molecular basis of substrate recognition and help delineate the catalytic mechanism of TcmN.

Keywords: Polyketide, Aromatase/cyclase, Conformational dynamics / **Supported by:** CNPq, CAPES, FAPEMIG

E.77 - Epitope mapping by NMR to identify biomarkers in the serum of patients with COVID-19

Pedro Gontijo Carneiro [1], Talita Steling Araújo[2], Adolfo Henrique de Moras Silva[3], Mariana Torquato Quezado de Magalhães[1]
[1]Departamento de Bioquímica e Imunologia, Universidade Federal de Minas Gerais (Minas Gerais, Brazil),
[2]Plataforma Avançada de Biomoléculas, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil),
[3]Departamento de Química, Universidade Federal de Minas Gerais (Minas Gerais, Brazil)

INTRODUCTION: COVID-19 is a disease caused by the single-stranded sense RNA virus SARS-CoV-2. Among its four structural proteins, the nucleocapsid protein (N), conserved among beta-coronaviruses, is highly immunogenic and abundantly expressed during infection. This protein is a representative antigen for T cell response in vaccine development and biomarker discovery. Its N-terminal domain (NTD) packages and protects RNA, which plays an essential role in viral replication. Downstream, there is a rich serine and arginine region, an intrinsically disordered region, responsible for the N protein multimerization, interaction with RNA, and modulation of the host cellular metabolism. OBJECTIVES: A comprehensive molecular understanding of how human SARS-CoV-2 antibodies recognize the N protein is still needed and may lead to vaccine targeting, diagnosis, and antiviral therapies. MATERIALS AND METHODS: We used nuclear magnetic resonance (NMR) spectroscopy to study the interaction between the Sars-Cov-2 N protein and antibodies in the serum of convalescent patients with COVID-19. The N-NTD and SR region proteins were subjected to titration with reactive polyclonal antibodies purified from the sera of patients from the Belo Horizonte metropolitan region. Epitope mapping was performed using a heteronuclear NMR [^{1} H, ^{15} N]-HSQC experiment to observe antigen NMR signals changes upon antibody titration. These changes are known as chemical shift perturbation (CSP). Also, the ^{15} N transverse relaxation (R2) was measured to characterize the SARS-CoV-2 N-NTD backbone molecular dynamics in the presence of the antibodies, on a time scale from microseconds to milliseconds. DISCUSSION AND RESULTS: We observed significant changes when the protein is in complex with the antibodies. The β-hairpin (β2-β3) and the second alpha-helix of NTD emerge as the main epitope. Validation experiments were performed with the MERS-CoV N protein, and no CSP was observed. CONCLUSION: Our data described new epitopes in the SARS-CoV-2 N-protein and demonstrated the importance of studying the molecular dynamics involved in epitope recognition of the N protein by antibodies.

Keywords: Epitope Mapping, NMR, Nucleocapsid / **Supported by:** FAPEMIG

E.78 - Catechol-O-Methyltransferase from Paracoccidioides spp.: Advances in Biochemical and Structural Characterization

Marielly Moura Martins[1], Tayssa Kama Vitoria[1], Fabiano Torres Cruz[1], Alexandre Martins Costa Santos[1], **Juliana Barbosa Coitinho Gonçalves [1]**
[1]Programa de Pós-Graduação em Bioquímica, Universidade Federal do Espírito Santo (Vitória, Brazil)

INTRODUCTION: Fungi of genus Paracoccidioides are causative agents of systemic mycosis Paracoccidioidomycosis (PCM), which primarily affects lungs and may spread to other tissues. PCM is endemic in Latin America and Brazil accounts for 80% of reported cases. The treatment with antifungal agents is prolonged, expensive and with side effects, and there are reported cases of resistance. Thus, the search for new pharmacological targets remains important. The protein catechol O-methyltransferase (COMT) may be a possible pharmacological target because, in other fungi, it seems to participate in its survival in the host, however, in Paracoccidioides, it has never been studied so far. OBJECTIVES: The aim of this work was to characterize biochemically and structurally the recombinant COMT from Paracoccidioides spp. MATERIALS AND METHODS: The protein was expressed in E. coli Arctic Express and Rosetta followed by affinity purification, activity assays and in silico analyses. Different expression, lysis and affinity purification conditions were evaluated. DISCUSSION AND RESULTS: COMT was obtained at the soluble fraction, albeit in small amounts, in both strains. In E. coli Arctic Express, expression was confirmed by Western blot using anti-His antibody. In addition, samples obtained from the two strains and purified by affinity chromatography showed enzymatic activity. In silico analyzes showed that the 3D structure predicted by the I-TASSER server presents the typical folding of O-methyl-transferases (O-MTs) with little divergence between different MTs identified in the PDB. Furthermore, by structural alignments, amino acid residues that interact with substrate and cofactors were also predicted and can guide docking studies for the design of inhibitors. CONCLUSION: With the characterization of COMT, it will be possible to better understand the biology of fungi of the genus Paracoccidioides and create solid knowledge that can be used for the development of new and more powerful drugs, contributing to diversify the available antifungal arsenal.
Keywords: catechol-O-methyl-transferase, Paracoccidioides, heterologous expression. / **Supported by:** Fapes, Capes and CNPq

E.79 - Membrane interaction of Golgi Reassembly and Stacking Protein from S. cerevisiae

Carolina Gimenes Oliveira [1], Luis Felipe dos Santos Mendes[1], Natália Ap. Fontana[1], Antônio José da Costa Filho[1]
[1]Physics Department, University of São Paulo (São Paulo, Brasil)

INTRODUCTION: The Golgi reassembly and stacking proteins (GRASPs) are related to the structure and function of the Golgi apparatus. GRASPs were initially identified by *In vitro* experiments as one of the main factors necessary for stacking of the Golgi cisternae in mammalian cells. Over the years, data about GRASPs have shown their participation of this family of proteins in many cellular processes, including the unconventional protein secretion. OBJECTIVES: GRASPs are found in membrane-bound organelles that participate in the early and late secretory pathway, and even though we already have knowledge about their structure and function, there are no reports yet providing a detailed description of their interaction with membranes, which is our main goal. To understand more about how these proteins anchor to the membranes of organelles, the yeast *Saccharomyces cerevisiae*, a model organism, was used in this work. This budding yeast has a single homolog of GRASP65, which is called Grh1. We chose this yeast due to the acetylation of Grh1 N-terminal that is necessary for its interaction with the membrane. MATERIALS AND METHODS: We used heterologous expression, fluorescence spectroscopy, circular dichroism, and other biophysical tools to investigate the mechanisms of interaction between GRASP (Grh1) and model membranes. In additional we propose to show that Grh1 is capable to undergo liquid-liquid phase separation (LLPS), when purified and placed with buffer that mimetic the physiological conditions. This suggest that the protein can act in tethering and organization of the Golgi. DISCUSSION AND RESULTS: The results of fluorescence spectroscopy and circular dichroism showed that Grh1 protein does not interact with membranes without the N-terminal acetylation, suggesting that this post-translational modification is important for this interaction. Moreover, under several experimental conditions, Grh1 underwent LLPS, giving rise to liquid droplets that will be further characterized. CONCLUSION:
Keywords: GRASP65/55, Membrane, protein
Supported by: CAPES

E.80 - Structural and Functional Characterization of Three Key Enzymes in the Biosynthesis of Vitamin C from Myrciaria dubia

Jhon Antoni Vargas Santillan[1], Diego Antonio Leonardo Cabrejos[1], Humberto D´Muniz Pereira[1], Juan Carlos Castro Gómez[2], Richard Charles Garratt[1]

[1]Grupo de Biofísica e Biologia Estrutural, Instituto de Física de São Carlos, Universidade de São Paulo, São Carlos-Brasil (São Paulo, Brasil), [2]Unidad Especializada del Laboratorio de Investigación en Biotecnología, Centro de Investigaciones de Recursos Naturales de la UNAP, Universidad Nacional de la Amazonia Peruana (Loreto, Perú)

INTRODUCTION: *Myrciaria dubia* (Kunth) McVaugh, known as camu-camu or aracá, is a fruit native to the Amazon, characterized by its high content of L-Ascorbic Acid (Vitamin C or AsA). For this reason, the enzymes that participate in AsA biosynthesis may have potential biotechnological applications for the artificial production of AsA. However, up until now the structural biology of these enzymes has been poorly explored OBJECTIVES: Here, we describe the biophysical, functional, and structural properties for three enzymes of the Smirnoff/Wheeler pathway (*Md*GME, *Md*GDH and *Md*GalDH) MATERIALS AND METHODS: All enzymes were purified by affinity chromatography followed by molecular exclusion DISCUSSION AND RESULTS: L-GDP-D-mannose 3´,5´ epimerase (*Md*GME), which catalyzes a double epimerization of GDP-mannose to produce GDP-L-galactose and GDP-L-gulose, proved to be a dimer in solution and its crystal structure was solved at 1.3 Å resolution. L-galactose dehydrogenase (*Md*GDH) is monomeric in solution with a Km of 0.128 mM and an optimal pH of 7. The activity of the enzyme was not affected by high concentrations of AsA as has been reported for its homolog from spinach (*Md*GDH). This, we show to be due to a change in pH. The crystal structure of *Md*GDH was solved to 1.4 and 1.75Å in its apo and holo (NAD+ bound) forms respectively. The structure allows us to rationalize the use of NAD+ (and not NADP+) as the cofactor and to describe differences within the active site when compared to other aldo-keto reductases, which are related to substrate specificity. Finally, L-galacton-1,4-lactone dehydrogenase (*Md*GalDH), presents a Km of 0.041 mM and an optimal pH of 8 and its crystal structure was solved at 2.4 Å. CONCLUSION: The present work contributes to a broader understanding of structure-function relationships of enzymes involved in the synthesis of vitamin C.

Keywords: Crystallographic structure, Vitamina C, Myrciaria dubia “camu-camu” / **Supported by:** CAPES

E.81 - Prospection, Structure and Interaction Studies of NS2B protein and NS3 protease of Zika Virus

Thamires Moreira [1], Larissa Cardoso Silva Marques[1], Beatriz Rosa Penna[2], Danielle Maria Perpetua de Oliveira Santos[1], Ana Paula Valente[2], Cristiane Dinis Anobom[1]

[1]Departamento de Bioquímica, Instituto de Química, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brazil), [2]Instituto de Bioquímica Médica, Centro Nacional de RMN Jiri Jonas, Universidade do Federal do Rio de Janeiro (Rio de Janeiro, Brazil)

INTRODUCTION: Flavivirus are arthropod-borne pathogens responsible for significant medical diseases, like, Dengue (DENV), yellow fever (YFV) and Zika (ZIKV) capable to generate significant neurological disorders and high mortality. The NS2B of ZIKV is a transmembrane non-structural protein that interacts with NS3 protease domain, through the soluble portion, acting as a cofactor to the catalytic activity. The NS2B-NS3-complex is responsible by processing the viral polyprotein having an important role in viral replication, being an attractive target for the development of antiviral drugs. OBJECTIVES: The objective of this work is to determine the structure and dynamics of the NS2B-NS3 complex and to select new inhibitor compounds through fragment screening by Nuclear Magnetic Resonance (NMR) in solution. MATERIALS AND METHODS: The NS2B-NS3-complex was cloned in a specialized company using pET-Duet as a vector. Expression tests were performed using different parameters. All experiments were monitored by SDS-PAGE. In parallel, prospecting studies of NS2B and NS3 proteins from other flaviviruses were performed using bioinformatics tools. DISCUSSION AND RESULTS: The best NS2B-NS3 expression condition was observed in E. coli Rosetta with 1 mM IPTG at 37ºC for 16h. Purification of NS2B-NS3 will be performed using affinity and size-exclusion chromatography. For structural determination, the complex will be isotopically labeled with15N e 13C for usual experiments by NMR. Interaction studies and fragment screening will be performed using a library of 536 compounds and analyzed by STD and CSP experiments. The prospection studies showed a slight modification in the NS2B protein of YFV when compared to other flaviviruses and studies are ongoing. CONCLUSION: The structural and interaction studies with NS2B-NS3 complex and integral NS2B from different flavivirus could bring important information about new specific inhibitors.

Keywords: flavivirus, protein, structure / **Supported by:** FAPERJ, CNPq and CAPES

E.82 - Biochemistry studies of a hypothetical protein LIC_11920 that contains a DUF1577 and a PILZ domains

Aline Biazola Visnardi [1], Tania Churasacari Vinces[2], Edgar Llontop[3], Lucas Souza dos Santos[1], Cristiane Rodrigues Guzzo Carvalho[1]
[1]Depto de Microbiologia , Instituto de Ciências Biomédicas – Univ. de São Paulo (SP, Brasil), [2]Depto de Parasitologia, Instituto de Ciências Biomédicas – Univ. de São Paulo (SP, Brasil), [3]Depto de bioquímica , Instituto de Quimica – Univ. de São Paulo (SP, Brasil)

INTRODUCTION: Bacteria from the *Leptospira genus* are responsible for leptospirosis, a zoonotic disease of public health importance. The *Leptospira interrogans* genome encodes diguanylate cyclases (DGC) capable of synthesizing the cyclic dinucleotide c-di-GMP, known to regulate various processes within the cell, such as biofilm formation, motility, and virulence factors. The protein LIC_11920 of *L. interrogans* contains a PilZ domain in its C-terminal, known to bind c-di-GMP, and a Domain of Unknown Function (DUF1577) in its N-terminal. Although c-di-GMP binding residues are conserved in the PilZ of LIC_11920, we don't know if LIC_11920 is a c-di-GMP receptor and the functional role of the DUF1577. OBJECTIVES: Functional and structural characterization of LIC_11920 and its interaction with the signaling molecules such as c-di-GMP. MATERIALS AND METHODS: The protein was cloned fused to a 6×His tag in a pET28a vector, expressed in E. coli BL21(DE3) cells, and purified using conventional chromatograph techniques. To test if the protein is monomeric in solution, we applied the samples to a SEC-MALS (size exclusion chromatography – multiple angle light scattering) system to determine its molecular mass. The interaction with possible ligands, including different nucleotide molecules and divalent cations, was tested by thermal denaturation analysis monitored by circular dichroism (CD) spectroscopy and ThermoFluor assay. DISCUSSION AND RESULTS: The protein migrated as a monomer in the SEC-MALS analyses, with an experimental molecular weight of 46 ± 2 kDa versus a theoretical mass of 46.6 kDa (considering the 6×His tag). In the interaction analyses, no evidence of binding with nucleotides was observed, but calcium-binding was detected. CONCLUSION: The PilZ domain of LIC_11920 appears to have lost its nucleotide-binding function and the reason is under investigation. The LIC_11920 crystallized, and its X-ray diffraction data is now being analyzed for the determination of its three-dimensional structure.
Keywords: c-di-GMP, PilZ, Leptospira interragons / **Supported by:** FAPESP, CAPES, MAXXIDATA

E.83 - Functional and Structural Studies of Two Activators of Hsp90 ATPase (Aha) Cochaperones Uncover Novel Mechanisms for Hsp90 Regulation in Plasmodium falciparum.

Thiago V. Seraphim[1], Marcela da Silva Torricillas[1], Karine Minari[1], Leandro R. S. Barbosa[2,3], Júlio César Borges[1]
[1]Dept of Molecular Physics and Chemistry, São Carlos Institute of Chemistry, University of São Paulo (São Paulo, Brazil), [2]Dept of General Physics, Physics Institute, University of São Paulo (São Paulo, Brazil), [3]Brazilian Synchrotron Light Laboratory, Brazilian Center for Research in Energy and Materials (São Paulo, Brazil)

INTRODUCTION: Hsp90 molecular chaperone is a central player for proteostasis maintenance, being involved in processes such as cell signaling, genome stability, RNA processing, among others. This chaperone also plays critical roles in protozoa and the unbalancing of its functions leads to disruption of parasites life cycle. Hsp90 is a flexible dimer that undergoes large conformational rearrangements orchestrated by auxiliary proteins called cochaperones. Aha is a cochaperone that stabilizes Hsp90 in catalytically active conformation, stimulating its ATPase activity. Two domains, Aha1N and AHSA1, are organized to give rise to four Aha isoforms (named Aha1 to 4). In *Plasmodium falciparum*, we identified two Aha isoforms: PfAha2 and PfAha4. The most well-characterized Aha cochaperones are Aha1 and Aha3, in contrast to Aha2 and Aha4. OBJECTIVES: This work aimed to characterize the structure, interaction, and function of two novel Aha activators of Hsp90 in *P. falciparum*. MATERIALS AND METHODS: Structural properties were investigated by circular dichroism, analytical ultracentrifugation, and small angle X-ray scattering. Isothermal titration calorimetry and ATPase assays were performed to investigate the interaction between PfAha2/4-PfHsp90 as well as their functions. DISCUSSION AND RESULTS: Structural analyzes revealed that PfAha2 is an elongated monomer formed by a duplication of Aha1N domains. Our data showed, however, that these domains are not equivalent and suggested the existence of interdomain contacts. PfAha4 is formed by a single well-folded monomeric AHSA1 domain. In interaction assays, two PfAha2/4 bound to one PfHsp90 2-mer with similar affinities. Interestingly, formation of PfAha2-PfHsp90 complexes was entropically driven and did not hinder PfHsp90 conformational changes, whereas PfHsp90-PfAha4 interaction was enthalpically driven and induced a decrease in PfHsp90 conformational flexibility. As consequence, PfAha2 marginally stimulated PfHsp90 activity while PfAha4 stimulated it 7-fold. CONCLUSION: Two novel activators of Hsp90 with different structures and regulatory roles were identified in *Plasmodium falciparum*.
Keywords: Hsp90, Molecular chaperones, Plasmodium falciparum / **Supported by:** FAPESP, CNPq

E.84 - Structural Characterization of a Cell-Penetrating Peptide Derived from the SARS-CoV-2 Spike Protein

Renata Naporano Bicev [1], Louíse Eloá Araujo Souza[1], Lucas Rodrigues de Mello[1], Thiago da Costa Lourenço[1], Emerson Rodrigo da Silva[1]
[1]Biofísica, Universidade Federal de São Paulo (São Paulo, Brazil)

INTRODUCTION: The COVID-19 pandemic has recorded over than 500 million cases and 6 million deaths, according to WHO data from April 2022. Due to the high infectivity of SARS-CoV-2, its proteome will be a valuable source of new cell-penetrating peptides (CPPs) and the next years surely will bring interestingly discoveries. OBJECTIVES: To characterize nanoscale structure of chimeric peptides derived from the SARS-CoV-2 and SV40 viral proteomes. To determine the organization of peptide/DNA complexes with potential for gene therapy and to investigate the elastic properties of lipid vesicles in the presence of these complexes. MATERIALS AND METHODS: A chimera peptide was designed combining a short CPP sequence from the spike protein of SARS-CoV-2 with another segment from the SV40 virus. The chimera peptide was associated with nucleic acids to produce self-assembly nanocomplexes. In addition, the interaction of this peptide and the binary system (peptide and nucleic acids) with lipid membranes was investigated. CryoTEM and CD experiments were used to reveal the finer structure of these particles and their interactions. The antimicrobial capacity of the chimera peptide was also evaluated regarding *E.coli* bacteria. DISCUSSION AND RESULTS: The characterization performed indicate that these chimeric sequences present coiled secondary structures and can strongly interact with DNA. Also, interaction with biomembranes has been evidenced through cryoTEM imaging that has shown membrane disruption upon contact with the peptides, but multilamellar transition upon mixture with peptide/DNA. Interestingly, a strong antimicrobial activity has been identified against *E. coli* strains. SAXS measurements will be carried out to refine the strategies used in the formulation of the complexes. CONCLUSION: Our results, along with biological assays that revealed antimicrobial properties against bacterial strains, have potential to disclose a new class of cell-penetrating peptides derived from the SARS-CoV-2 proteome and shed light on their interaction with biomembranes.

Keywords: SARS-CoV-2, Cell-penetrating peptide, nanocomplexes

E.85 - Antibody characterization against SARS-CoV-2 nucleocapsid protein through mass spectrometry

Camila Akemi Oliveira Yamada [1], Bruno de Paula Oliveira Santos[1], Pedro Gontjo Carneiro[1], Mariana Torquato Quezado de Magalhães[1]
[1]Bioquímica e Imunologia, Universidade Federal de Minas Gerais (MG, Brazil)

INTRODUCTION: Antibodies are glycoproteins that are part of the humoral immune response, an important component of the adaptive response of the immune system against various diseases. The antigens of pathogens are the main target of the biological activity of antibodies, and the reason for the diversity of their primary structure. The main function of antibodies is to neutralize antigens and kill microorganisms by opsonization. Because of this high recognition capacity and specificity, studies focusing on antigen-antibody interaction have become an important tool for the pharmaceutical industry. Recently, strategies for the prevention and treatment of various diseases have leveraged knowledge of antigen-antibody to develop new biopharmaceuticals such as protein-based vaccines and the use of epitope peptides for diagnostics. The current Covid-19 pandemic is an example of the urgent need for the scientific community to identify new targets for vaccines, therapeutics, and diagnostics. OBJECTIVES: Here, we propose the characterization of a polyclonal pool of antibodies by mass spectrometry to identify the fragments responsible for antigen recognition, using the SARS-CoV-2 nucleocapsid protein as a model. MATERIALS AND METHODS: To perform these analyses, we expressed and purified the SARS-CoV-2 nucleocapsid (N) protein. In parallel, plasma from several Covid-19 convalescents was subjected to affinity chromatography with Ptn-G. The polyclonal pool of IgGs was loaded into an NHS column containing the N protein. DISCUSSION AND RESULTS: The specific group of IgGs was cross-linked to the N protein. The samples were treated with trypsin to identify the fragments responsible for antigen recognition by MALDI-TOF/MS-MS. CONCLUSION: The next step of this work is to investigate if there is a cross-reaction between the antibodies of the same pool towards other nucleocapsid proteins of the coronavirus. All this information will allow us to better understand the antibody-antigen relationship and provide insights for vaccine development.

Keywords: Antibody characterization, COVID, MALDI-TOF /MS-MS / **Supported by:** CAPES, FAPEMIG and CNPq

E.86 - Evaluation of the Effect of pH in Phosphatidic Acid-Induced Prion Conversion into Amyloid Fibrils

Cyntia Alves Conceição [1,2], Jerson Lima Silva[1,2], Tuane Cristine Ramos Gonçalves Vieira[1,2]
[1]Structural Biology, National Institute of Science and Technology for Structural Biology and Bioimaging (Rio de Janeiro, Brazil), [2]Structural Biology, Institute of Medical Biochemistry Leopoldo de Meis (Rio de Janeiro, Brazil)

INTRODUCTION: Prion protein (PrP^{C}) is composed of structured C-terminal and unstructured N-terminal domains. The C-terminal domain is comprised of three alpha-helices and a small beta-sheet. Its misfolded form, prion scrapie (PrP^{Sc}) is enriched in beta-sheet content and, in most cases, is resistant to proteinase K (PK) digestion. PrP^{C} conversion mechanism is not fully understood. Our group showed that phosphatidic acid (PA) vesicles interacted with recombinant PrP (rPrP), leading to its aggregation at pH 5,5 and pH 7,4 at 25 °C, to a greater extent at pH 5,5. The characteristics of these aggregates and how the acid pH affects PrP:PA interaction is still unclear. OBJECTIVES: We aimed to elucidate PA-induced rPrP conversion into amyloid fibrils at neutral and acid pH, characterizing this aggregate's structure and resistance to PK. MATERIALS AND METHODS: We used light scattering, circular dichroism, fluorescence spectroscopy, and PK at neutral and acid pH to obtain information about two rPrP constructs – PrP23-231 (full-length) and PrP90-231 (truncated N-terminal). We then evaluated the resistance of the fibrils to PK digestion. DISCUSSION AND RESULTS: PA vesicles induced aggregation of both rPrP in all temperatures and both pH, but to a less extent for PrP 90-231 at pH 7,4. PrP23-231 tryptophan fluorescence in the presence of PA shifted to blue values in all temperatures and both pHs. PrP90-231 tryptophan fluorescence changed only by the influence of temperature in acid and neutral pH, but PA interaction had no significant effect. PA-induced fibrillization of both constructs in all temperatures, at both pH. This fibrillization was temperature-dependent: the lower the temperature, the greater the effect. PK fully digested the fibrils formed at the lower temperature at pH 7,4. CONCLUSION: Our results suggest that PA may induce fibrillization of both PrP constructs in a pH-independent manner. This fibrillization is more prominent in the lower temperature tested, and the fibrils formed in this condition are PK-sensitive.

Keywords: Amyloid fibrils, Prion, Phosphatidic acid
Supported by: CNPq, FAPERJ, INBEB, CAPES

E.87 - Insights About the Function of SH3-like Tandem Domain of Human KIN17 Protein: NMR Structure, RNA Binding Site, and Protein-RNA Affinity

Isabella Ottenio de Lourenço[1], Flávio Augusto Vicente Seixas[2], Maria Aparecida Fernandez[3], Fabio Ceneviva Lacerda Almeida[4,5], Marcelo Andres Fossey[1], Fátima Pereira de Souza[1], Ícaro Putinhon Caruso[1,4,5]
[1]Physics, São Paulo State University (SP, Brazil), [2]Technology, State University of Maringá (PR, Brazil), [3]Biotechnology, Genetics and Cell Biology, State University of Maringá (PR, Brazil), [4]National Center for Structural Biology and Bioimaging, Federal University of Rio de Janeiro (RJ, Brazil), [5]Institute of Medical Biochemistry Leopoldo de Meis, Federal University of Rio de Janeiro (RJ, Brazil)

INTRODUCTION: KIN17 participates in several processes in DNA metabolism. This protein up-regulates DNA damage caused by ionizing radiation in all cells, as well as being one of the proteins in spliceosome complexes that mediate different types of nucleic acid transition. A KOW *motif* (338-364) is localized on the second of the two SH3-like subdomains linked by a short loop in the C-terminal region of the full protein. This domain can bind differently with RNA homopolymers, preferring poly(rG) and poly(rU) *in vitro*. OBJECTIVES: This study consists of resolving the structure in solution, determining the affinity between this domain with RNA homopolymers, and showing the binding site on the domain. MATERIALS AND METHODS: The recombinant domain was expressed in M9 labeled and LB medium for *Escherichia coli* culture, and it was purified by chromatography methods. The ^{1}H, ^{15}N, and ^{13}C resonance assignments for the domain were obtained from a multidimensional heteronuclear NMR experiment. The NMR structure was determined via constraints acquired from NOESY experiments using the program ARIA. The CSP from the 2D ^{15}N-^{1}H HSQC NMR experiments were used to identify the domain binding site. The k_D was estimated by Trp quenching at 25 °C for yeast RNA, RNA homopolymers, and poly(A) DNA. DISCUSSION AND RESULTS: The backbone atoms ^{13}CO, $^{15}N/^{1}H^{N}$, $^{13}C\alpha$, and $^{1}H\alpha$ of the domain were 98.4% assigned. The structure in solution is similar to the crystal structure already solved. The binding region is shown between the subdomain units with an important residue in the KOW *motif*. The direct measure of the affinity constant shows that this domain has a preference to poly(rG) and poly(rC), k_D on order of 10^{-8} M^{-1}, when compared to the k_D on order of 10^{-6} M^{-1} for poly(rU) and poly(rA). CONCLUSION: The different affinities for the homopolymers would suggest new insights about the contribution of this domain on DNA metabolism.

Keywords: KIN protein, NMR structure, SH3-like domain / **Supported by:** CNPq

E.88 - Effects of Aripiprazole on Female Reproductive System

Aline Gabrielle Gomes Da Silva[1,2], Artemia Kelly Holanda Pereira[2], Renata Gleysiane De Sousa Felix[2], Caio Cesar Araújo Dos Santos[2], Ana Beatriz Silva Angelo[2], Maria Joana Nogueira De Moura[1,2], Cibele Dos Santos Borges[2]
[1] Biochemistry and Molecular Biology, University of Rio Grande do Norte (Rio Grande do Norte, Brazil), [2]Bioscience, Biological and Health Sciences Center - CCBS, Federal University of the Semi-Arid Region (Rio Grande do Norte, Brazil)

INTRODUCTION: Aripiprazole, an antipsychotic drug used to treat mental disorders, has high affinity for serotonin, dopamine and noradrenaline receptors. It is known that the 5-HT system can directly influence the reproductive function of animals. However, there are few reports of the effects of aripiprazole on female reproductive system. OBJECTIVES: Evaluate the impact of exposure to different doses of aripiprazole on female reproductive system, mainly estrous cyclicity. MATERIALS AND METHODS: Adult female Wistar rats (n=11/group) from control group (CTRL), group treated with 0.3mg/kg (EXP1), 3.0mg/kg (EXP2) 6.0mg/kg (EXP3) of aripiprazole were treated during 15 days and the estrous cycle of the rats was monitored. After the end of the treatment, in the first estrus, six female rats/group were weighted and euthanized by decaptation. Blood samples were collected to hormonal levels. Vital organs (kidney, adrenal, heart, liver, brain, pituitary and thyroid) were collected and weighed. The ovaries and uterus were collected, weighed and fixed for further histopathological processing. Ethics Committee (UFERSA-23091.014949/2019-90). Statistical analysis: ANOVA (P< 0.05). DISCUSSION AND RESULTS: No alterations were observed in the final body, absolute and relative weights of reproductive and vital organs of the rats exposed to different doses of aripiprazole when compared to the CTRL group. However, when compared between the experimental groups, the relative weight of the pituitary was significantly increased in the EXP1 group (0.07+0.01mg/g) in relation to the EXP2 group (0.04+0.01mg/g). The evaluation of the estrous cycle showed a significant decrease in the number of estrus in the EXP3 group (2.4+0.5) when compared to the CTRL group (5.2+0.7). On the other hand, when performing sexual behavior, the only group that showed a significant reduction in relation to the CTRL group was the EXP1 group (62% x 34%). CONCLUSION: The exposure to aripiprazole during adulthood, although subtle, it can cause impairment on female cyclicity and/or libido.

Keywords: Antipsychotics, endocrine system, reproduction / **Supported by:** PIVIC

E.89 - [PROJECT] Structural Characterization of the C-terminal Domain of the VipA Protein from Klebsiella pneumoniae Type VI Secretion System and the search for virulence blockers

Izabelle Oyamada dos Santos [1], Gisele Cardoso de Amorim[1,2]
[1]Multidisciplinary Center for Research in Biology (Numpex-Bio), Federal University of Rio de Janeiro - Campus Duque de Caxias (Rio de Janeiro, Brazil), [2]National Center for Structural Biology and Bioimaging (CENABIO)/National Center , Federal University of Rio de Janeiro (Rio de Janeiro , Brazil)

INTRODUCTION: *Klebsiella pneumoniae* is a Gram-negative bacterium that causes pneumonia and other serious infections. Its hypervirulent and multiresistant strains are a public health challenge, given its wide circulation. Thus, understanding its virulence mechanisms is of great importance for comprehending the cause and progression of the infection. Among these mechanisms, there is the secretion of effectors and toxins by the type VI secretion system (T6SS), a contractile machine formed by at least 13 protein families. The effectors can be secreted into an eukaryotic target cell, in the process of infection, or a prokaryotic one, during competition. In this project, we aim to characterize the C-terminal domain of the protein VipA (VipA_CTD) which, together with VipB, is responsible for the formation of the contractile sheath that drives the secretion of effector proteins. OBJECTIVES: To determine the structure of VipA_CTD by Nuclear Magnetic Resonance (NMR); to perform fragment-based screening using VipA_CTD as a target and evaluate the potential of these ligands to inhibit the interaction of VipA and VipB MATERIALS AND METHODS: The CTD of VipA will be obtained by heterologous expression in E. coli BL21 strain. After this step, cells will be lysed and the protein purified by nickel affinity chromatography. For the structural determination, a set of classical 2D and 3D NMR experiments will be acquired. The drug screening will be based on 1D 1H experiments, such as CPMG and water LOGSY. The residues of VipA_CTD interacting with the selected ligands will be mapped by NMR chemical shift perturbation (CSP). These data will offer a new target for drug development against resistant bacteria infections.

Keywords: Klebsiella pneumoniae, VipA, RMN
Supported by: Faperj, CNPq

E.90 - Project: Structural Characterization of the Hcp2 hexameric rings of the Type VI Secretion System of Klebsiella pneumoniae

Maria Eduarda Ferreira do Rosário [1], Débora Caroline Amorim de Souza [1], Marcos Caique Santana Silva[1], Gisele Cardoso de Amorim[1]
[1]Campus Duque de Caxias Prof. Geraldo Cidade, Núcleo Multidisciplinar de Pesquisa UFRJ-Xerém em Biologia (RJ, Brasil)

INTRODUCTION: Klebsiella pneumoniae is a Gram-negative bacterium from the Enterobacteriaceae family. It is responsible for urinary and respiratory tract infections. Multiple K. pneumoniae antibiotic multiresistant strains have emerged, including resistance to the latest generation carbapenems, which makes the study of its virulence factors of great interest. Among these factors, the type six secretion system (T6SS) is noteworthy. It is an important protein apparatus for the secretion of effector molecules inside the target cell. T6SS is composed of 13 structural protein families. Its mechanism of action is evolutionarily derived from T4 bacteriophage and is based on the contraction of an external sheath and injection of the inner tube into the host cell. The internal tube is assembled by hexameric rings of the Hemolysin co-regulated protein (Hcp). It has an inner diameter of about 40 Å that can fit secreted effector proteins. OBJECTIVES: Our main objective is to characterize these Hcp2 hexameric rings by Negative-Staining Electron Microscopy and Single Particle Analysis by Cryoelectron microscopy (SPA). MATERIALS AND METHODS: The wild type Hcp2 and its designed mutants were expressed in E. coli Rosetta pLysS strains and purified by nickel affinity chromatography. These mutants were designed to impair the interaction between the rings, inhibiting the assembly of the tube. Samples with different concentrations will be analyzed by negative-staining using uranyl acetate as the contrasting agent for electron microscopy. The SPA experiments will help us to resolve the conformation of the Hcp2 hexameric rings and tube. DISCUSSION AND RESULTS: Our previous results with electron microscopy show rings with different and bigger diameters than the expected, which led us to design these mutants. We hope to see more homogeneous structures. And also that SPA results will give us a better understanding of the structural organization of these important T6SS structures. CONCLUSION:

Keywords: T6SS, hexameric rings, electron microscopy / **Supported by:** FAPERJ

E.91 - Structural and Dynamic Characterization of Evolved VgrG Protein of the Klebsiella pneumoniae Type 6 Secretion System

Jairo Herlan Dominguez Antty[1], Gisele Amorim[1]
[1]Numpex-Bio, Universidade Federal de Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: *Klebsiella pneumoniae* presents a variety of virulence factor, including the Type 6 Secretion System (T6SS), which allows the translocation of effectors from the cytoplasm to the extracellular medium or into target cells. Some of the components of T6SS are the Hcp, PAAR and VgrG proteins. The C-terminal region of VgrG (VgrG-CTD) has been related to the recruitment of effector molecules and can also have effector activity. The VgrGs that show effector activity are known as evolved VgrG. In the kp52.145 strain of *K. pneumoniae*, it has been shown that the VgrG4-CTD interacts with actin and other proteins, modulating the cytoskeleton structure in favor of the infection process. OBJECTIVES: The objective of this project is to express, purify and study the structure and dynamics of the VgrG4 protein of kp52.145, as well as its interaction with hypothetical effectors. MATERIALS AND METHODS: To obtain VgrG, the expression vector pET28a-VgrG4 will be used to transform *E. coli* BL21 cells by heat-shock. The cells will be grown in LB culture medium, and protein expression will be induced by IPTG at 37°C. Subsequently, the supernatant from cell lysis will be purified by affinity chromatography (HisTrap FF column). We will perform 19F NMR experiments to study the dynamics and interaction of VgrG with putative effectors. DISCUSSION AND RESULTS: We expect to express, purify and study the dynamics of VgrG, and also map its interaction with other effectors. These data could improve the understanding of the role of this important protein in T6SS activity.

Keywords: T6SS, Klebsiella pneumonia, VgrG
Supported by: FAPERJ

E.92 - Structural and functional characterization of the N-terminal domain of the human coronavirus HCoV-HKU1 nucleocapsid protein

Aline de Luna Marques [1], Ícaro Caruso[2,3], Peter Bezerra[2], Fábio Almeida[2], Gisele Amorim[1,2]
[1]Multidisciplinary Center for Research in Biology (Numpex-Bio), Federal University of Rio de Janeiro - Campus Duque de Caxias, RJ, (, Brazil), [2]2National Center for Structural Biology and Bioimaging (CENABIO)/National Center, Federal University of Rio de Janeiro, RJ (, Brazil), [3]Multiuser Center for Biomolecular Innovation (CMIB), São Paulo State University (, Brazil)

INTRODUCTION: Since 2002, three human coronaviruses (HCoVs) have gained notoriety: SARS-CoV, MERS-CoV, and SARS-CoV-2, the latter being responsible for Covid-19 pandemic. Besides them, there are endemic HCoVs, such as HCoV-HKU1, associated with mild respiratory tract disease, that can progress to acute respiratory failure. During infection, the transcription of the viral genome generates full-length and subgenomic RNAs, through a discontinuous process regulated by transcriptional regulatory sequences (TRSs). Nucleocapsid (N) protein of CoVs acts as an RNA chaperone and its N-terminal domain (N-NTD) binds to TRS, forming a high-affinity complex. The specific binding of dsTRS and single-stranded RNA (TRS+ and TRS-) to N-NTD is still poorly understood. The comprehension of the mechanisms involved in the pathogenesis of endemic HCoVs contributes to the understanding of the SARS-CoV-2 infection and the development of therapeutic strategies. OBJECTIVES: Probing HKU1-N-NTD melting activity of dsDNA/RNA, determining the dissociation constant (Kd) of its interaction with RNA TRS+, TRS- and dsTRS, mapping the residues involved in this interaction and comparing these results with those obtained for SARS-CoV-2-N-NTD. MATERIALS AND METHODS: HKU1-N-NTD melting activity towards double-stranded DNA/RNA was assessed by FRET fluorescence. The Kd of HKU1-N-NTD interaction with TRS was obtained by anisotropy and the interacting residues were determined by protein NMR titration with DNA/RNA. DISCUSSION AND RESULTS: HKU1-N-NTD melting activity was greater for dsRNA than for dsDNA, unlike SARS-CoV-2, which presents similar activities for both. The SR region, contiguous to N-NTD, contributes to this process. The affinity of N-NTD is greater for TRS(-) than for TRS(+) and it increases in the presence of SR region. NMR data indicate that the main binding region is located between the central loop and the β-sheet core. CONCLUSION: HKU1-N-NTD interacts with DNA/RNA fragments, promoting double-strand melting, being more specific to RNA. These results may help better understand the role of N protein in the discontinuous transcription, enabling new approaches to fight CoVs.
Keywords: HCoV-HKU1, nucleocapsid protein, TRS / **Supported by:** FAPERJ

E.93 - [PROJECT] Expression, Purification and Biochemical Characterization of a putative Effector of the Type VI Secretion System (T6SS) from Klebsiella pneumoniae

Felipe Henrique Peçanha [1], Gisele Cardoso Amorim[1]
[1]Campus Duque de Caxias, Multidisciplinary Center for Research in Biology (Numpex-Bio), UFRJ (RJ, Brasil)

INTRODUCTION: Klebsiella pneumoniae is a Gram-negative bacterium known for causing nosocomial infections. In the last years, new hypervirulent and multiresistant K. pneumoniae strains emerged worldwide. Among its identified virulence factors, the Type VI Secretion System (T6SS) is a protein complex also present in other pathogenic Gram-negative bacteria. The T6SS mediates the injection of effector molecules into a target cell or extracellular milieu. In the genome of the kp52.145 strain, three T6SS loci were identified. At locus III, there is a gene that codifies to a hypothetical phospholipase D (PLD1), that seems to be involved in pathogenicity. Considering the importance of understanding K. pneumoniae virulence mechanisms and find ways to fight them, this research project aims to establish a method for obtaining the PLD1 protein by heterologous expression, and characterize its activity, elucidating its function during bacterial infection. OBJECTIVES: Express and purify PLD1 and determine its activity and structure in order to understand its importance to kp52.145 virulence. MATERIALS AND METHODS: The pET-28a(+) plasmid will be employed as an expression vector and used to transform E. coli BL21 (DE3) by heat-shock .Different expression conditions will be tested, as temperature and IPTG (inducer) concentration. Polyacrylamide gel electrophoresis will be used to qualitatively analyze the production of the protein and also the purification steps. Protein purification and refolding steps will be carried-out by in-column refolding using an affinity chromatography column. The EnzyChrom™ Phospholipase D Assay Kit will be used for the activity assay. DISCUSSION AND RESULTS: CONCLUSION: Expression and correct refolding of PLD1. Confirmation of its phospholipase enzyme activity. With these, we aim to determine the exact role of PLD1 in the virulence of K. pneumoniae. These results might pave the way for the development of virulence inhibitors for the treatment of infections caused by multiresistant bacteria.
Keywords: phospholipase D (PLD1), heterologous expression, in-column refolding
Supported by: FAPERJ, CNPq

F - Biotechnology and Biomaterials

F.01 - Study of Encapsulation of Antineoplastic Drugs in Nanostructured Systems: Structural Characterization and Synergistic Effect of Drugs

Amanda Bellini[1], Raquel Frenedoso da Silva[2], Iris Renata Ribeiro[2], Leandro Ramos Souza Barbosa[2]
[1]Biochemical-Pharmaceutical Technology Department, Faculty of Pharmaceutical Sciences - USP (São Paulo, SP), [2]LNLS, Brazilian Synchrotron Light Laboratory (São Paulo, Brasil)

INTRODUCTION: Nanotechnology field has been growing in the past decades, improving studies of several compounds. Among these technologies, the drug delivery systems are being largely used, whose objective is increase pharmaceutical substances efficacy, and this may includes a more selective distribution within the sick organism, the molecules' liberation time fine tuning and/or attenuation of adverse effects. To make this possible, drugs are nanoencapsulated within synthetic origin or biodegradable structures, necessarily compatible with the active principle. Their composition may vary between polymers, lipid particles, emulsions, membranes, hydrogels, microspheres, dendrimers and other molecular complexes, such as metals (silver, silica and gold). Among the nanoparticles responsible for this systems are cubosomes, which are complex nanostructures that successfully encapsulate hydrophilic and hydrophobic actives. OBJECTIVES: The main objective is to study the effect of the antineoplasic actives against cervical cancer cells (HeLa) and human epidermal keratinocyte (HaCat), the synergic/antagonic effects of drugs, cubosomes synthesis and characterization for further encapsulation of actives inside them. MATERIALS AND METHODS: The chosen molecules are doxorubicin, cisplatin, vemurafenib and curcumin. In this research project, we will broaden knowledge of antineoplastic activity of these compounds, testing them on HeLa and HaCat cells, quantifying the post-exposure cell viability. The cubosomes were synthesized with phytantriol and tested by dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA). DISCUSSION AND RESULTS: The cell viability test demonstrated that HeLa is more resistant to the effect of drugs, the IC50 and IC25 of the samples were determinated and the combination of cisplatin and vemurafenib looks promising. The cubosomes presented a DLS 175nm, PDI 0,08 and NTA 1,8 x 1012 particles/mL. CONCLUSION: The synergy between cisplatin/curcumin and cisplatin/vemurafenib looks promising and the cubosomes were efficiently synthesized and seem to be stable.
Keywords: Cubosomes, Antineoplasic, Drug delivery / **Supported by:** CAPES

F.02 - RT-LAMP: an emerging assay for SARS-CoV-2 detection

Carolina Ribeiro Córdula [1], Gabriela Calixto Ribeiro Holanda[1,2], Valéria Visani[1], Sandra Elizabeth Silva[1], Carlos Henrique Madeiros Castelletti[1], José Luiz Lima-Filho[1], Danyelly Bruneska Gondim Martins[1]
[1]Laboratory of Immunopathology Keizo Asami Institute (iLIKA), Universidade Federal de Pernanbuco (, Brazil), [2]Institute of Tropical Medicine, Nagasaki University (, Japan)

INTRODUCTION: When new viruses emerge on a pandemic scale, authorities must respond rapidly to control the fast spread that can threaten human life. The World Health Organization recommends nucleic acid amplification tests for infection virus disease diagnosis, mainly reverse transcription-quantitative polymerase chain reaction (RT-qPCR), a sensitive and specific method for early detection, and fast immunological tests, less sensitive but cheaper. Loop-mediated isothermal amplification with reverse transcription (RT-LAMP) is an emerging technique that amplifies cDNA at a constant temperature. It explores the polymerase displacement activity of *Bst* DNA polymerase and a set of 4 to 6 primers, showing up faster and cheaper than RT-qPCR. OBJECTIVES: This work aimed to evaluate an RT-LAMP for SARS-CoV-2 detection shown in Yoshikawa R et al. (PLoS Negl Trop Dis. 2020 Nov 4;14(11)). MATERIALS AND METHODS: Nasopharyngeal samples from patients were submitted to automated RNA extraction, followed by RT-qPCR, using 2019-nCoV_N1, 2019-nCoV_N2, and RNAseP primers/probe sets according to CDC 2019-nCoV protocol (2020), and RT-LAMP, using a set of 6 primers based on the sequences of the ORF1b region of SARS-CoV-2, as described by Yoshikawa R et al. (PLoS Negl Trop Dis. 2020 Nov 4;14(11)). DISCUSSION AND RESULTS: Among 107 samples analyzed, 59 were positive for SARS-CoV-2, and 48 were negative by the RT-qPCR technique. Results showed a limit of detection (LoD95) equal to 100 copies/µL. For positive tests, including only samples whose concentrations were above LoD95, sensitivity was 100%; specificity was 100%; the positive predictive value was 100% (for every 100 positive tests, 100 individuals would be sick); the negative predictive value was 100% (for every 100 negative tests, 100 would be healthy); and the accuracy (correct classification rate) was 100%. CONCLUSION: In conclusion, the RT-LAMP test in the evaluation was as sensitive and specific as RT-qPCR when viral concentrations were above 100 copies/µL, representing a potential tool for emergent viruses' diagnosis SARS-CoV-2. **Keywords:** RT-LAMP, diagnosis, SARS-CoV-2

F.03 - Rheological and Toxicological Characterization of Hydrogels from Guanosine Derivatives and the Impact of KCl and NaCl Salts on These Properties.

Rodrigo Fernandes de Almeida [1]
[1]DFAP, Instituto de física, Universidade de São Paulo (São Paulo, Brazil), [2]Centro de ciencias naturais e humanas, Universidade federal do ABC (São Paulo, Brazil), [3]CLA, Instituto de Pesquisas Energéticas e Nucleares (São Paulo, Brazil)

INTRODUCTION: Given the growing interest in biomaterials such as hydrogels, this work is part of a project to develop and characterize hydrogels from guanosine derivatives to be used as a methylene blue delivery system which act as a photosensitizer in fungal photoinactivation. As seen previously in the literature, it is known that potassium Guanosine monophosphate (K-GMP) salts form a stable self-assemble hydrogel that can have its consistency raised by the addiction of guanosine (Gua). OBJECTIVES: The objective of this work is to investigate the impact of NaCl and KCl salts on the rheological properties of these gels and the use of them to stabilize sodium guanosine monophosphate (Na-GMP) hydrogels in differents Gua:GMP molar ratios and different salts concentrations to be able to compare with Gua:K-GMP hydrogels without chlorides. MATERIALS AND METHODS: For such characterization rheological test by frequency scan. In addition, we seek to study the cytotoxicity of the hydrogels of interest through a colorimetric assay in lysosomes culture in order to be able to use the gels as a vehicle for drug delivery in animals and humans in future research. DISCUSSION AND RESULTS: The results observed for the rheological assays is the increase in elastic module and consistency coefficient and also the increase of viscosity for most of the samples and the verification of the stabilization role of KCl for the Na-GMP hydrogels. CONCLUSION: The use of KCl salt in the hydrogels seems to increase stability and consistency for both GUA:GMP-K and GUA:GMP-Na gels when subjected to stress and temperature variation.

Keywords: Biomaterials, Guanosine, Hydrogels

F.04 - Lignin Modulates the Activity of AA9 Lytic Polysaccharide Monooxygenases

Ellen Karen Barreto Roman [1], Lúcia Daniela Wolf[1], Leandro Vieira dos Santos[2], George Jackson de Moraes Rocha[1], Thamy Lívia Ribeiro Corrêa[1], Mário Tyago Murakami[1]
[1]LNBR, Centro Nacional de Pesquisa em Energia e Materiais (SP, Brazil), [2]Genetics and Molecular Biology Graduate Program, University of Campinas (SP, Brazil)

INTRODUCTION: Lytic polysaccharide monooxygenases (LPMOs) represent a major breakthrough in the lignocellulosic biomass deconstruction field by boosting classical enzyme cocktails. LPMOs depends on external electron donors by a mechanism yet not completely understood. OBJECTIVES: In this work, we show how macromolecular lignin and some of its building blocks affect the activity of an AA9 LPMO, the *Af*LPMO9A from *Aspergillus fischeri*, which is active on cellulose and hemicellulose. MATERIALS AND METHODS: The products released by *Af*LPMO9A from PASC and xyloglucan were analyzed by high-performance anion-exchange chromatography/pulsed amperometric detection (HPAEC-PAD). DISCUSSION AND RESULTS: Lignin obtained from alkali pretreatment showed the best results, which can be associated with its lower molecular weight and solubility. Amongst the 33 lignin building blocks evaluated, only dimethoxylated monophenols and diphenols showed to be effective electron donors to *Af*LPMO9A. Interestingly, lignin-derived compounds did not promote activity on xyloglucan, except when PASC was added in the reaction. In conclusion, our study points out how industrially relevant lignin preparations and an extensive list of lignin building blocks act as electron donors to AA9 LPMOs, altering its activity. CONCLUSION: These results are relevant for biotechnological applications of LPMOs and point to still elusive mechanisms of interaction between electron donors and LPMO function.

Keywords: AA9, Electron donors, LPMOs

F.05 - Preliminary analysis of coagulotoxic profile from individual venoms of seven Bothrops species.

Nathália da Costa Galizio [1], Caroline Serino-Silva[1], Weslei da Silva Aguiar[1,2], Luccas Daniel Padueli[1], Giovanni Perez Machado da Silveira[1], Sávio Stefanini Sant'Anna[1], Kathleen Fernandes Grego[1], Anita Mitico Tanaka-Azevedo[1], Karen de Morais Zani[1]
[1]Laboratório de Herpetologia, Instituto Butantan (São Paulo, Brasil), [2]Programa de Pós-Graduação Interunidades em Biotecnologia, Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: Snake envenomation is a neglected tropical disease responsible for approximately 2.5 million envenomation and 150,000 deaths per year. In Brazil, the Viperidae family includes the majority of species of highest medical importance, as they are responsible for most of snakebites accidents. Among the effects resulting from these accidents, haemostatic disorders are believed to play a central role in the pathophysiology of envenomation and prey capture. The intraspecific variability of snake venoms associated with their functional diversity is already known and it is particularly critical when considering their reactivity with antivenom. Considering the complexity of the envenomation caused by snake species from Viperidae family and the relevance of these species to public health, a multifaceted study comparing the coagulotoxic profile of these species and their neutralization by the antivenom produced is of extreme importance. OBJECTIVES: Thus, the present work proposes the characterization of the coagulotoxic profile of individual venoms of the *Bothrops atrox* (Bax), *B. neuwiedi* (Bn), *B. pauloensis* (Bp), *B. diporus* (Bd), *B. pubescens* (Bpu), *B. marmoratus* (Bmm) and *B. matogrossensis* (Bmt) snake species. The venom was collected individually. MATERIALS AND METHODS: The composition of individual venoms was evaluated by SDS-PAGE (15%). PLA2, LAAO and azocasein activities were performed. DISCUSSION AND RESULTS: Preliminary results in SDS-PAGE showed minor individual variation in Bax while Bn demonstrated the major individual variation among all species. LAAO and proteolytic activity on casein showed slight individual variation among species. Bd and Bpu showed the higher LAAO activity, whereas the PLA_2 activity demonstrated major individual variation. CONCLUSION: The next steps will allow the evaluation of coagulotoxic profile in a broad and specific way, exploring the different areas of the coagulation cascade. This work is supported by FAPESP (2020/07268-2) and CAPES.

Keywords: Coagulopathy, Bothrops, Viperidae
Supported by: Capes

F.06 - Biopharmaceutical evaluation of nanostructured biomaterial for cartilagino tissue regeneration

Mônica Helena Monteiro do Nascimento [1], Aryane Alves Vigato[1], Daniele Ribeiro de araújo[1]
[1]Centro de Ciências Naturais e Humanas, Universidade Federal do ABC (São Paulo, Brazil)

INTRODUCTION: Cartilage tissue pathologies have been the subject of relevant studies, once they represent an important health problem worldwide. The articular cartilage is known to have a poor intrinsic capacity for repair, due to the limited regenerative capacity. For this reason, minor injuries may progress to cartilage tissue damage and degeneration. A variety of scaffolds, such as polymers of natural origin, have been investigated for the expansion of chondrocytes *in vitro*, aiming at the repair of injured cartilage. The use of gelatin-hyaluronic acid (GE-HA) blends has been investigated in cartilage tissue engineering applications, with promising results. Glycyrrhizic acid (GA) is the main active component of the licorice root GA and has been reported to have multiple therapeutic properties, including anti-inflammatory effects. OBJECTIVES: Based on this information, this project aimed the preparation and physico-chemical characterization of a polymeric biomaterials blends based on GE and HA as a carrier for GA. MATERIALS AND METHODS: The blends were prepared by mixing GE (5 m/v%) with HA (1 m/v%) to obtain three different ratios of GE-HA (50:50; 30:70; 70:30 (v/v)). The GA concentration was 0.1 % for all biomaterials. Physicochemical and morphological characterizations were performed by different techniques. DISCUSSION AND RESULTS: The blends resulted in a biomaterial with a high swelling ratio that can allow nutrient distribution and absorption. The presence of HA increased the swelling capacity, which varied from 976% to 1420%, for GE-HA 70:30 and 30:70 respectively. The blends morphology in the highest ratio of HA (70%) presented a highly porous structure observed by SEM. The DSC analyzes showed an increase in the melting temperature of biomaterials in the presence of GA (154°C to 173°C for GE-HA 50:50). CONCLUSION: The GE-HA blend obtained has a great potential to sustain and prolong the GA release and to support and interact with cells in biological assays, this analyzes will be performed in sequence.

Keywords: Tissue Engeneering , Polimeric biomaterials, Cartilage Tissue
Supported by: CAPES, FAPESP, CNPq and UFABC

F.07 - E12F Peptide Exhibits Antitumor Effect *In vitro* and *In vivo* .

Agnes Kobayashi Calvo de Santana [1], Maria Carolina Mariano Cesar[1], Victória Santos Souza[1], Fernanda Fernandes Miranda da Cunha[1], Juliana Machado Anastácio[1], Denise Costa Arruda[1]
[1]Núcleo Integrado de Biotecnologia, Universidade de Mogi das Cruzes (São Paulo, Brasil)

INTRODUCTION: Melanoma is considered the most aggressive skin cancer due to its metastatic potential. Given the high mortality rate and the disadvantages of current treatments, it is imperative to study new compounds for the treatment of melanoma. BRN2 is a transcription factor that is overexpressed in melanoma and is related to cell migration and invasion. Peptides derived from transcription factors may be a good strategy for treating melanoma. In previous results, E12F peptide showed antitumor effect on B16F10-Nex2 murine melanoma cells. OBJECTIVES: Verify the antitumor effect of E12F in human melanoma cells and determine its effectiveness in murine melanoma cells *In vivo* . MATERIALS AND METHODS: The viability was determined by the Trypan Blue and MTT assays. The analysis of cell migration was done using a Wound-Healing (scratch) assay, and the invasion assay was performed with transwell inserts with a Matrigel layer. *In vivo* experiments were carried out in C57BL/6 male mice, which were intravenously inoculated with B16F10-Nex2 and treated with E12F peptide for ten days. Then, the lungs were removed and metastatic nodules counted. *In vivo* experiments were approved by the UMC Animal Care & Use Committee (CEUA; Protocols 005/2015 and 003/2018). DISCUSSION AND RESULTS: E12F was not found cytotoxic in either murine or human melanoma cells. The peptide inhibited migration in B16F10-Nex2 cells and SK-Mel-25 cells. Invasion also was inhibited in B16F10-Nex2 cells and SK-Mel-25 cells. In addition, E12F reduced the number of tumor nodules by 88% in metastatic model. CONCLUSION: Our results demonstrated that the E12F peptide has antitumor effects that inhibit migration and invasion in murine and human melanoma cells, and shows a protective effect *In vivo* in the metastatic model.

Keywords: Melanoma, Peptide, Metastasis
Supported by: FAPESP

F.08 - Bioluminescent bionsensor plate using Amydetes viviani firefly luciferase for antimicrobial bioprospection

Alessandra Freitas Serain [1], Marcos José Salvador[2], Aislan Cristina Rheder Fagundes Pascoal[3], Alexandra Christine Helena Frankland Sawaya[2], Vadim Viviani[1]
[1] Physics, Chemistry and Mathematics, Universidade Federal de São Carlos, Brazil (SP, Brasil), [2]Vegetal Biology, Universidade Estadual de Campinas (SP, Brasil), [3]Basic Sciences, Universidade Federal Fluminense (RJ, Brasil)

INTRODUCTION: Bioluminescence is defined as the light emission in living organisms, being used as a signaling system between them, as with fireflies. Firefly luciferases are responsible for catalyzing the bioluminescent oxidation of luciferin in the presence of adenosine triphosphate (ATP), hence ATP-based bioluminescence assays have been developed. OBJECTIVES: Aiming at the bioprospection of natural products with antimicrobial activity and knowing that the search for new natural antimicrobial substances demands optimization of bioprospecting assays, we propose the development of plates with immobilized biosensor bacteria, based on a former protocol used to detect toxic agents such as heavy metals. MATERIALS AND METHODS: *E. coli* BL21-DE3 cells transformed with plasmid pCold containing the luciferase gene from *Amydetes viviani* (pC-Amy) were immobilized in 96-well plates. The assay was performed in triplicate with 30 samples at concentrations of 5000, 1000, 200, 40, 8 and 1.6 µg/mL, applied to the plate in four replicates for 6 and 24 hours of contact between the samples and the cell culture. The plates were revealed by bioimaging with NightOwl CCD-refrigerated photodetection system (Berthold). DISCUSSION AND RESULTS: The main substances that showed antimicrobial activity after 24 hours of contact were essential oil of rosemary (*R. officinalis*) IC_{50} 224,110 ± 0,203 µg/mL, eucalyptus (*E. globulus*) IC_{50} 152,18 ± 0,202 µg/mL, and cypress (*C. semperverens*) IC_{50} 133,323 ± 0,186 µg/mL, *Sinningia schiffneri* crude extract IC_{50} 83,825 ± 0,183 µg/mL and red propolis IC_{50} 190,127 ± 0,167 and 199,313 ± 0,151 µg/mL. CONCLUSION: The results allowed a quick, accurate and quantitative screening, wherein the result is based on IC_{50}, an accurate concentration measurement, allowing a reliable comparison between the prospected samples.

Keywords: Bioluminescence, Bioprospection, Antimicrobial activity / **Supported by:** FAPESP

F.09 - Antibodies mimetic for CHIKV neutralization

Antonio Purificação Júnior[1], Matheus Ferraz [1,2], Marjorie Freire[3], Isabelle Viana[1], Roberto Lins Neto[1]
[1]Virologia, Aggeu Magalhaes Institute, Oswaldo Cruz Foundation (Pernambuco/PE, Brazil), [2]Dep. of Fundamental Chemistry,, Federal University of Pernambuco (PE, Brazil), [3]Physics Institute of São Carlos, University of São Paulo, Brazil (SP, Brazil)

INTRODUCTION: CHIKV infection causes a severe febrile illness and is characterized by debilitating polyarthritis. The E2 envelope protein is the main site of interaction with cellular receptors for the infection of host cells. Furthermore, epitope mapping of CHIKV-induced antibodies showed that the E2 protein is the main target of neutralizing antibodies. OBJECTIVES: In this context, we hypothesized that the development of molecules inspired by neutralizing antibodies directed against specific epitopes of the B domain of the E2 protein may be able to bind and neutralize CHIKV in *In vitro* assays. To this end, this project explored the structural characteristics of epitopes in E2B protein, to computer-design high affinity aptamers based on hot spots grafting. MATERIALS AND METHODS: The structural and dynamic properties of the most promising designed proteins were evaluated through molecular dynamics simulations, and as from the theoretical predictions, three the aptamers CETZDT-2, CETZDT-3, CETZDT-7 were was selected to be expressed in E. coli. DISCUSSION AND RESULTS: Using microscale thermophoresis assays, the binding affinity of these aptamers to CHIKV was determined, and the CETZDT-2 protein was able to recognize the antigenic target with affinity comparable to that of natural antibodies (k_D= 100nM). The evaluation of the maintenance of structural properties, by circular dichroism, showed that CETZDT-2 is higly stable, preserving its secondary structure content even at high temperatures. Neutralization assays (plaque reduction and microneutralization) demonstrated that CETZDT-2 was able to neutralize CHIKV infection *in vitro*, with an EC50 of 31.44µM (PRNT) or 107.4µM (MN). Due to the similarity of E2B epitopes between alphaviruses, we have tested the neutralization potential of CETZDT-2 against MAYV, and it was observed an EC50 of 79.45µM (PRNT), indicating its neutralizing potential *in vitro*. CONCLUSION: Therefore, our results indicate that the designed protein has potential application as a diagnostic marker for acute infections, as well as in new therapeutic strategies against CHIKV and MAYV infection.
Keywords: Chikungunya Virus, Aptamers,, Therapeutic, / **Supported by:** FACEPE

F.10 - Development of Synthetic Proteins for Zika Virus Neutralization

Lícya Samara da Silva Xavier [1], Danilo Fernandes Coêlho[1,2], Matheus Vitor Ferreira Ferraz[2], Tayná Evily de Lima[1], Isabelle Freire Tabosa Viana[1], Roberto Dias Lins Neto[1]
[1]Department of Virology and Experimental Therapy, Aggeu Magalhães Institute, Oswaldo Cruz Foundation (Pernambuco, Brasil), [2]Department of Fundamental Chemistry, Federal University of Pernambuco (Pernambuco, Brasil)

INTRODUCTION: Given the lack of effective therapeutic strategies against Zika virus (ZIKV), we have engineered, *in silico*, proteins that target an epitope of neutralizing antibodies on the DIII domain of ZIKV envelope protein (ZIKV-E). OBJECTIVES: To evaluate the binding affinity of the synthetic proteins to their target, as well as their ability to neutralize ZIKV infection *in vitro*. MATERIALS AND METHODS: The proteins were expressed in competent *Escherichia coli* cells and purified by affinity chromatography. Binding affinities were measured by microscale thermophoresis, and ZIKV (PE243-Asian strain) *In vitro* neutralization assays were assessed by plate viral microneutralization. DISCUSSION AND RESULTS: It was demonstrated that one of the engineered proteins was capable of bind to ZIKV-E with high affinity (KD = 7.8 x 10^{-9} M) and neutralize the viral infection *In vitro* with an EC50 of 0.9 µM. The binding affinity results obtained in our study were similar to the values presented by highly specific neutralizing antibodies described in the literature. CONCLUSION: Among the proteins synthesized and tested *in vitro*, one of them showed a high binding affinity to ZIKV-E protein, and it was capable to neutralize ZIKV infection *in vitro*. Therefore, the engineered protein comprises of a promising candidate for therapeutic strategies against ZIKV infections *In vivo* .
Keywords: Zika virus, Protein engineering, Viral neutralization
Supported by: FACEPE, CNPq, Fiocruz, Instruct-Ultra, CYTED.

F.11 - Heterologous Expression and Characterization of PETase in Eukaryotic System for Plastic Upcycling

Iara Ciancaglini [1], Ellen Karen Barreto Roman[1], Thamy Lívia Ribeiro Corrêa[1], Mario Tyago Murakami[1]
[1]Brazilian Biorenewables National Laboratory (LNBr), Brazilian Center of Research in Energy and Materials (SP, Brasil)

INTRODUCTION: Polyethylene terephthalate (PET) is a synthetic polymer used in the production of plastics with high durability, lightness, and/or transparency, giving this material a variety of industrial applications. PET is chemically inert and has high resistance to microbial degradation because of the aromatic hydrocarbons in its structure. A recent study characterized a new species of bacteria, *Ideonella sakaiensis* 201-F6, capable of using PET as a carbon source through the action of a dual-system enzyme (PETase and MHETase). The study of biological catalysts emerges as a biotechnological route to assist the circular economy of plastics. OBJECTIVES: Introduce genetic modifications in a fungal strain to express a codon-optimized PETase and to evaluate the deconstruction of PET by recombinant lineages. MATERIALS AND METHODS: The PETase gene was incorporated into a specific locus of an Ascomycota strain using the CRISPR/Cas9 system. The quantification of PETase in the recombinant lineages was carried out and the protein profile was evaluated by SDS-PAGE. A screening for activity will be performed using esters substrates by biochemical methods, and the best strain will be used for application essays. DISCUSSION AND RESULTS: After successive rounds of monosporic purification, seven recombinant lineages showed the presence of different growth responses compared to the native strain. Besides, plastic films will be treated with the enzyme, and the release of BHET, MHET, and TPA will be evaluated. CONCLUSION: Through the results to be achieved in this work with the expression of PETase, we seek to contribute to the development of new microbial platforms aiming industrial application for plastics upcycling.

Keywords: Ascomycota strain, CRISPR/Cas9-based genetic engineering, PETase

F.12 - 3D bioprinting of active scaffolds based on starch/collagen/hydroxyapatite for bone regeneration

Maryanne Trafani de Melo [1], Bianca Maniglia[1], Ana Paula Ramos[1]
[1]Department of Chemistry- FFCLRP, University of São Paulo (São Paulo, Brasil)

INTRODUCTION: 3D scaffolds able to sustain cells proliferation and function during bone regeneration has been used as an strategy for reconstructing and repairing damaged tissues. The aim is to offer mechanical support for the host also stimulating the synthesis of mineralized extracellular matrix (ECM). Biodegradable and biocompatible polymers, such as starch, have been exploited for this purpose. The addition of hydroxyapatite (HAp) and collagen, the main components of the bone ECM, to the starch-based matrix may improve the mechanical and osteogenic properties of the scaffolds. Aligned to this, 3D bioprinting is an innovative process able to create biomaterials with superior and personalized properties. OBJECTIVES: In this scenario, this study aimed to produce scaffolds based on starch/collagen/HAp by 3D printing extrusion (BioedPrinterV4, BioEdTech - Brazil). Different composition of these starch-based hydrogels were evaluated in regards to their 3D printing performance (rheology and reproducibility). MATERIALS AND METHODS: The printed scaffolds were characterized concerning morphology by SEM, swelling, biodegradability, mechanical properties, cytotoxicity by MTT assay using MC3T3-E1 pre-osteoblasts cells, and confocal fluorescence microscopy. The alizarin red assay evaluated the ability of the membranes to induce mineralization after 10 and 14 days of culture. DISCUSSION AND RESULTS: The scaffolds have shown to be biodegradable and non-toxic to the cells, with increased ability to mineralize in higher concentrations of collagen and HAp. Moreover, the mechanical properties of the scaffolds were impaired by the addition of 1% of collagen (g/100 g starch). However, the addition of HAp improved the mechanical properties of the materials. CONCLUSION: The 3D printed composite scaffolds share similar mechanical properties with the cancellous bone. Finally, this study revealed the potential of starch as an alternative and cheap source for the synthesis of 3D printed bioactives scaffolds when combined with collagen and HAp.

Keywords: starch, collagen, 3D printing / **Supported by:** PIPAE – Portaria PRP 822/2021 (#21.1.829.59.8); FAPESP (#2020/08727-0, #2021/05947-2)

F.13 - Selection Of Specific Inhibitors for The Main Protease (Mpro) Of Sars-Cov-2 By Phage Display

Graziele Cristina Ferreira [1], Camila Tita Nogueira[1], Gabriel Cerqueira Alves Costa[1], Fernando Allan Abreu Silva [1], Verônica de Moraes Manzato [1], Fabio Antonio Vaz Busoli [1], Ricardo José Soares Torquato[1], Débora Noma Okamoto[2], Maria Aparecida Juliano [3], Aparecida Sadae Tanaka [1]
[1]Departamento de Bioquímica - Escola Paulista de Medicina, [2]Departamento de Ciências Farmacêuticas, [3]Departamento de Biofisica - Escola Paulista de Medicina, Universidade de Federal de São Paulo (São Paulo, Brasil)

INTRODUCTION: Main protease (Mpro) is a nonstructural protein (NSP) from SARS-CoV-2 that processes the polyprotein during viral replication in 11 cleavage sites, which is a cysteine protease with a strong preference for the amino acid residue glutamine (Gln) in the substrate P1 position. Inhibitors of this protease may be a powerful therapeutic target. The phage display can contribute by selecting proteins/peptides that have high binding affinity to the target protein OBJECTIVES: The purpose of this work is to identify specific inhibitors for the Mpro of SARS-CoV-2 through the phage display technique using peptide libraries. MATERIALS AND METHODS: The recombinant plasmid, pGEX-Mpro was obtained commercially, and the enzyme was expressed in *E. coli* BL21(DE3) Star strain. After expression, lysis, and centrifugation, the protein was purified by nickel affinity chromatography and eluted with imidazole. The active protease without the His-tag was obtained after being processed with the HRV 3C Protease and followed by another purification step to remove HRV 3C and Mpro with the His-tag. Mpro activity assay was performed using the FRET based substrate Abz-SAVLQ↓SGFRK(Dnp)NH2 in the presence of DTT. Mpro was used to select the mutant from the SFTI (*Sunflower Trypsin Inhibitor*) library by Phage Display. The plasmid DNA mini preparations of clones were performed and then sequenced. DISCUSSION AND RESULTS: So far, we have obtained the purified Mpro with enzymatic activity. Then, a library based on the sequence of the trypsin inhibitor SFTI was used to select mutant peptides with binding affinity with the enzyme. We randomly selected a few clones from the third generation for sequencing. Among the 67 sequenced clones, 5 most frequent were synthesized and will be tested as an inhibitor. CONCLUSION: In our project, we expressed and purified the SARS-CoV-2 Main protease Mpro and used to select peptides that can have binding affinity with Mpro.
Keywords: Main protease SARS-CoV-2, Inhibitors , Phage Display / **Supported by:** FAPESP

F.14 - Ranking and Biotechnological Application of New Fungi Isolated: Production of Cuticle-Degrading Enzymes and Potential for Pest Biocontrol.

Fabiane Cruz Bezerra [1], Aline Pereira de Almeida[1,2], Camila Langer Marciano[1,2], Fabiana Fonseca Zanoelo[1,2], Giovana Cristina Giannesi[1,2], Elisângela Souza Loureiro[3], Douglas Chodi Masui[1,2], Roberto Ruller[1]
[1]Laboratório de Bioquímica Geral e de Microrganismos-LBq, [2]Programa Multicêntrico Pós-Graduação Bioquímica e Biologia Molecular-PMBQBM, [3]Laboratório de Biotecnologia, Universidade Federal de Mato Grosso do Sul-UFMS/INBIO (Mato Grosso do Sul, Brasil)

INTRODUCTION: Fungi are a diverse group of microorganisms important in the biological control of arthropod populations. Significant progress has been made in understanding the fungal enzymes involved in penetrating and degrading of cuticle of "target" hosts. The cuticles of pest-arthropods are composed of chitin, proteins, and lipids; and cuticle-degrading enzymes (CDE) are essential for the effectiveness of biocontrol action in mycoparasitic processes. OBJECTIVES: To classify the best fungi as pest biocontrol and understand their potential for producing CDE, thirteen new fungi isolated were individually cultivated in a minimal medium. MATERIALS AND METHODS: After then, all crude enzyme extracts were, separated and evaluated for proteolytic, lipolytic, and chitinolytic activities. Subsequently, samples of 10^8 spores/mL of the best enzyme-producing microorganisms were used for confrontation tests and biological control against *Ripicephalus sanguineus*, *Tytus paraguayensis*, and *Spodoptera frugiperda*. The entomopathogenic fungus, *Beauveria bassiana* strain IBCB66 (commercial biocontrol) was used as a standard. DISCUSSION AND RESULTS: The results showed that *Metarhizium anisopliae* strain E9, *Metarhizium rileyi* strain UFMS03, and *Purpureocillium lilacinum* strain UFMS10 were the best producers of extracellular proteases, lipases, and chitinases. These results of CDE production were 1.5 to 2.5-fold higher than the commercial fungus. Tests to evaluate the potential of these three microorganisms as biocontrol for *R. sanguineus* and *T. paraguayensis* indicated 100% of efficiency and deaths of all samples submitted to mycoparasitism. However, for the biologic control tests against *S. frugiperda*, only the fungus *M. anisopliae* strain E9 showed no mortality for the samples submitted to the bio-treatment. For the microbial confrontation tests, the *B. bassiana* strain IBCB66 was compatible together *M. anisopliae* strain E9 and *M. rileyi* strain UFMS03. CONCLUSION: Thus, it is suggested that high CDE secretion can improve the effectiveness of mycoparasitism of fungi on their pest hosts. For the next steps, the biopesticide potentials of co-cultures of the best fungi compatible will be evaluated.
Keywords: Biological Control, Cuticle-Degrading Enzymes, Spodoptera frugiperda / **Supported by:** CAPES AND CNPq

F.15 - Amyloidogenic Cell-Penetrating Peptides: Opportunities for a New Class of Peptide-Based Therapeutics?

Emerson Rodrigo Da Silva [1]
[1]Biofísica, Universidade Federal de São Paulo-Escola Paulista de Medicina (SP, Brasil)

INTRODUCTION: Cell-penetrating peptides (CPPs) are very promising compounds in the future development of nanotherapeutics. They are usually composed of 5-30 amino acids, exhibiting a strong translocating ability toward biomembranes. These features hint at their suitability for producing nonviral vectors for a variety of applications, including gene therapy. Recently, a new class of amyloid-forming CPPs has emerged as a potential candidate for creating hybrid materials that enhance the range of uses of these species. We present recent studies from our laboratory on the structure of CPP-based scaffolds designed for the transport of nucleic acids. We highlight the characteristics of different classes of CPPs and pay special attention to hydrophobic CPPs which simultaneously show very low cytotoxicity and high amyloidogenic propensity. OBJECTIVES: We aimed at analyzing and comparing both spatial organization and cell response of CPPs to establish structure-activity correlations envisaging the optimization of nonviral vectors based on these species. MATERIALS AND METHODS: The self-assembly of short CPPs (6-16 aa) was investigated by means of SAXS, cryo-EM, and AFM-IR. Internalization capacity was assessed by confocal fluorescence microscopy imaging in HeLa cells. DISCUSSION AND RESULTS: The formation of amyloidogenic assemblies was ascertained by the presence of classical features exhibited by these structures, namely organization into unbranched β-sheet-enriched nanofibrils and sensitiveness to thioflavin and Congo red staining. The presence of aromatic residues was found to be a determinant of the appearance of amyloid fibrils, and vectorization assays revealed that, although these species are efficient to promote cytoplasm internalization, the formation of amyloidogenic aggregates limits translocation of the eukaryotic barrier. CONCLUSION: Amyloid forming CPPs are versatile molecules that exhibit very low cytoxicity in comparison to traditional CPPs. These species can easily translocate the cytoplasm barrier; however, amyloidogenic aggregates seem to trigger endosomal entrapment limiting their usage for targets at cell nuclei.

Keywords: amyloids, peptide self-assembly, drug delivery / **Supported by:** FAPESP, Proc. 19/20907-7

F.16 - Sequence Optimization and Expression Efficiency Evaluation of DNA Vaccines Against Zika Virus

Bruno Henrique de Sousa Leite [1], Emerson Moreira[1,2], Isabelle Viana[1,1], Roberto Lins[1]
[1]Virologia, Instituto Aggeu Magalhães - Fundação Oswaldo Cruz (PE, Brasil), [2]Department of Fundamental Chemistry, Federal University of Pernambuco (PE, Brasil)

INTRODUCTION: The Zika virus (ZIKV) is an emerging flavivirus that has been associated with several neurological disorders. In the absence of virus-specific therapeutics and unequivocal diagnostic tests, the development of vaccine strategies capable to prevent the infection remains as a public health priority. To this aim, the virus surface proteins (envelope – E and pre-membrane/membrane – prM) are often considered the most immunogenic, and therefore the best candidates as vaccine antigens. OBJECTIVES: To evaluate two developed DNA vaccine formulations against ZIKV covering the E and prM/M proteins. In these vaccines, the viral antigens are fused to molecular adjuvants aiming to enhance antigen expression and presentation to the immune system. MATERIALS AND METHODS: The DNA sequences coding for the ZIKV E and prM/M proteins were synthesized in frame with molecular adjuvants and cloned into two DNA vaccine plasmid vectors (p43.2 ZIKV/ENV_Lamp and pCDNA 3.1 ZIKV_ENV). HEK293T cells were transfected with the vaccine formulations and appropriate controls. Immunofluorescence (IF) and western-blot (WB) assays were used to assess the expression efficiency and cellular trafficking of the vaccine antigens *in vitro*. DISCUSSION AND RESULTS: The ZIKV vaccine antigens were successfully expressed in human cell lines *In vitro* upon cell transfection. Our IF data show that the virus antigens are homogeneously found in the cellular cytoplasm, which indicates appropriate antigen production and localization. WB analyses are currently ongoing to assess the cell secretion of the ZIKV vaccine antigens as folded proteins *in vitro*. CONCLUSION: The ZIKV vaccine proteins encoded by our DNA vaccine formulations are properly expressed *In vitro* and present a homogenous localization pattern throughout the cellular cytoplasm. The association of these proteins to molecular adjuvants has proven to increase antigen production *in vitro*, in comparison to assay controls. Together, our data suggest that our DNA vaccines are suitable candidates for ZIKV prophylaxis. *In vivo* assays are currently being performed.

Keywords: ZIKV, DNA vaccine, Molecular adjuvants / **Supported by:** FACEPE, CNPq, CAPES, Fiocruz.

F.17 - Project: Metagenomic Analysis Of The Intestinal Microbiota In Chickens Treated With Phytobiotics

Alessandra da Silva Santos [1], Diko Holger Becker[3], Lenita de Cássia Moura Stefani[2], Aleksandro Schafer da Silva[2], Camila Ceccato Ferreira [2], Tayse Burger Neto Zanin[2], Miklos Maximiliano Bajay [1]
[1]Bioquímica e Biologia Molecular, Universidade do Estado de Santa Catarina (Santa Catarina, Brasil), [2]Depto de Bioquímica e Biologia Molecular - PMBqBM, Universidade do Estado de Santa Catarina (Santa Catarina, Brasil), [3]Phytobiotec Agroindustrial LTDA (Santa Catarina, Brasil)

INTRODUCTION: Antimicrobials were important to aviculture development through its action on intestinal biota which led to better performance and productivity. However, the search for alternatives is increasing due the transmission and proliferation of resistant bacteria. One alternative are phytobiotics that can act on microorganisms found in the intestines of birds. OBJECTIVES: Assess the effects of two commercial phytobiotics (Acidosan® and Enterosan®) on intestinal microbiota of chickens though biochemical analysis based on the taxonomy of identified microbiota. Specifically, perform the characterization of intestinal microbiota of phytobiotic supplemented chickens; assess the effect of increased concentrations of phytobiotics on diet; evaluate the impact of the predominant intestinal microbiota organisms on animal production. MATERIALS AND METHODS: 140 birds of 15 weeks (5 birds/cage) were used, randomly divided into 4 treatments with seven replications. Twenty stool samples were collected for each of the following treatments: 1. Negative control; 2. Positive control (Enrammycin); 3. Acidosan® (250g/tonne of feed); 4. Enterosan® (500g/ton of feed). Bacterial DNA was extracted through the feces pool of each cage. In high performance sequencing, the V3/V4 regions of the 16S rRNA gene were used to identify bacteria. Sequencing was performed in the MiSeq equipment, using the V2 kit, with coverage of 50,000 readings for each sample. Bacterial groups were identified using the NCBI Blast function in specialized databases. Statistical analyses were performed in R software. DISCUSSION AND RESULTS: Through sequencing of the samples of the intestinal contents it was possible to access preliminary information of the microorganisms present for each treatment and its diversity.
Keywords: Phytobiotics, Microbiota, Aviculture
Supported by: FAPESC

F.18 - Two-photon fluorescence excitation cross sections of Resveratrone Compound.

Livia Maria Barros Campos [1], Bárbara Regina Melo Ribeiro[1], Gladystone Rocha da Fonseca[1], Mychel Gonçalves Silva[1], Ana Maria de Paula[1]
[1]Física, Universidade Federal de Minas Gerais (MG, Brasil)

INTRODUCTION: Resveratrol is a compound found on grapes skin and known by its benefits for cardiovascular diseases. Resveratrone is a new compound formed by the UV radiation of trans-resveratrol. It has already been determined to have large values of absorption cross-section making it suitable for bio-imaging and multi-color labeling [1]. In this work we study the nonlinear properties of resveratone by two-photon fluorescence spectroscopy in order to measure the excitation cross-section. The results were compared with well-known dyes with data from literature such as Lucifer Yellow, Alexa 488 and DAPI. OBJECTIVES: Determine the two-photon excitation cross-section of Resveratrone in a 0,5 g/L ethanol solution. MATERIALS AND METHODS: We implemented an experimental setup for the two-photon absorption fluorescence spectroscopy using a pulsed Ti-sapphire laser. We measured the spectrum of a resveratrone solution and also for a rhodamine 6G solution on the same conditions to be used as reference, from 720 nm to 960 nm at a constant average excitation power. DISCUSSION AND RESULTS: A set of measurements were obtained for each excitation wavelength and the Photoluminescence Excitation (PLE) curve was used to calculate the excitation cross-section by comparison with the rhodamine 6G PLE reference values [2]. The results shown by the PLE indicate a high intensity fluorescence at 720 nm and the maximum cross-section at 820 nm. CONCLUSION: The obtained two-photon excitation cross section values are compared to the values of the typical dyes. In addition we observed a large Stoke's Shift. This optical characterization of resveratrone confirmed it as appropriate for multicolor labeling and *In vivo* imaging.
Keywords: Two Photon Absorption, Resveratrone, Spectroscopy

F.19 - Promising Biological Properties of Secondary Metabolites Produced by the Fungus Penicillium maximae Isolated from Caatinga Biome

Rafael Conrado[1], Tainah Colombo Gomes[1], Itamar Soares de Melo[2], Welington Luiz de Araújo[3], **Ana Olívia de Souza [1]**

[1]Development and Innovation Laboratory, Instituto Butantan (São Paulo, Brazil), [2]Environmental Microbiology Laboratory, EMBRAPA Meio Ambiente (SP, Brazil), [3]LABMEM, Department of Microbiology, Universidade de São Paulo (São Paulo, Brazil)

INTRODUCTION: The discovery of bioactive molecules produced by microorganisms, mainly from not very well studied environments, has great biotechnological relevance. The Brazilian biodiversity is very promising in the drug discovery process, and the fungi isolated by our group, from the Caatinga biome, are being studied as source of compounds with antimicrobial, antitumor or wound healing properties. OBJECTIVES: In this study, the main objective was to evaluate the antimicrobial and cytotoxicity properties of secondary metabolites produced by a fungus strain isolated from the Caatinga biome. MATERIALS AND METHODS: The fungus strain coded as AG2511, isolated from the Caatinga biome soil, was taxonomically identified based on morphology by scanning and transmission electron microscopy, and molecular analysis (ITS-1 and 5.8S-ITS-2 sequences). For secondary metabolites biosynthesis, the fungus was cultivated in potato dextrose broth for 15 days at 28 oC and 150 rpm. The metabolites were obtained by solid phase extraction (C18 cartridge and methanol as solvent). The crude extract was fractionated by high performance liquid chromatography (HPLC) in a C18 column and isocratic method with methanol. The cytotoxicity of the fractions (F1-F7) was evaluated from 125 to 500 µg/mL, on the human breast adenocarcinoma cell lines MCF-7 (ATTC HTB-22) and MDA-MB231 (ATCC HTB-26), on the pancreatic cancer cell line MIA PaCa-2 (ATCC CR61420), and on the human melanoma cell line SK-MEL-28 (ATCC HTB-72) by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The antimicrobial property was assayed by antagonism or minimal inhibitory concentration assay (125-500 µg/mL), on at least fifteen species of phytopathogens. DISCUSSION AND RESULTS: The fungus AG2511 was identified as Penicillium maximae, and the fractions inhibited the growing of the phytopathogens Bipolaris sorolaniana, Fusarium oxysporum, Fusarium solani and Fusarium verticilloides. The fractions F6 and F7 were more effective and cytotoxic on all tumor cell lines. CONCLUSION: Due to its promising biological effect, the fractions are under purification process for obtaining the secondary metabolites.

Keywords: Secondary metabolites, Penicillium maximae, Caatinga

F.20 - Using TiO2-Diatomite for Photodisinfection in Contaminated Wastewater

Paloma Otsuka Kotani [1], Daphne Montesuma Ferro[1], Laura Pavarin Tavares[1], Regina Affonso[2], Nilce Ortiz[1]

[1]Centro de Química e Meio Ambiente, Instituto de Pesquisas Energéticas e Nucleares (São Paulo, Brasil), [2]Centro de Biotecnologia, Instituto de Pesquisas Energéticas e Nucleares (São Paulo, Brasil)

INTRODUCTION: The urban pressure reduced water availability and quality through the years, promoting the development of water treatment and disinfection processes such as the Advanced Oxidation Process (AOP). A highly efficient catalyst with Diatomite as biotemplate and solar energy can enhance hydroxyl radicals (OH) production for disinfection and pollutants degradation. Preliminary TiO_2-Diatomite experiments with *Escherichia coli* provided valuable insights on its photodisinfection efficiency, legitimating its usage in the wastewater samples presented in this study. OBJECTIVES: Evaluate the use of TiO_2-Diatomite in photodisinfection process in contaminated wastewater. MATERIALS AND METHODS: TiO_2 synthesis used titanium isopropoxide sol-gel process with Diatomite powder, the filtration step followed the mixed water suspension, and the drying process lasted overnight. The wastewater samples were collected from a household washing machine and 0.05 g of TiO_2-Diatomite were added in the photodisinfection reactor. The total reaction lasted for 90 minutes in the solar chamber with all parameters controlled. The suspension aliquots were collected after 30 minutes of agitation and plated on LB agar at Petri plates. After incubation, the emerged colonies were counted through software (OpenCFU) and the data processed using R programming language. DISCUSSION AND RESULTS: The Scanning Electron Microscopy (SEM) micrograph of TiO_2-Diatomite presented enhanced surface area and microstructure obtained by biotemplate addition. The bacterial inactivation percentage was above 75 % for 1 hour of solar radiation exposure. Kinetics models indicated better correspondence with interparticle reaction. CONCLUSION: Photodisinfection kinetics studies provided more efficient bacterial inactivation with the addition of 0.05 g of TiO_2-Diatomite in the sample. The study presents an affordable and sustainable treatment using a viable renewable energy source for application in distant areas with contaminated effluents with the addition of a reagent easily obtained by government agencies.

Keywords: Diatomite, Photodisinfection, Solar energy / **Supported by:** CNPq

F.21 - Surface Analysis and *In vivo* Effect of the New Alloy Co63Cr28Ta9

Beatriz da Silva Batista [1], Luzeli Moreira da Silva[1], Ralph Santos-Oliveira[2], Luciana Magalhães Rebêlo Alencar[3,1]
[1]Centro de Ciências Sociais, Saúde e Tecnologia, Universidade Federal do Maranhão (Maranhão, Brasil), [2]Comissão Brasileira de Energia Nuclear, Instituto de Engenharia Nuclear (Rio de Janeiro, Brasil), [3]Departamento de Física, Universidade Federal do Maranhão (Maranhão, Brasil)

INTRODUCTION: Cobalt-based alloys are used in the biomedical sector for their excellent properties, such as wear and corrosion resistance and biocompatibility. It is necessary to develop new cobalt-based alloys that provide its improvement and consequent improvement in the quality of life of patients, such as through the addition of a new alloy element. OBJECTIVES: Therefore, a new alloy $Co_{63}Cr_{28}Ta_9$ (Cobalt-Chromium-Tantalum) was synthesized and its surface and effect *In vivo* were evaluated for possible application as a biomaterial. MATERIALS AND METHODS: The metal alloy $Co_{63}Cr_{28}Ta_9$ was synthesized by melting in an arc furnace and subjected to heat treatment at 1000 °C for 48 h, then it was cut into transverse slices which in turn were sanded. The samples were characterized by X-Ray Diffraction and Atomic Force Microscopy and analyzed in animals (edema formation in mice and leukocyte and cytokine counts). DISCUSSION AND RESULTS: The structural analysis showed that the sample crystallized in the cobalt allotropes, with the εCo (P63/mmc) and αCo (Fm-3m) phases, both admitting chromium and tantalum in solid solution and that tantalum induced formation of a new $TaCo_2$ phase (P63/mmc). The surface analysis showed the sample with a nanometric roughness of 18.64 nm and adhesion of 8.37 nN, together with the topographic and adhesion maps, the influence of the distribution of elements on the surface was noted. Meanwhile, *In vivo* analysis showed that the sample did not induce significant edema formation or leukocyte migration, in addition to showing a reduction in IL-1β and IL-6. CONCLUSION: The new alloy $Co_{63}Cr_{28}Ta_9$ is a promising material for the biomedical sector.
Keywords: anti-inflammatory, cobalt, metal alloy

F.22 - Identification and Quantification of Coronavirus (SARS-CoV-2) Circulating in Brazil by Mass Spectrometry

Beatriz Pereira de Morais [1], Guilherme Lanfredi[1], Ana Paula Masson[1], Vitor Marcel Faça[1], Eurico de Arruda Neto[2], Ronaldo Martins[2]
[1]Biochemistry Department, Biochemistry Department, Ribeirão Preto Medical School, USP, RP, Brazil (São Paulo, Brazil), [2]Virology, Virology Research Center, USP, RP, Brazil (São Paulo, Brazil)

INTRODUCTION: SARS-CoV-2 structural proteins SPIKE (S), envelope (E), and nucleocapsid (N) assist assembly and fusion with the host cell. Furthermore, through them it is possible to characterize viral particles and perform diagnostic strategies in patient samples. OBJECTIVES: The aim of our work is to develop a method establishment for detection and quantification of SARS-CoV-2 viral particle proteins from *In vitro* and clinical samples by liquid chromatography coupled with mass spectrometry (LC-MS/MS). MATERIALS AND METHODS: SARS-CoV-2 genomic sequences analysis was used to select S, N and E representative peptides. Vero E6 cells were used to establish a viral expansion model for stock development used later for LC-MS/MS analysis. Among representative peptides, 12 were selected for final panel composition. Five of these peptides were synthesized through solid phase approach. Sensibility evaluation and method optimization was performed for each peptide. Collision energy optimization was performed using Skyline software. Virus inactivation was performed in patient positive RT-PCR nasopharyngeal swab samples. Samples were inactivated in duplicate through four different treatments: 8M Urea; 100% Ethanol; 50% Ethanol, 50% Acetone, 0,1% Acetic Acid; 6M Guanidine Hydrochloride. Samples were then digested and analyzed by Targeted Proteomics. DISCUSSION AND RESULTS: Sensibility and reproducibility were evaluated considering its use in viral particle detection for different samples. Inactivated samples were analyzed through two targeted proteomics approaches for each inactivation treatment. It was possible to observe variations between treatments involving signal intensity among approaches. The method was efficient to detect part of the peptides designed. CONCLUSION: Sample inactivation and preparation protocols were key to achieve acceptable results. Viral particle enrichment in patient samples is important for efficiency detection by MS methods. Results suggest that shorter runs can improve signal intensity, exhibiting great advantages in clinical settings, and could be considered as an alternative for routine clinical samples analysis.
Keywords: Coronavirus, Mass Spectrometry, Proteomics / **Supported by:** CNPq, FAPESP

F.23 - Short-Term Intake of Theobroma Grandiflorum Juice Fermented With Lacticaseibacillus rhamnosus ATCC 9595 Amended The Outcome Of Endotoxemia Induced By Lipopolysaccharide

Adrielle Zagmignan [1], Gabrielle Damasceno Costa dos Santos[2], Cláudia Zeneida Gomes Parente Alves Lima[2], Yasmim Costa Mendes[1], Gabrielle Pereira Mesquita[1], Lucas dos Santos Silva[1], Camila Caetano da Silva[1], Katia Sayuri Aragão Aguiar[1], Aléxia Figueiredo Ferreira[1], Washington Kléber Rodrigues Lima [1], Simeone Julio dos Santos Castelo Branco[2], Luís Cláudio Nascimento da Silva[1]

[1]Departamento de Patogenicidade Microbiana, Universidade CEUMA (Maranhão, Brasil), [2]Departamento de Bioquímica, Universidade Federal de Pernambuco (Pernambuco, Brasil)

INTRODUCTION: Endotoxemia is a condition caused by increasing levels of lipopolysaccharide (LPS) characterized by an impaired systemic response that causes multiple organ dysfunction. Lacticaseibacillus rhamnosus ATCC 9595 is a strain with probiotic potential which shows immunomodulatory properties. The incorporation of this bacterium in food rich in bioactive compounds, such as cupuaçu juice (Theobroma grandiflorum could result in a product with interesting health properties. OBJECTIVES: This work evaluated the effects of the oral administration of cupuaçu juice fermented with L. rhamnosus in the outcome of LPS-induced endotoxemia in mice. MATERIALS AND METHODS: The study was approved by CEUA-UNICEUMA (Process nº 68/17). C57BL/6 mice (12/group) received oral doses (100 µL) of saline solution, unfermented or fermented cupuaçu juice (108 CFU/mL). After 5 days, the endotoxemia was induced by an intraperitoneal injection of LPS (10 mg/kg). The endotoxemia severity was daily evaluated using a score based on grooming behavior, mobility, presence of piloerection and weeping eyes. After 6 h and 120 h, the mice (6/group) were euthanized for analysis of cell counts (in peritoneal lavage and serum) and organs weight. DISCUSSION AND RESULTS: . rhamnosus grew in cupuaçu juice and produce organic acids without the need for supplementation. The bacteria counts were stable in the juice during storage at 4º C for 28 days. In general, the administration of L. rhamnosus-fermented juice allowed significant improvement in several characteristics of endotoxemic status (weight loss, hypothermia, severity index, cell migration). In addition, treatment with fermented juice significantly reduced the weight of the spleen, liver, intestine and kidneys when compared to the saline-treated endotoxemic group. CONCLUSION: Taken together, our data show that short-term intake therapy of cupuaçu juice fermented with L. rhamnosus ATCC 9595 can reduce systemic inflammation in an experimental model of LPS-induced endotoxemia in mice. **Keywords:** Endotoxemia, Fruit juice, probiotic / **Supported by:** FAPEMA

F.24 - Hyperspectral Microscopy Applied to Biological Tissue Imaging

Gladystone Rocha da Fonseca[1], Mychel Gonçalves Silva[1], Bárbara Elza Nogueira de Faria[1,2], Bárbara Regina Melo Ribeiro[1], Livia Maria Barros Campos[1], Ana Maria de Paula[1]

[1]Departamento de Física, Universidade Federal de Minas Gerais (Minas Gerais, Brasil), [2]Departamento de Química, Heinrich-Heine-Universität Düsseldorf (Nordrhein-Westfalen, Deutschland)

INTRODUCTION: Hyperspectral Imaging (HI) is a technique used to obtain the spectral signature of a sample with spatial resolution. HI is used on macroscopic scale in a wide range of applications from remote sensing to art conservation and restoration. Recent developments achieved measurement of spectral signatures with spatial resolution about microns, adapting Fourier-Transform Spectrometers (FTS) to microscopes (Hyperspectral Microscopes). Currently, diagnostic methods are mainly based on tissue visual evaluation, demanding sample preparations routines frequently involving staining methods. Literature points out advantages in the use of optical techniques, specially optical spectroscopy associated to microscopic imaging as a possible diagnostic tool, in particular for pre-cancer diagnosis, the stage close to the beginning of cancer development where tissue morphology does not present observable alterations when analyzed by the methods widely used for diagnosis. OBJECTIVES: In this work, we present an application of HI at the microscopic scale to observe tissue biopsies. MATERIALS AND METHODS: The experimental setup consists in a Fourier-Transform spectrometer made with a birefringent plate (α-BBO crystal) and two wedges (TWINS – Translating-Wedge-based Identical pulses eNcoding System) adapted to a horizontal custom-made optical microscope (a microscope objective, a tube lens and a monochrome CCD camera). We also developed an appropriate system for uniform illumination on the sample plane, both for transmission and reflection microscopy based on Kȍhler Illumination. DISCUSSION AND RESULTS: We present space resolved spectra of fluorescence images from tissue biopsies, identifying the fluorescence spectrum of Eosin, obtained with a Hyperspectral Microscope. We also present space resolved spectra of autofluorescence images from tissue biopsies and compare with the autofluorescence of collagen from literature. We compare the spectrum obtained using the Hyperspectral Microscope with the spectrum obtained using a commercial spectrometer. CONCLUSION: We present the development and application of a Hyperspectral Microscope based on a birefringent interferometer in the study of tissue fluorescence images. **Keywords:** Hyperspectral Imaging, biophotonics, birefringence / **Supported by:** CAPES, FAPEMIG, CNPq

F.25 - Nucleotide-based Nanoparticles for Intracellular Delivery of Quantum Dots

Renata Lara Mori [1], Lucas Rodrigues de Mello[1], Emerson Rodrigo da Silva[1]
[1]Departamento de Biofísica, Universidade Federal de São Paulo - Escola Paulista de Medicina (São Paulo, Brasil)

INTRODUCTION: Nucleotides are excellent metal ligands capable of establishing coordination interactions with metal ions, especially with transition metals. The co-assembly of nucleotides and metal ions through coordination interactions at specific sites gives rise to nanoscopic aggregates called metal-organic frameworks (MOFs). These materials exhibit a range of applications in biomedicine, including drug delivery and bioimaging. Quantum dots (QDs) are semiconductor nanoparticles presenting unique characteristics regarding their optical properties. Therefore, the combination of these systems with biocompatible nanostructures presents great potential for biomedical application. OBJECTIVES: To develop a synthesis protocol to produce supramolecular complexes involving guanosine-based nucleotides, metal ions and graphene quantum dots. Our goal is to provide a detailed characterization of the structure of these nanostructured materials and to test their behavior *In vitro* against tumor cell lines. MATERIALS AND METHODS: The synthesis protocol consists of solubilizing guanosine monophosphate (GMP) in the presence of metal ions such as Zn, Mg, Mn, Fe, Co, Cu and Ni. Graphene quantum dots are co-solubilized with GMP and metal ions, leading to spontaneous formation of nanoparticles. Morphology characterization was performed with atomic force microscopy, whereas the emission of the nanostructures was investigated through steady-state fluorimetry. Cytotoxicity features were investigated by incubating the complexes with HeLa cells. DISCUSSION AND RESULTS: We have been successful at producing ordered, crystal-like, GMP/QD nanoparticles in the presence of the above-mentioned metals. Interestingly, MOFs prepared with Fe3+ were found to modulate the emission properties of graphene QDs, either by shifting the absorption/emission maxima or by modifying the fluorescence yield. The MOFs were well-tolerated by cells up to concentrations in the mg/ml range. CONCLUSION: A easy protocol to produce nucleotide-based nanoparticles embodying QDs has been developed. The resulting particles are well-tolerated by HeLa cells and able to modulate the fluorescence capabilities of QDs, indicating promising of these MOFs in the development of biomaterials.

Keywords: metal-organic frameworks, nucleotides, metal ligands
Supported by: To FAPESP (grant n°19/20907-7) for financial support. To CNPq for a MSc. fellowship.

F.26 - Avaliação de parâmetros cinéticos de fermentação com cepas de levedura do gênero Saccharomyces em simulações de processo descontínuo alimentado

Gabriel Alves de Jong [1], Taísa Magnani Dinamarco[1]
[1]Departamento de Química, Universidade de São Paulo (SP, Brasil)

INTRODUCTION: An efficient fed-batch fermentation requires determination of the theoretical transition time (t) which translates to an operational start time of substrate feeding flow rate. This variable can be estimated from kinetic parameters (μ_{max} and $Y_{X/S}$). STECKELBERG (2001) characterized 19 different *Saccharomyces* strains isolated from alcoholic fermentation processes in different regions of Brazil OBJECTIVES: The main objectives are to compare industrial strains in fed-batch process simulations, and to obtain an estimate of the transition time associated with the μ_{max} and $Y_{X/S}$ in MATLAB MATERIALS AND METHODS: The strains and culture medium are as described in STECKELBERG (2001). The conditions for the process simulation (flow rate (l/h), initial volume (l)) were fixed according to usual procedures from the prior art. The step-by-step performed by MATLAB was (A) to plot a cell growth curve (X·V (g/l) vs t (h)) using Monod model and μ_{max}; (B) to plot a cell growth curve (X·V (g/l) vs t (h)) using mass balance and Y; (C) to determine the intersection time of plots from steps A and B (transition time t); and (D) to plot the fed-batch simulation (X, S, V vs t) for the regions above and below transition time *t* found in step C. DISCUSSION AND RESULTS: The strain obtained from *"Cia. Açucareira Vale do Rosário"*, from Morro Agudo/SP, showed the longest transition time, of 13 hour and 8 minutes. While the shortest transition time was observed in *"Destilaria de Álcool California"* (DACAL) strain, from Parapuã/SP, with a 6 hours and 25 minutes period. Kinetic parameter μ_{max} of strains impacted more significantly on transition time than $Y_{X/S}$. Strains isolated from continuous and fed-batch process were found to be not statistically different ($p < 0,05$). CONCLUSION: It was possible to calculate the transition time of different strains observed in Brazilian industries, and to compare the strains based on the growth profile obtained.

Keywords: bioprocessos, fermentação, *Saccharomyces cerevisiae*
Supported by: CAPES

F.27 - Expression of Catalase Recombinant Enzyme from Trichoderma reesei

Amanda Cristina Esteves Leite [1], Patrícia Pereira Adriani[1], Felipe Santiago Chambergo Alcalde[1]
[1]Escola de Artes, Ciências e Humanidades, Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: Catalases (EC 1.11.1.6) are heme-containing proteins which belong to the oxidoreductase enzyme class and convert hydrogen peroxide into water and molecular oxygen. Due to their function, they present important applications such as in textile, food, paper and pharmaceutical industries. The filamentous fungus *Trichoderma reesei* has been used for the production of cellulases and hemicellulases enzymes, demonstrating its potential use as an enzyme source. OBJECTIVES: Express and purify the catalase recombinant enzyme from *T. reesei* (TrCat), using *E. coli* BL21 as host. MATERIALS AND METHODS: The gene sequence was synthetized and cloned into pET303/CT-His, pTrc-HisA and pFLAG-CTS expression vectors. The expression was evaluated. Later, the TrCat/pTrc-HisA construction was coexpressed with pT-GroE vector and TrCat expression was evaluated. The expressed recombinant enzymes were purified through different chromatographic methods: immobilized-metal ion affinity chromatography (IMAC), ion exchange chromatography or the combination of both. The enzyme activity was evaluated using 3% hydrogen peroxide. DISCUSSION AND RESULTS: The expression with the pET303/CT-His vector combined with purification through IMAC resulted in a small quantity of pure and active catalases. The expression with pFLAG-CTS vector combined with ion exchange chromatography presented similar results. The expression with pTrc-HisA vector required a two-step purification, using IMAC followed by ion exchange chromatography, resulting in pure but inactive catalases. Due to the presented issues in obtaining significant amount of functional catalases, the later construction was coexpressed with GroES/GroEL chaperonins by the use of pT-GroE vector. This method presented better results in the expression of active catalases and was followed by a combination of IMAC and ion exchange chromatography, since the use of this coexpression system generated greater expression of non-specific proteins. CONCLUSION: The obtained results indicate that the bacterial absence of chaperonins can influence their ability in expressing the recombinant protein TrCat and that adding GroES/GroEL chaperonins may help this expression.

Keywords: catalase, recombinant enzyme, Trichoderma reesei / **Supported by:** FAPESP

F.28 - Changes in the collagen organization may be related to lymph node metastasis in canine mammary carcinomas

Daiana Yively Osorio Taborda[1], Ana Paula Vargas Garcia[1], Luana Aparecida Reis[2], **Bárbara Regina Melo Ribeiro** [2], Geovanni Dantas Cassali[1], Ana Maria de Paula[2]
[1]Laboratório de Patologia Comparada, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (MG, Brazil), [2]Departamento de Física, Universidade Federal de Minas Gerais (MG, Brazil)

INTRODUCTION: In Brazil 66,280 new cases of breast cancer were estimated in 2022. Canine mammary tumors present many similarities with breast neoplasms in women and can be considered as a comparative model of study. The carcinoma in mixed tumor (CMT), that is the most frequent type in dogs, has a good prognosis and rarely presents lymph node metastasis. However, some cases evolve to lymph node metastasis with a worse prognosis. It is believed that changes in the extracellular matrix, specifically in collagen fibres, may be responsible for this more aggressive behavior. OBJECTIVES: We used nonlinear microscopy imaging to analyze samples of normal mammary gland tissue (NMT), benign mixed tumours (BMT), carcinoma in mixed tumour without metastasis (CMT) and carcinoma in mixed tumour with metastasis (CMTM). MATERIALS AND METHODS: Second harmonic generation (SHG) and two-photon excited fluorescence (PL) microscopy images allowed the evaluation of tissue collagen and cell segments, that were extracted by an automated software developed for image analysis. That allows to measure the parameters of the collagen fibres and cellular regions: Cell Segment Circularity, Cell Segment Coverage, Fibre Segment Coverage, Fibre Segment SHG Coherence, Mean Fibre Length and Number of Fibres. DISCUSSION AND RESULTS: The results show that the BMT presents greater fibre coverage, lower cell coverage, lower number of fibres and longer average fibre length as compared to the NMT. The CMT and CMTM showed greater cell coverage, lower fibre coverage, lower number of fibre, longer average fibre length and greater circularity of the cell segment in comparison to MBT and NMT. When comparing the carcinomatous groups, the CMTM presented less fibre organization in relation to the CMT. Also, the number of fibres in the MTCM is smaller compared to the MTC. CONCLUSION: Thus, changes in the collagen characteristics of the MTC and MTCM may be responsible for the different behavior of the two tumors.

Keywords: mammary tissue, nonlinear microscopy, collagen fibres
Supported by: FAPEMIG, CNPq and CAPES

F.29 - THE PEPTIDE C9K SHOWS ANTITUMOR EFFECT AGAINST MURINE MELANOMA CELLS

Maria Carolina Mariano Cesar[1,3,4], Andrey Senos Dobroff[2,3,4], Denise Costa Arruda[1]
[1]Núcleo Integrado De Biotecnologia, Univ. De Mogi Das Cruzes (SP, BRAZIL), [2]Target Identification And Validation, Nextrna Therapeutics (Massachusetts, United States Of America), [3]Department Of Internal Medicine, University Of New Mexico (New Mexico, United States Of America), [4]Comprehensive Cancer Center, University Of New Mexico (New Mexico, USA)

INTRODUCTION: Melanoma is the deadliest form of skin cancer due to its high metastatic potential. Proteins involved in the regulation of metastasis are considered attractive targets for the development of novel therapeutic modalities. Thus, peptides designed to interfere or block the expression of such proteins have been extensively evaluated by our group. In fact, the BRN2-derived peptide, C9K, has shown significant antitumor activity by modulating the expression of proteins involved with melanoma motility. OBJECTIVES: To determine the *In vitro* and *In vivo* antitumor activity of the C9K peptide in murine melanoma model. MATERIALS AND METHODS: The viability, migration, and the capacity of B16F10 cells to form spheroids after C9K treatment were assessed by the xCELLigence and the Incucyte Zoom® live-cell imaging platform. The expression of proteins of interest was analyzed by Western blot before and after C9K treatment. The potential of C9K in reducing lung metastasis was evaluated in C57BL/6 mice intravenously inoculated with B16F10-Nex2 and treated with C9K for ten days. *In vivo* toxicity studies were carried out by injecting healthy animals with an excess of C9K for up to three days followed by histopathological analysis. Statistical analyses were performed using Student's t-test. DISCUSSION AND RESULTS: C9K decreased cell viability after 29 hours ($p < 0.05$), and inhibited cell migration ($p < 0.01$) and spheroid formation ($p < 0.01$) after 24 and 12 hours, respectively. Additionally, we observed a decrease on MITF and Vimentin levels after treatment with C9K. More importantly, C9K showed a protective effect *In vivo* , reducing the number of metastatic foci by 70-fold ($p < 0.001$), with no off-target or tissue toxicity-related issues. CONCLUSION: Although more studies are necessary to establish C9K as a new therapeutic to treat melanoma metastasis, our results indicate that C9K disturbs the expression of metastasis-related proteins, thus leading to a reduction in cell viability, migration, and spheroid formation of melanoma cells.
Keywords: Melanoma, Peptide, Metastasis / **Supported by:** CAPES; FAPESP

F.30 - Delivery of superoxide dismutase by TAT and abalone peptides for the protection of skin cells against oxidative stress

Gustavo Roncoli Reigado [1], Patricia Adriani[2], Viviane Nunes[3]
[1]Department of Biotechnology, Universidade de São Paulo (São Paulo, Brazil), [2]Department of Pharmacology, Universidade de São Paulo (São Paulo, Brazil), [3]Arts, Sciences and Humanities Department, Arts, Sciences and Humanities Department - Universidade de São Paulo (São Paulo, Brazil)
INTRODUCTION: *Trichoderma reesei* superoxide dismutase (TrSOD) is a well-characterized enzyme being stable between 30 and 90ºC for 1 h with activity at pH between 2.6 and 9.0. OBJECTIVES: This work aimed to clone, express, purify, and evaluate the protective effect antioxidant of this enzyme on skin cells when fused to transactivator of transcription (TAT) protein transduction domain of HIV-1 and abalone (Ab) peptides to allow cell penetration. MATERIALS AND METHODS: TrSOD, TAT-TrSOD-Yfp (fused to yellow fluorescente protein), and Ab-TrSOD were expressed in E. coli and purified as soluble proteins. The cytotoxicity of the enzymes, at the concentrations of 1, 3, and 6 µmol/L was evaluated for a period of 24 and 48 h of incubation, with no cytotoxic effect on 3T3 fibroblasts. The 3T3 cells were exposed to the oxidant agent tert-butyl hydroperoxide and evaluated for reactive oxygen species (ROS) generation, in the presence or not of the recombinant enzymes. DISCUSSION AND RESULTS: TAT-TrSOD-Yfp was able to decrease the generation of ROS by 15% when used in the concentrations of 3 and 6 µmol/L in comparison to the control without any enzyme, but there was no difference in relation to the effect of TrSOD. Ab-TrSOD, when compared to TrSOD, promoted a decrease in the formation of ROS of 19% and 14% at the concentrations of 1 and 6 µmol/L, respectively, indicating that this recombinant form of the enzyme was more effective in reducing oxidative stress compared to SOD without the cell-penetrating peptide (CPP). CONCLUSION: Together, these results indicate that the fusion of SOD with these CPP increased the antioxidant capacity of fibroblasts, identified by the reduction in the generation of ROS. In addition, such molecules, in the concentrations initially used, were not toxic to the cells, opening perspectives for the development of products for antioxidant protection of the skin that may have therapeutic and cosmetic application.
Keywords: antioxidants, cell-penetrating peptide, superoxide dismutase / **Supported by:** CAPES

F.31 - Production of recombinant protein using the microalgae Chlamydomonas reinhardtii

Guilherme Henrique Bittencourt [1], Giovanni Montovaneli[1], Breno Santos[1], Silas Rodrigues[1]
[1]Nucleo multidisciplinar de Pesquisa em Biologia, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: The microalgae *Chlamydomonas reinhardtii* is a model for studies on photosynthetic organisms in general, due to its rapid reproduction and the inexpensive and easy laboratory culturing. They are pointed out as promising for the expression of recombinant proteins, as they can do post-translational modifications (PTMs), and have little maintenance requirements. OBJECTIVES: This project aims at establishing a method for *C. reinhardtii.* chloroplast genome transformation using a L-Asparaginase enzyme (L-Asp) encoding DNA sequence as target. MATERIALS AND METHODS: The TAP-medium grown CC-125 *C. reinhardtii.* strain was used for transformation experiments. P-322 vector was chosen based on literature data. A DNA construct composed of codon optimized ansB gene, which encodes a L-Asp isoform that is prescribed to treat acute lymphoblastic leukemia (LLA) in Brazil, and 3'- and 5'-regulatory sequences was chemically synthesized and subcloned at P-322. After sequence validation by DNA sequencing and restriction enzymes treatment assays, tungsten microparticles were coated with the vector. Then, the cells were submitted to biolistics experiments followed by erythromycin-based transformat selection. The cells treated with P-322 without the expression cassette or treated with microparticles without the vector were used as controls. DISCUSSION AND RESULTS: After incubating the cells in TAP-solid medium containing antibiotics, it was possible to observe four positively selected *C. reinhardtii.* colonies. Based on the current results, the cells could be either transformed or natural mutants, which were already reported from studies using the same vector and selection antibiotic. Experiments of PCR and DNA sequencing are in progress for molecular validation of transformation events. CONCLUSION: It was possible to obtain four positively selected *C. reinhardtii.* The result requires further validation.

Keywords: L-Asparaginase, heterologous protein, protein expression. / **Supported by:** CNPq and FAPERJ.

F.32 - Production, characterization and application of a chymotrypsin-like from Pycnoporus sanguineus

Hugo Juarez Vieira Pereira [1], Alexsandra Ferreira[1]
[1]Instituto de Química e Biotecnologia, Universidade Federal de Alagoas (Alagoas, Brasil)

INTRODUCTION: In cheese production, animal rennet is often used to coagulate milk. Rennet is an enzyme complex derived from the abomasum of weaned calves, consisting mainly of chymosin. The increased demand for cheese production by dairies is not compatible with the availability of rennet of animal origin, in addition, these coagulants are expensive and are not well accepted by groups that do not consume them for reasons religious, ethical and dietary restrictions. Therefore, we present in this work the production, characterization and application of a protease from Pycnoporus sanguineus with the property of coagulating casein. OBJECTIVES: Identify, produce, characterize and apply a new chymotrypsin-like enzyme produced by Pycnoporus sanguineus. MATERIALS AND METHODS: The filamentous fungus Pycnoporus sanguineus was inoculated in a potato-Dextrose-Agar culture medium. After the mycelial growth of the cultures, discs were removed to be used in the solid state fermentation. After fermentation sodium phosphate buffer (0.1 mol L-1, pH 7.0) with 0.6% NaCl were added and subjected to agitation. Subsequently, this extract was filtered, the filtrate supernatant was called enzymatic extract. The extract was characterized in relation to the specificity in the hydrolysis of the specific substrates of trypsin, chymotrypsin and elastase-2 and the inhibition by PMSF, benzidine, SBTI and DTT. After the characterization of the enzymatic activity was applied in milk coagulation assays. DISCUSSION AND RESULTS: The protease showed specificity to hydrolyze the chymotrypsin substrate and was totally inhibited by PMSF (serine protease inhibitor), partially by DTT (thiol protease inhibitor and reducing agent) and no inhibition by benzamidine and SBTI (trypsin specific inhibitors). The chymotrypsin-like activity was able to hydrolyze casein in enzymatic assays and in zymographies and demonstrated the ability to clot casein in pasteurized milk samples. CONCLUSION: It was possible to produce a new chymotrypsin-like protease with caseinolytic activity. The enzyme produced showed promise as an agent for coagulation of milk and cheese production.

Keywords: Protease, Coagulant, Caseinase

F.33 - UNG AND MUG GLYCOSYLASES ARE PRESERVED IN CORYNEBACTERIUM PSEUDOTUBERCULOSIS AND *IN VITRO* ASSAYS INDICATED BOTH PROTEINS ARE INVOLVED IN THE URACIL REMOVAL

Cássio Siqueira Souza Cassiano [1], Bruno Carvalho Resende [1,2], Thalia Queiroz Ladeira[1], Diego Lisboa Rios[2], Carlos Renato Machado [2], Vasco Azevedo [2], Luciana Santos[1], Débora De Oliveira Lopes [1]
[1]Federal University of São João del-Rei (MG, Brazil), [2]ICB, Federal University of Minas Gerais (Minas Gerais, Brazil)

INTRODUCTION: Corynebacterium pseudotuberculosis, the etiological agent of caseous lymphadenitis, is a facultative intracellular microorganism vulnerable to DNA damage. Since inefficiencies in the DNA damage repair system can lead to death, the characterization of repair genes may provide valuable molecular targets for caseous lymphadenitis therapy. Uracil DNA N-glycosylase (Ung) is an efficient enzyme, which can remove uracil from any context and mismatch-specific uracil DNA glycosylase (Mug) is known for exhibiting glycosylase activity on three mismatched base pairs, T/U, G/U and C/U. OBJECTIVES: The purpose of this study was to characterize and compare the Ung and Mug proteins from C. pseudotuberculosis and evaluate their glycosylase activity. MATERIALS AND METHODS: The ung and mug gene sequences were obtained from GeneBank and analyzed using bioinformatics tools. DISCUSSION AND RESULTS: The domains and catalytic residues important for the enzymatic activity are preserved in both proteins. To verify the glycosylases activity, *In vitro* assays were performed, where both genes were cloned in the pET-21a vector and the proteins were expressed in Escherichia coli and purified. An enzymatic activity assay, performed using an oligonucleotide (labeled with 6-carboxyfluorescein) substrate containing single uracil conducted by fragment analysis in an automatic sequencer, showed glycosylase activity for both proteins. A Mug glycosylase model was built and Molecular Docking analysis indicated, by comparing the mean distances in angstroms, that the catalytic residues had more affinity for the DNA strand containing the uracil lesion than for other lesions. An Ung glycosylase model and Molecular Docking will be built. Phylogenetic analysis was performed to evaluate the presence of mug and ung genes in 100 species of Corynebacterium and an ancestrality pattern between the pathogenic and non-pathogenic species was not verified. CONCLUSION: To investigate the effect of the absence of these genes in this organism, knockout cells have been constructed through CRISPR-technical, to perform the *In vivo* assays.
Keywords: DNA repair, Corynebacterium pseudotuberculosis, Glycosylase Assay / **Supported by:** Capes

F.34 - Partial *In vivo* Protection Against Lethal Effects of Peruvian Spider Loxosceles laeta Venom by Immunization of Mice with a Multiepitopic Protein (rMEPLox)

Ruth Liliam Quispe[1], Michael Lorenz Jaramillo[1], Frank Torres-Huaco[1,2], Cesar Bonilla[1,3], Jacqueline Isasi[1], Clara Guerra-Duarte[4], Carlos Chávez-Olórtegui[5,1]
[1]Laboratorio de Toxinología, Centro Nacional de Productos Biológicos (CNPB), Instituto Nacional de Salud (Lima, Peru), [2]Coordinación de Investigación, Universidad Continental (Arequipa, Peru), [3]Facultad de Odontologia, , Universidad Nacional Mayor de San Marcos (Lima, Peru), [4]Diretoria de Pesquisa e Desenvolvimento, Fundação Ezequiel Dias (Minas Gerais, Brazil), [5]Departamento de Bioquímica e Imunologia, ICB, Universidade Federal de Minas Gerais (Minas Gerais, Brazil)

INTRODUCTION: Loxoscelism is a serious public health problem in Peru, with approximately 2500 accidents reported per year. Biotechnological improvements have not yet been applied on the loxoscelic antivenom production in Peru. OBJECTIVES: To envision alternatives to cope with this health problem a neutralizing humoral immune response against the lethal effects of Peruvian spider *Loxosceles laeta* venom was evaluated in the mouse model by using a non-toxic multiepitopic chimeric protein (rMEPLox) containing epitopes from metalloprotease (LALP-1), hyaluronidase (LiHYAL) as well as from sphyngomielinase-D (SMase-D) from *Loxosceles intermedia* and from SMase-I from L. laeta venoms MATERIALS AND METHODS: An immunization schedule of 96 days (~14 weeks) using rMEPLox as antigen was initiated in mice (N=6 male Swiss mice weighing 18–20 g). The median lethal dose (LD50) of *L. laeta* (Peru) venom collected by electrical stimulation was 21.7 µg/20 g established in male Swiss mice (18 - 20 g). DISCUSSION AND RESULTS: After six injections of this protein rMEPLox, mice developed an IgG response and the anti-rMEPLox antibodies had high reactivity with *L. laeta* venom. *In vivo* protection assays showed that five out of six (~90 %) of mice immunized with rMEPLox resisted a challenge of 1.4 LD50 (intraperitoneal) of *L. laeta* venom, according to the mice's weight (28-weeks old). In the non-immune mice group, only two animals survived with this same venom dose. CONCLUSION: This study suggests that this multiepitopic protein can be a promising candidate for experimental vaccination approaches or for antivenom production against Peruvian *L. laeta* venom. **Keywords:** Loxosceles laeta, rMEPLox, spider venom
Supported by: FONDECYT grant: 021-2019-FONDECYT-BM-INCORPORACIÓN-DE-INVESTIGADORES-PERU and CNPq (Process: 406163/2018-9) and by CAPES, Brazil - (Program COFECUB Process: 88881.191812/2018-01).

F.35 - Optimization of the Process of Expression in E. coli and Purification of the Catalytic Sites of the ACE1 by the ELP-Intein System

Beatriz Angelo Prata[1], Carolina Machado dos Santos[1], **Regina Affonso [1]**
[1]Centro de Biotecnologia, Instituto de Pesquisas Energéticas e Nucleares (São Paulo, Brasil)

INTRODUCTION: Angiotensin I-converting enzyme (ACE) is a fundamental part of the renin-angiotensin system; this has two domains, N- and C-, each of which has a catalytic site that exhibits 60% sequence identity. Its actions are in the control of blood pressure, protection of the brain by cleavage of beta-amyloid bodies, cell proliferation, formation of hematopoietic stem cells, among others. OBJECTIVES: Obtaining the catalytic sites Ala361 to Gly468 (N domain region, csACEN) and Ala959 to Ser1066 (C domain region, csACEC) in pure form and with their correct structural conformation. MATERIALS AND METHODS: Expression conditions of pE1csACEN and pE1csACEC vectors in E. coli BL21(DE3) strain: cultures grown in Terrific Broth at 37ºC at 140 rpm for 20–24 h and 0.1 mM IPTG. Purification by Elastin-like Polypeptide (ELP) precipitation: ELP-bound catalytic sites were purified with two ammonium sulfate precipitations (ASp). Remotion of ELP: by autocleavage of the Intein sequence using the buffers: sodium phosphate, sodium cacodylate, MES and Tris-HCl. The ELP/Intein was removed from the sample by ASp. The analyzes of all stages of the process were performed by SDS-PAGE and Dot blotting. DISCUSSION AND RESULTS: The differential for obtaining the pure peptides was the temperature of 37ºC, with a significant increase in expression concerning the cultivation of 16ºC. In the ELP purification steps, ammonium sulfate buffer concentrations of 0.57 M and 0.8 M were the most efficient. Intein's self-cleaving was more efficient with MES buffers and Tris-HCl for ELPsACEN and ELPsACEC, respectively. Structural analysis by Circular Dichroism and Fluorescence confirmed the correct structure of the pure peptides. CONCLUSION: In the present work, we defined the most efficient conditions for expression, purification, and obtaining of ACE catalytic sites in pure form. The csACEN and csACEC peptides will allow greater assertiveness in obtaining and characterizing new hypertensive drugs and in the hydrolysis of substrates such as beta-amyloid.

Keywords: Catalytic sites of ACE, ELP/ Intein, high temperature of expression

F.36 - Biophysical and Structural Studies of a Novel Und-type Decarboxylase Active in Fatty Acids

Raul De Castro Cunha Claudino [1], Letícia Leandro Rade[1], Mayara Chagas de Ávila[1], Gabriela Felix Persinoti[1], Wesley Cardoso Generoso[1], Ricardo Rodrigues de Melo[1], Letícia Maria Zanphorlin[1]
[1]Brazilian Biorenewables National Laboratory, Brazilian Center for Research in Energy and Materials (SP, Brazil)

INTRODUCTION: Alkenes are an important platform for producing chemical building blocks and drop-in biofuels. After the discovery of the Und decarboxylase from *Pseudomonas sp*. that decarboxylates fatty acid into 1-undecene, the scientific and technological interest in this family of enzymes vastly increased. OBJECTIVES: Our research focuses on finding new Und-type decarboxylases with fatty acid decarboxylation activity to understand what drives that reaction and propose a biotechnology route to produce alkenes. MATERIALS AND METHODS: Herein, we explored a microbial consortium in the presence of different sources of fatty acids to identify a novel Und-type decarboxylase. The selected enzyme was expressed in a host cell (*E. coli*) and purified by chromatography methods. The purified enzyme was submitted to biophysical studies, crystallization assays and X-ray diffraction experiments to determine its molecular properties. DISCUSSION AND RESULTS: The results revealed that regardless of the source of fatty acid used, the predominance of microorganisms belonging to the phylum *Proteobacteria* was highlighted. The enzyme was purified in a folded form, presenting a Tm of 53.7 ºC. The elucidation of the tertiary structure shows the fatty acid molecule in the binding pocket oriented by the conserved catalytic residues (Glu103, His106, and His196). CONCLUSION: Taken together, these results will help us understand the relationship between the structure and function of these enzymes and their molecular mechanisms. Ultimately, this project has enormous potential to contribute to developing biological routes to produce drop-in biofuels and other bioproducts derived from terminal alkenes.

Keywords: *Alkenes, Decarboxylase, Drop-in biofuels*
Supported by: *FAPESP*

F.37 - Biopolymers Conjugated Cubosome for Delivery of Acemannan

Rafael Ricardo Madrid Martinez [1], Patrick Mathews[1], Omar Mertins[1], Barbara Pimienta[1]
[1]Biofisica, Universidad Federal de Sao Paulo (Sao Paulo, Brasil)

INTRODUCTION: Cubosomes are nanoparticles with crystalline structure formed by a lipid bilayer that provides a high hydrophilic and hydrophobic surface area for the entrapment of bioactives. OBJECTIVES: we develop a cubosome containing biopolymers shell for the delivery of acemannan, a bioactive extracted from aloe vera MATERIALS AND METHODS: The conjugated cubosome was prepared with monoolein as lipid and pluronic F127 as stabilizer, adding solutions of chitosan-N-arginine, alginate and acemannan. The mixture was subjected to 10 times vortex mixing (1 min) and bath sonication (5 min) following our previous described protocols.1 The samples were studied by means of dynamic light scattering and isothermal titration calorimetry for evaluation of the thermodynamic interaction of the conjugated cubosomes with bovine serum albumin. DISCUSSION AND RESULTS: The studies unveiled that the biopolymers shell provides a pH-responsive surface charge and structural modifications of the conjugated cubosome in pHs 2.5 and 7.4. These results indicate the potential of the conjugated cubosome for application in oral administration. The encapsulation of acemannan was effective, also evidencing the potential of the conjugated cubosome as a bioactive delivery system. The interaction of the conjugated cubosome with bovine serum albumin, as a physiologic protein, was besides studied with isothermal titration calorimetry The results evidenced and increased thermodynamic interaction with the protein, compared to the control plain cubosome, thanks to the biopolymers shell. CONCLUSION: The charge profile of the two biopolymers, which changes with pH, plays a role in a pH-dependent enthalpic and entropic contributions during the interaction, leading to decrease in Gibbs energy which is more intense in pH 2.5 relative to pH 7.4.

Keywords: Cubosomes, Acemannan, pH Responsive / **Supported by:** FAPESP-CNPq

F.38 - Evaluation of Exclusive CDSs as Potential Antifreeze Proteins from the Antarctic Yeast *Metschnikowia australis*

Duarte, J.C.[1], Hilário, H.O.[2], Macedo, A.M.[1], Machado, C.R.[1], Tahara, E.B.[1], Franco, G.F.[1]
[1]Departamento de Bioquímica e Imunologia, Universidade Federal de Minas Gerais
[2]Pós Graduação em Biologia de Vertebrados, Pontifícia Universidade Católica – Minas Gerais

Introduction. The harsh Antarctic environment led organisms to evolve interest features for their survival on the continent. One organism isolated from the Antarctic Seawater is the yeast *Metschnikowia australis*. Although the genus is present in different habitats all over the globe, *M. australis* is the only *Metschnikowia* species currently isolated from the Antarctic continent. **Objective.** To evaluate the ability of *M. australis* to produce antifreeze proteins (AFPs), a type of protein able to modulate ice growth and protect cells from freezing. **Material and Methods.** Our group sequenced and analyzed the *M. australis* genome and compared it with 35 genomes from other *Metschnikowia* species. We found 16 exclusive coding sequences (CDSs) classified as potential AFPs, which had their expression analyzed in this work. We first grew *M. australis* in temperatures of 4°C, 12°C, 20°C, and 28°C, then obtained their RNA and compared the expression of these 16 CDSs through conventional PCR and qPCR. **Results and Discussion.** *M. australis* presented a better growth at lower temperatures and no growth at 28°C. From PCRs results, we found one CDS with higher expression when the yeast was cultivated at 4°C, low expression at 12°C, and no expression at 20°C. The other 15 CDSs were amplified from all three temperatures analyzed, but the expression was higher at 12°C and 20°C. RT-qPCR, results showed the same expression pattern: one CDS with higher expression at 4°C (around 100-fold increase when compared to 12°C or 20°C) and 15 CDSs with expression around two times higher at 12°C and 20°C than at 4°C. **Conclusions.** Our results indicate this CDS could represent a potential AFP, and further studies are in progress to elucidate its involvement in the yeast protection against freezing.

Key words: Antifreeze protein; Antarctic yeast; *Metschnikowia australis*.
Acknowledges: CAPES, FAPEMIG, CNPq

F.39 - Diagnostic Potential Of A Recombinant Chimera From Leptospiral Membrane Proteins

Luana de Sousa Ramos[1], Elói Quintas Gonçalves da Silva[1], Larissa Coelho Pereira[1], Juliana Lopes Rangel Fietto[1], Abelardo Silva Júnior [2], Gustavo Costa Bressan[1]
[1]Departamento de Bioquímica e Biologia molecular, Universidade Federal de Viçosa (Minas Gerais, Brasil), [2]Departamento de Veterinária, Universidade Federal de Viçosa (Minas Gerais, Brasil)

INTRODUCTION: Leptospirosis is a neglected re-emerging zoonosis of worldwide importance caused by the pathogenic spirochete of the genus *Leptospira*, prevalent in tropical, temperate, and endemic regions of areas affected by heavy rains and floods. It is considered a serious public health problem in several countries and its epidemiology is strictly associated with the presence of susceptible hosts, both maintenance and accidental. Improvements in diagnostic methods are critically needed to understand the complex epidemiology of leptospirosis and help the control of transmission from subclinically infected reservoir hosts. Among the reservoir hosts, dogs are one of the main sources of transmission and, therefore, the development of point-of-care tests such as immunochromatographic tests are particularly useful in rapid diagnostic response and ease of execution, aiding in clinical management and epidemiology of the disease in these animals. OBJECTIVES: To produce a recombinant chimera from leptospiral membrane proteins for the development of a serological diagnostic test for canine leptospirosis. MATERIALS AND METHODS: The recombinant protein gene sequence was cloned into the pET28a expression vector, later inserted into *Escherichia coli* and expressed with IPTG. The purification of the recombinant chimera occurred by means of affinity chromatography using FPLC, characterized by western blot. Reactivity of the recombinant protein was evaluated by ELISA and by the rapid immunochromatographic assay (LFIA). DISCUSSION AND RESULTS: Expression and purification of the recombinant protein were successful and confirmed by Western blot. In both ELISA and LFIA, the chimera was able to discriminate sera samples from positive field dogs from sera samples from negative field dogs, evidencing its potential for diagnostic tests. CONCLUSION: The recombinant protein has the potential for the development of a low-cost rapid diagnostic test for canine leptospirosis.

Keywords: diagnosis, leptospirosis, recombinant
Supported by: CAPES, CNPq, FAPEMIG

F.40 - Coagulotoxic profile characterization of snake venoms from *Crotalus durissus terrificus* and *Crotalus durissus collilineatus*

Luccas Daniel Padueli [1,2], Nathália da Costa Galizio[1], Jarbas Prado Vidueiros[1], Anita Mitico Tanaka-Azevedo[1], Karen de Morais-Zani[1]
[1]Laboratório de Herpetologia, Instituto Butantan (São Paulo, Brasil), [2]Farmácia, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: Snakebites represent a serious public health problem for tropical and subtropical countries due to the range of snake species and the frequency of accidents. *Crotalus* venom is a mixture of biologically active peptides and proteins with enzymatic activity, mainly crotoxin, contabilizing 70 to 90% of this species's venom, and crotamine, an important myonecrotic toxin. *C. durissus* spp venom has high individual and geographic variability, which may represent a challenge to the production of new antivenoms. OBJECTIVES: This study aims to analyze and characterize the coagulotoxic profile of *C. d. terrificus* (Cdt) and *collilineatus* (Cdc) snake venoms. MATERIALS AND METHODS: The analysis included SDS-PAGE, HPLC, enzymatic activity and fibrinogen cleavage profile. DISCUSSION AND RESULTS: In the SDS-PAGE, it was noted that all of the Cdc and Cdt snake venoms presented main bands at approximately 30kDa associated with the presence of SVSP and 14kDa associated with the presence of phospholipase A2 (PLA2). Most notably, all three venoms that showed high PLA2 activity had bands slightly above the main 14kDa bands. It was also noted that most males and most Cdc venoms presented bands at approximately 10kDa, associated with the presence of crotamine, which was also observed in the HPLC chromatograms of these venoms. Regarding coagulation tests, fibrinogen cleavage profile showed that none of the venoms were able to completely cleave any of the three fibrinogen chains, in the time intervals evaluated (15 to 60 min). However it was noted a decrease in intensity of the alpha chain in most of the venoms and the beta chain in a lesser scale, observing that the alpha chain is degraded primarily by the venom; none of the venoms degraded the gamma chain. CONCLUSION: To conclude, the study and characterization of snake venom profiles are of utmost importance to the improvement of existing antivenoms and the discovery of new biologically active compounds.

Keywords: coagulant, antivenom, coagulopathology / **Supported by:** FAPESP (2020/07268-2 and 2020/08246-2)

F.41 - Peptide C15D Induces Cell Cycle Arrest In Murine Melanoma Cells

Juliana Machado Anastácio [1], Tiago Rodrigues[2], Renato Arruda Mortara[3], Claudia Alves da Costa[4], Maria Carolina Mariano César[1], Denise Costa Arruda[1]

[1]Núcleo Integrado de Biotecnologia, Universidade de Mogi das Cruzes (São Paulo, Brasil), [2]Centro de Ciências Naturais e Humanas , Universidade Federal do ABC (São Paulo, Brasil), [3]3Departamento de Microbiologia Imunobiologia e Parasitologia , Universidade Federal de São Paulo (São Paulo, Brasil), [4]Centro Interdisciplinar de investigação Bioquímica , Universidade de Mogi das Cruzes (São Paulo, Brasil)

INTRODUCTION: Melanoma is a skin cancer originated from malignant transformation. Intracellular signaling pathways regulate cellular essential processes, such as proliferation, adhesion, and survival. These pathways are altered in cancer and they might be activated by mutations in regulatory genes, which increases the expression of several proteins. BRN2 is a transcription factor expressed in melanocytes that is overexpressed in melanoma, and it is directly related to metastatic capacity of melanoma cells. Peptides derived from BRN2 could compete with the DNA BRN2 binding site and/or interact with regulatory proteins, representing a potential therapeutic strategy OBJECTIVES: To determine the effects of C15D peptide derived from BRN2 in murine melanoma cells *in vitro*, focusing on its interference in the cell cycle. MATERIALS AND METHODS: The viability of B16F10-Nex2 cells was determined by the trypan blue assay. Also, a clonogenic assay was performed by colony counting and area measurement. For cell cycle assay, cells were incubated with C15D, treated with RNAse, stained with PI, and analyzed by flow cytometry. Confocal microscopy was performed by treating cells with biotinylated C15D. After fixation, permeabilization, and staining, cells were analyzed to verify peptide internalization. Protein expression was analyzed by Western blotting. DISCUSSION AND RESULTS: C15D decreased the proliferation of murine melanoma cells (p<0.001). Although, this peptide did not interfere with colony number, colonies were smaller than control (p<0.05). Moreover, C15D induced cycle arrest (G2/M phase) (p<0.05), and a decrease in cyclin A levels and an increase in phospho-cyclin B and p53 levels were observed. Also, C15D was internalized by cells and exhibited a co-localization with actin filaments and nucleus CONCLUSION: Altogether, C15D peptide is internalized by cells and its main effect is to interfere with cell growth. These results point to the potential of C15D peptide as a promising molecule for melanoma therapy.

Keywords: Cell Cycle, Melanoma, Peptide / **Supported by:** CAPES & FAPESP

F.42 - DMPEI as a Biocidal Paint on Surfaces: The Technological Application in Health

Vinícius Souza Tarabal [1], Daniel Bonoto Gonçalves[2], José Antônio da Silva[1], Adriano Guimarães Parreira[1], Marcelo Gomes Speziali[3], Paulo Afonso Granjeiro[1]

[1]Laboratory of Biotechnological Processes and Purification of Macromolecules, Federal University of São João del Rei (MG, Brazil), [2]Department of Biosystems Engineering, Federal University of São João del Rei (MG, Brazil), [3]Chemistry Department, Federal University of Ouro Preto (MG, Brazil)

INTRODUCTION: Brazil has a prevalence of 14% of nosocomial infections (NIs) with more than 12 million hospitalizations per year, generating 100.000 annual deaths. Microorganisms present on hospital surfaces are the most common in NIs, remaining on surfaces after standard hospital cleaning procedures. DMPEI is a second-generation polymer, which is used in surface coating to obtain surfaces with antibiofilm properties. OBJECTIVES: The objective of this study was evaluate the anti-adhesive property of biofilm on stainless steel and polystyrene surfaces coated with a biocidal paint with DMPEI. MATERIALS AND METHODS: DMPEI was synthesized from poly(2-ethyl-2-oxazoline). DMPEI was solubilized in isopropyl alcohol at a concentration of 2 mg/mL. The antibiofilm activity against *Escherichia coli* was evaluated on stainless steel and polystyrene surfaces by serial dilution and plating. Paper surfaces were coated with the paint and the antibiofilm property was evaluated by serial dilution and plating. A commercial antibacterial paint was used as a positive control. Stainless steel surfaces were coated with DMPEI and the shelf life was evaluated over 12 weeks. DISCUSSION AND RESULTS: The synthesis of DMPEI was visually evaluated, attesting to the synthesis of a yellowish powder. The DMPEI coated stainless steel and polystyrene surfaces showed a reduction in biofilm adhesion of 87.16% and 86.16%, respectively. The paint with DMPEI showed an antibiofilm property of 93.96%, while the positive control showed a reduction of 100%. Shelf life has been evaluated for 12 weeks, presenting an average reduction of 93% over the weeks. CONCLUSION: DMPEI has potential as an additive in paints and varnishes, coating of hospital surfaces and in the creation of a self-disinfecting environment. **Keywords:** Biofilm, DMPEI, Nosocomial Infections

Supported by: CAPES, CNPq, FAPEMIG and UFSJ

F.43 - Synthesis and characterization of metallic nanoaggregate-in-polymeric hybrid hydrogels for tooth whitening applications

Vinicius Ikezu Saito [1], Ian Machado[2], Aryane Vigato[1], Anderson do Espírito Santo Pereira[3], Leonardo Fraceto[3], Estefania Campos[4], Renata de Lima[5], Daniele Ribeiro de Araujo[1]
[1]CCNH - Centro de Ciências Naturais e Humanas, Fundação Univ. Federal do ABC (SP, Brasil), [2]Chemistry, University of Turku (, Finlândia), [3]Engenharia Ambiental, Univ. Estadual de São Paulo Júlio de Mesquita Filho (, Brasil), [4]Bioscience and Biotechnology, French Alternative Energies and Atomic Energy Commision (França), [5]Biotecnologia, Univ. de Sorocaba (Brasil)

INTRODUCTION: The search for teeth whitening and its aesthetic character has led scientists to develop new materials to increase the whitening effect and minimise the side effects of enamel wear and tooth sensitivity OBJECTIVES: Then, new systems have been produced associating bleaching agents in nanomaterials. MATERIALS AND METHODS: Here, we prepared commercial titanium salts in hydrogels systems (poloxamer 407-P407/10 or 90 kDa hydroxypropylmethylcellulose-HPMC (20/0.5% w/v)), and the biogenic-based synthesis process was assessed for titanium nanoaggregates production. Nanoaggregates were obtained by associating titanium isopropoxide with Myrrh (Commiphora myrrha SISGEN-ADF2523) aqueous extract at different pH and temperatures. DISCUSSION AND RESULTS: The sol-gel transition temperatures (Tsol-gel) were determined by rheological analysis, ranging from ~24.5 to 26.1 °C, after titanium isopropoxide and myrrh extract were incorporated into the gels. 10 kDa or 90 kDa HPMC-P407 hydrogels showed an increase inTsol-gel compared to the commercial titanium salts-loaded hydrogels (Tsol-gel=18.8-24.9 °C). The elastic (G') and viscous (G") moduli relationships were ca. 5-7-fold higher than PL-based hydrogels. The incorporation of Myrrh extract + Ti-isopropoxide solution influenced hydrogels' structural organization considering the P407 or P407/HPMC presence (10 or 90 KDa). DLS (Dynamic Light Scattering) analysis (25ºC, pH 7) revealed variations in the nanoaggregates dimensions from 100 to 400 nm. Nanoaggregates obtained from synthesis processes at 60 ºC (for 12h, pH 7), and the hydrodynamic diameter showed similar results (100-400 nm) with a polydispersion index of 0.2 to 0.5. By NTA (Nanoparticle Tracking Analysis) were also obtained hydrodynamic diameters from 80 to 400 nm. Topographical analysis by AFM (Atomic Force Microscopy), revealed better parameters for the synthesis at pH 7, after Polyethylene glycol 400 addition as stabilizing agent. CONCLUSION: The results so far showed that titanium isopropoxide is an adequate precursor for metallic nanoaggregates biogenic synthesis, which must occur at pH 7, or with slight variations, avoiding aggregation and high polydispersion corroborating the oral cavity biological conditions. - **Keywords:** Metallic nanoaggregates, Hydrogels, Hybrid systems

F.44 - Characterization of a Bifunctional α-L-Arabinofuranosidase/β-Xylosidase from functional Metagenomics

Brisa Moreira Gomes [1], Vandierly Sampaio de Melo[1], Felipe Santiago Chambergo Alcalde[1]
[1]Biotecnologia, Escola de Artes, Ciências e Humanidades - Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: Environmental problems caused by the release of greenhouse gases (GHG) in the burning of fossil fuels have motivated the search for ecological and sustainable consumerism. Exploit renewable and sustainable biomass resources for biofuels and biochemicals is a strategy. Lignocellulosic materials are renewable, low cost and abundantly available on the planet, and their product is widely used in various industrial sectors. One way to converting this material is through the biochemical route, based on hydrolysis and fermentation processes. This process is carried out by several lignocellulolytic enzymes responsible for the degradation of lignocellulosic biomass in fermentable sugars. Some of this enzymes are α-L-arabinofuranosidases and β-xylosidases, responsible for the degradation of hemicellulose. OBJECTIVES: This work seeks to characterize an bifunctional α-L-arabinofuranosidase/β-xylosidase (TerARA) from termite intestine metagenoma. MATERIALS AND METHODS: The putative enzyme gene were selected through the CAZy database, synthesized and cloned, and the recombinant protein were expressed in E. coli BL21. The enzyme was purified by affinity chromatography on a nickel-sepharose column. In silico analysis of the amino acid sequence, analysis of enzyme activity in different synthetic and natural substrates, and the influence of pH, temperature and ions on the enzyme activity were carried out. DISCUSSION AND RESULTS: The bifunctionality of the enzyme was verified to pNP-X and pNP-A, with diferents values to pH and temperature for the two substrates: 7,0 and 40 °C to pNP-A and 5,5 and 30 °C to pNP-X. Different results were shown on the ion assay as well, with copper and iron presenting positive effects on pNP-X and inhibition effects on pNP-A. Calcium and manganese showed the same results in both substrates, a slightly positive effect. TerARA didn't show efficient thermal stability on neither substrates. CONCLUSION: The results obtained show that TerARA has interesting characteristics for application in the deconstruction of plant biomass.
Keywords: hemicellulases, glycosyl hydrolases, bifunctional enzymes

F.45 - Effects of Microemulsion-Melatonin Bioconjugation on Breast Cell Oxidative Stress

André Henrique Furtado Torres [1], Tatiane Araújo Soares[2], Eliane Trovatti[2], Saulo Santesso Garrido[1]
[1]Bioquímica e Química Orgânica, Universidade Estadual Paulista "Júlio de Mesquita Filho", Instituto de Química, Araraquara/SP (, Brasil), [2]Biomateriais e Biotecnologia, Universidade de Araraquara, Biotecnologia em Medicina Regenerativa e Química Medicinal, Araraquara/SP (SP, Brasil)

INTRODUCTION: Currently, several biomaterials have been developed in order to reduce the adverse effects and increase the therapeutic effects of various drugs and hormones. The hormone melatonin has the function of modulating the circadian rhythm and the immune system, protecting cells from endogenous and exogenous factors. Aiming at the potential of biomaterials and the therapeutic effects of melatonin, the bioconjugation of the microemulsion-melatonin biomaterial can be fundamental for the treatment of several cytopathological diseases, including breast cancer. OBJECTIVES: The study aims to evaluate the oxidative stress effects of microemulsion-melatonin bioconjugation in breast cells. MATERIALS AND METHODS: The hormone melatonin was solubilized at a concentration of 10% in distilled water, then it was conjugated with a microemulsion containing isopropyl myristate, oil phase, melatonin solubilized in distilled water, aqueous phase, Polysorbate 80 and polyethylene glycol 400, surfactants and stabilized with the aid of an agitator. Superoxide anion and superoxide dismutase (SOD) enzyme assays were performed on MCF-10 cells treated with microemulsion-melatonin bioconjugation. DISCUSSION AND RESULTS: The microemulsion-melatonin bioconjugation modulated the oxidative stress of the cells, enhancing the antioxidant effect in the cells, keeping the superoxide anion level constant (> 0.05) and increasing the action of SOD (< 0.05), resulting difference was observed in cells treated with melatonin, with the level of superoxide anion increased (< 0.05) and the action of SOD was not modulated, when compared to cells treated with melatonin and microemulsion-melatonin bioconjugation. if the antioxidant action of bioconjugation prevailed (< 0.05). A similar result was observed in the study by Torres et al. (2021) with MCF-7 cells treated with melatonin associated with a modified delivery system, on the other hand, low levels of the SOD enzyme were observed in MCF-7 cells stimulated with nanocomposite (Solairaj;Rameshthangam;Arunachalam, 2017). CONCLUSION: We can conclude that the microemulsion-melatonin bioconjugation with its antioxidant effect may be an alternative prophylaxis for the development of breast cancer.
Keywords: Biomaterial, Breast cancer, Melatonin / **Supported by:** CAPES foundation for Ph.D. fellowship

F.46 - Development of Nanostructured Lipid Carriers Containing Doxorubicin and Chloro-Aluminum Phthalocyanine for the Treatment of Breast Cancer Applied to Photodynamic Therapy

Talita Cesarim Mendonça [1], Ludmilla David de Moura[1], Fabíola Vieira de Carvalho[1], Gabriela Gerônimo [1], Gustavo Henrique Rodrigues da Silva[1], Marcia Cristina Breitkreitz[2], Eneida de Paula[1]
[1]Bioquímica e Biologia Tecidual, Univ. Estadual de Campinas (SP, Brasil), [2]Instituto de Química, Univ. Estadual de Campinas (SP, Brasil)

INTRODUCTION: Since the 90s, breast cancer is the most common solid neoplasm among women in the world, being the second most common cause of death from cancer in this gender. Doxorubicin (DOX) is an antineoplastic antibiotic of the anthracycline group, isolated from cultures of Streptomyces peucetius. Nanostructured Lipid Carriers (NLC) are drug delivery carriers that can improve the bioavailability, stability and potentiate the action of lipophilic drugs, such as DOX. Photodynamic therapy (PDT) is another useful tool in oncology, in which a photochemical reaction causes selective tissue destruction after irradiation with a photosensitizing agent. OBJECTIVES: Our work aimed at the development and characterizing NLC containing DOX and the photosensitizer chlorine-aluminum phthalocyanine (ClAlPc) (NLC-DOX/ ClAlPc) for the treatment of breast cancer. MATERIALS AND METHODS: The formulation was developed using Design of Experiments (DoE), by facecentered central composite design (α=1). An optimized formulation, containing 10% total lipids, 65%:35% Myristyl myristate : DhayKol, 7.5% Pluronic 188, DOX (100μM) and ClAlPc (100μM) was obtained. The formulation was followed for 6 months of storage at 25oC, regarding size, polydispersity index (PDI), zeta potential (ZP), particle concentration, pH and morphology (by transmission electron microscopy) DISCUSSION AND RESULTS: Nanoparticles of 160.8-198.0 nm average diameter, PDI < 0.20, negative ZP (|15.2 - 38.8| mV, 5.01 ± 0.18x1013 particles/mL, spherical shapes and regular contours were obtained at pH=5.30 CONCLUSION: The optimized (NLCDOX/ ClAlPc) formulation showed good self stability. In the next step its pharmaceutical potential, by associating DOX and photodynamic therapy will be tested *In vitro* aiming the treatment of breast cancer.
Keywords: Doxorubicin, Chloro-aluminum phthalocyanine, , nanostructured lipid carriers / **Supported by:** FAPESP

F.47 - Construction and characterization of microreactors of Chlorocatechol 1,2-dioxygenase using low complexity domains as molecular adhesives

Nathan Nunes Evangelista [1], Mariana Micheletto[1], Luis Mendes[1]
[1]Departamento de Física da FFCLRP, Universidade de São Paulo (, Brasil)

INTRODUCTION: Low Complexity Domains (LCDs) can be used as molecular adhesives on proteins and are capable of undergoing Liquid-Liquid Phase Separation (LLPS). LLPS has emerged as an interesting strategy to produce microreactors useful in vaccines development and tissue engineering. Choloratechol 1,2-Dioxygenase (CCD) is an enzyme with an increasing potential for bioremediation. Therefore, the construction of the CCD-LCD [2] chimera could lead to the development of microreactors capable of spatially and temporally controlling reactions for application in environmental decontamination. OBJECTIVES: To determine expression and purification protocols for CCD-LCD [2] ; to characterize the structure, stability, and LLPS formation for CCD-LCD [2] and functionalize it as microreactors. MATERIALS AND METHODS: The initial step was based on constructing the DNA coding for chimera protein (CCD-LCD [2]) with subcloning in a vector containing the LCDs codes. The protocol for expression in E. coli and purification was established. DIC and Fluorescence Microscopies determined the behavior for LLPS. The stability of the proteins was determined by optical absorption, fluorescence, circular dichroism, and DLS. DISCUSSION AND RESULTS: The CCD-LCD [2] gene code was successfully constructed, and a heterologous expression produced the recombinant protein. The expression and purification of CCD-LCD [2] resulted in a protein with 43 kDa, capable of undergoing LLPS at low pHs and salt absence. The secondary and tertiary structures were conserved with a slight increase in the content of other structures, such as intrinsically disordered regions. The protein also showed lower thermal stability than its wild-type sequence. CONCLUSION: The chimera protein showed a very conserved structure to its wild-type version, capable of phase separating at low pHs and allowing the microreactor functionalization in soil pollution conditions. However, this process must be made at pHs where the chimera protein has lower efficiency in its catalysis.

Keywords: Biorremediation, 1,2-CCD, LCDs
Supported by: FAPESP

F.48 - Improving the refolding efficiency and purification of SA-TRIM21 to the secondary antibody

Anelize Felicio Ramos [1], Luísa Fontes Giachini[1], Bruna Andersen Pereira de Jesus[1], Gustavo Fellipe da Silva[1], Maria de Lourdes Borba Magalhães[1]
[1]Bioquímica e Biologia Molecular , Universidade do Estado de Santa Catarina (Santa Catarina, Brasil)

INTRODUCTION: Biotechnical research frequently requires purifying recombinant proteins in the simplest and most efficient manner possible. Bacterial systems facilitate the expression of proteins on a large scale, nevertheless, limitation of this process is often the insolubility of the protein, which may be expressed largely in inclusion bodies. The development of a strategy for the efficient refolding of the proteins from inclusion bodies has become an important issue in the recovery of soluble active protein. Previous studies have developed a chimeric protein consisting of the use of the protein TRIM21 fused to protein streptavidin (SA-TRIM21) for the detection of IgG-type antibodies. OBJECTIVES: In these studies, the protein proved to be functional, but with low productive yield, generating the need for the prospection of processes that enable the production of protein on an industrial scale. Optimization of the refolding process was carried out to enhance the yield of active SA-TRIM21 for cost-effectiveness. MATERIALS AND METHODS: SA-TRIM21 was expressed in Escherichia. coli (BL21 (DE3) pLysS. The inclusion bodies containing the protein were denatured using 6M of Guanidine Hydrohydrochloride and refolding by dialyzes followed by purification using Ni-NTA resin, compared to a simplified alternative process in which basically is the removal of denaturant. In the direct method, the solubilized protein is also refolded by continuous exchange of dialysis buffer with a semipermeable membrane, and concentration occurring by Amicon. DISCUSSION AND RESULTS: The purification using Ni-NTA resin had an average yield of 0.99% of functional proteins recovered after refolding evaluated by ELISA and cost per mg of R$372.20. With the method developed in this study, the yield was 16.32% with a cost of R$17.02. CONCLUSION: Obtaining a protein on an industrial scale and in favorable stability conditions makes the SA-TRIM21 promising as a bioactive of market activity such as secondary antibodies, easy to obtain, low cost, and without the need for immunization in animals.

Keywords: Immunoglobulin G, inclusion bodies, refolding yield / **Supported by:** CNPQ

F.49 - Functional Validation of Chimera Defensins with Antimicrobial Potential

Ian Alejandro Fernandez Soliz[1], Vivian de Jesus Miranda[2], Octávio Luiz Franco[1,3], Fançoise Gosti[2], M. N Dam[2], C. Nespoulous[2], P. Berthomieu[2], Cristiane dos Santos[1]

[1]S-Inova, Programa de Pós-Graduação em Biotecnologia, Universidade Católica Dom Bosco (Mato Grosso do Sul, Brasil), [2]BPMP, SupAgro, CNRS, INRAE, Montpellier SupAgro, Univ Montpellier (Montpellier, França), [3]Centro de Análises Bioquímica (CAPB), Pós-Graduação em Genômica e Biotecnologia, Universidade Católica de Brasília (Distrito Federal, Brasil)

INTRODUCTION: Defensins are cationic peptides that belong to the antimicrobial peptides (AMPs) family. In plants, AMPs display multifunctional potential in biotic and abiotic stress control. OBJECTIVES: This work aim is to evaluate chimeric defensin (ChD1) multifunctionality obtained by in silico modeling from a previous study in our lab. MATERIALS AND METHODS: The vector pYX212 with ChD1 gene (pYX212:ChD1) was introduced into *Saccharomyces cerevisiae* genome by electroporation. The yeast carrying empty vector was the negative control. The PCR product was run on a 1.0 % agarose gel and was used to sequencing analysis. cDNA synthesis was performed from RNA extracted, to detected transcript. After protein extraction, bands (< 10 kDa) from SDS-PAGE were cut for peptide extraction to evaluation by MALDI. The ChD1 protein isolation was performed by HPLC. Clones expressing ChD1 gene were used for zinc tolerance assay. DISCUSSION AND RESULTS: The plasmid, pYX212:ChD1, was introduced into yeast and colonies resistant to ampicillin were selected. In order to confirm the vector insertion, PCR products yielded by two specific primers were analyzed in 1.0% agarose gel electrophoresis. Gel showed two expected bands about 130 and 700 bp fragments corresponding to the ChD1 gene and vector region, respectively. Nucleotide sequences generated by such products, showed full identity ChD1 gene region. The positive clones were used to zinc (ZnSO4) bioassay, using microdrop method, and two different zinc concentration (22.5 mM/ 25 mM). The results showed that yeast, expressing ChD1 gene, keep the zinc tolerance ability, when compared to control. CONCLUSION: These results demonstrated that ChD1 can provide zinc tolerance to yeast in *In vitro* tests. Thus, it may be a potential candidate to be tested in a transgenic plant. The heterologous ChD1 production, will be possible verify the effect of the changes in the primary sequence and to study the functional promiscuity of AMPs.

Keywords: Antimicrobial peptides, Plant defensins, Chimera

F.50 - Preparation and Characterization of Iron Oxide Magnetic Particles with Specific Ligand for Proteases Purification

Layla Carvalho Mahnke[1], **Maria Helena Menezes Estevam Alves** [1], Mariana Paola Cabrera[1], José Antônio Teixeira[2], Luiz Bezarra de Carvalho Jr[1]

[1]Laboratório de Imunopatologia Keizo Asami (LIKA), Universidade Federal de Pernambuco (Pernambuco, Brasil), [2]Centro de Engenharia Biológica, Universidade do Minho (Braga, Portugal)

INTRODUCTION: The usual enzyme purification techniques cover several steps, are expensive and time-consuming. Therefore, Magnetic Particles (MPs) represent a very promising alternative for purification as low-cost, high reusability and recovery. The MPs developed for our research group proposed a one-step collagenase and trypsin purification based on affinity binding by using a magnetic composite containing specifics ligand: azocoll (MPs-Azocoll) and azocasein (MPs-Azo). OBJECTIVES: The present study aimed to synthetize and characterize the MPs-Azo and MPs-Azocoll. MATERIALS AND METHODS: MPs Synthesis: An aqueous mixture containing $FeCl_3$ and $FeCl_2$ was added to azocasein or azocoll, and ammonium hydroxide 28.0–30.0%. The mixture was heated at 50°C, collected by an external magnet, dried at 50ºC and kept at 25°C for later use. MPs Characterization: The MPs were characterized using Scanning Electron Microscopy (SEM), Energy Dispersive X-ray (EDX), X-Ray Diffraction (XRD), Fourier Transform Infrared (FTIR) and Magnetometer. DISCUSSION AND RESULTS: SEM analysis show the MPs-Azo and MPs-Azocoll as agglomerates. Further analysis by EDX displayed as expected that the carbon element is only present in the MPs-Azo (7%) and MPs-Azocoll (23%). In the DRX, a slight angular displacement and a decrease of the peaks intensity for MPs-Azo and MPs-Azocoll were observed. These small natural offsets occurs when Fe_3O_4 are synthesized with other elements. The presence of azocasein and azocoll in the MPs are showed by FTIR characteristic absorption bands. The results of Magnetometer suggest that both MPs present superparamagnetic properties. CONCLUSION: SEM conclude that MPs with the ligand presented larger size variation. The presence of azocasein and azocoll in the magnetic particles is suggested by carbon present in EDX, FTIR characteristic absorption bands and by a broad band in each spectrum obtained from XRD. Therefore, both magnetic particles can be proposed as alternative for proteases purification.

Keywords: Magnetic composite, Protease, Enzyme purification

Supported by: CNPq, CAPES and FACEPE

F.51 - What do we know about mead fermentation by S. cerevisiae? An extensive systematic literature review and NMR metabolomics of honey fermentation.

João Vitor Rios Mayrinck [1], Werner F. Brandão[1], Gisele Cardoso de Amorim[1], Marcel Menezes Lyra da Cunha[1]
[1]Núcleo Multidisciplinar de Pesquisa UFRJ – Xerém em Biologia, Univ. Federal do Rio de Janeiro (RJ, Brasil)

INTRODUCTION: Mead is an alcoholic beverage made from fermented honey. Publications about meads are growing in number since 2016, and many publications focus on fermentation improvements featuring molecules produced by HPLC. OBJECTIVES: Our work has as objective the characterization and quantification of molecules produced and consumed during the fermentation of mead by NMR. A systematic search was performed in Google Scholar, PubMed and Web Of Science databases. Keywords used were NMR (Nuclear magnetic resonance), HPLC (High performance liquid chromatography), Fermentation, Yeast, *Saccharomyces cerevisiae*, QA23, Honey, Mead, Beer, Wine. MATERIALS AND METHODS: For NMR analyses, 60 µL of D2O were added to the samples for calibration and adjustment of the magnetic field and 60 µm DSS (4,4-dimethyl-4-sila pentane-1-sulfuric) at 10 MM for internal reference, totaling 600 µL of final volume in all samples. Samples were analyzed in a 500 mhz Bruker Nuclear Magnetic Resonance apparatus (Bruker, Germany) at a temperature of 20°C (293 K) and TOCSI, HSQC and NOISY spectra were acquired. DISCUSSION AND RESULTS: The results were extracted and submitted on online Rayyan QCRI organization software. 147 articles were found that describe the state of the art on the subject. Fermentations were carried out in real time and also carried out on specific days to evaluate the molecules produced and consumed during the fermentation. The consumption and production of some important molecules for the taste of mead were our interest, among them, sugars and alcohols. CONCLUSION: The later stages of our project, which will be carried out until the date of presentation at the SBBq congress, are the analysis of the NMR spectra, characterizing some molecules and quantifying them from an analysis with quantified maleic acid as an external reference, so to ensure the quantification of the molecules present in the mead

Keywords: Mead, Fermentation, NMR
Supported by: faperj

F.52 - Use of Immobilized Calotropis procera Cysteine Peptidases (CpCPs) for Cheesemaking

Cleverson Diniz Teixeira de Freitas [1], João Pedro Brasil Oliveira[1], Marcio Viana Ramos[1]
[1]Bioquimica e Biologia Molecular, Universidade Federal do Ceará (Ceara, Brazil)

INTRODUCTION: Calotropis procera cysteine peptidases (CpCPs) have presented several biotechnological potentialities. Recently, we reported the immobilization of CpCPs on different supports. Among all supports tested, the peptidases immobilized on glyoxyl-agarose exhibited the best results (Glyoxyl-CpCPs). OBJECTIVES: The aim of this study was to evaluate the action of Glyoxyl-CpCPs on cow's milk-clotting activity and cheesemaking. MATERIALS AND METHODS: The proteolytic assays were performed using azocasein as substrate at pH 5.0. For casein hydrolysis, casein solution (10 mg/mL, in 200 mM sodium phosphate buffer, pH 6.5) was incubated with Glyoxyl-CpCPs at 37 °C, 130 rpm for 120 min, and the casein hydrolysates were monitored spectrophotometrically by the increment in the absorbance at 550 nm and by SDS-PAGE. Casein micelle aggregation was measured by atomic force microscopy. Cheeses made using Glyoxyl-CpCPs were submitted to the following analyzes: moisture, protein, ash, fat, and carbohydrate. DISCUSSION AND RESULTS: Spectrophotometric assays and SDS-PAGE showed that the casein hydrolysis by Glyoxyl-CpCPs was similar to soluble CpCPs. In addition, Glyoxyl-CpCPs exhibited similar ratio of milk-clotting activity to proteolytic activity (1494.33) when compared to soluble CpCPs (1541.90) and a commercial chymosin (1359.08). Atomic force microscopy (AFM) showed that the process of casein micelle aggregation after treatment with Glyoxyl-CpCPs was very similar to its soluble form and a commercial chymosin. Finally, Glyoxyl-CpCPs performed well after five reaction cycles (78% of residual proteolytic activity) producing cheeses with yield (11 g of cheese to 100 mL of milk) similar to commercial chymosin (10 g/ 100 mL) and soluble CpCPs (9.8 g/ 100 mL). CONCLUSION: All results support the biotechnological potential of immobilized CpCPs as an alternative biocatalyst for cheesemaking with the advantage of drastically reducing costs through five cycles of reuse.

Keywords: Atomic force microscopy, Casein micelles, Glyoxyl-agarose / **Supported by:** CNPq, CAPES, FUNCAP.

F.53 - Photodynamic effect of the xanthene Rose Bengal on colorectal cancer-derived cells

André Satoshi Ferreira [1], Mirella Boaro Kobal[1], Lucas Gontijo Moreira[1], Sabrina Aléssio Camacho[1,2], Karina Alves de Toledo[3], Pedro Henrique Benites Aoki[1]

[1]Department of Biotechnology, São Paulo State University (São Paulo, Brazil), [2]São Carlos Institute of Physics, University of São Paulo (São Paulo, Brazil), [3]Department of Biological Sciences, São Paulo State University (São Paulo, Brazil)

INTRODUCTION: Colorectal cancer is the third with the highest occurrence and the second with the highest mortality. Traditional cancer treatments such as chemotherapy and radiotherapy cause severe side effects, demanding new therapeutic strategies. In this context, photodynamic therapy (PDT) stands out owing to its effectiveness in reducing tumor cell viability and as a non-invasive therapeutic modality. Rose Bengal (RB) is a promising PS of the xanthene family, presenting a higher quantum yield for $^1O_2$ generation. OBJECTIVES: Evaluate the efficiency of PDT mediated by the xanthene Rose Bengal on colorectal cancer cells (Caco-2). MATERIALS AND METHODS: The photodynamic efficiency of RB was evaluated at different concentrations (from 0.25 to 25×10^{-6} mol/L) and different incubation periods (0.5, 3 and 24 hours) in *In vitro* culture of cells derived from colorectal carcinoma (Caco-2). The cells were irradiated for 1 hour at 525 nm (32,3 mW/cm^2 of irradiance) and after 24 hours of treatment the cell viability was evaluated via MTT assays. DISCUSSION AND RESULTS: When irradiated, the minimum concentration of RB did not show a great impact on cell viability leading to reductions in cell viability of 13.91%, 18%, and 25% when incubated for 30 minutes, 3 hours, and 24 hours, respectively. On the other hand, the concentration of 5 μM of RB showed a greater effect causing a reduction of Caco-2 viability of 87.48%, 82.14%, and 88.15% for 30 minutes, 3 hours, and 24 hours, respectively. Finally, the maximum concentration of 25 μM of RB leads to a reduction of 89.8%, 89.45%, and 87.95% in the same periods. When not irradiated, the RB did not affect the cells viability indicating that the irradiation of the RB is responsible for the treatment effectiveness. CONCLUSION: The xanthene RB has demonstrated great potential and effectiveness as PS in the PDT of colorectal cancer, being able to significantly decrease the viability of Caco-2 cells.

Keywords: Caco-2, PDT, Xanthene / **Supported by:** FAPESP and CNPq

F.54 - Screening of antigenic salivary proteins from Rhipicephalus microplus by Phage Display technique

Gabriel Cerqueira Alves Costa [1], Verônica de Moraes Manzato[1], Itabajara da silva Vaz Junior[2,3], Ricardo José Soares Torquato[1], Aparecida Sadae Tanaka[1,3]

[1]Department of Biochemistry - Escola Paulista de Medicina, Universidade Federal de São Paulo (São Paulo, Brazil), [2]Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul (Rio Grande do Sul, Brazil), [3]Entomologia Molecular , Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular (Rio de Janeiro, Brazil)

INTRODUCTION: *Rhipicephalus microplus*, also known as cattle tick, is one of the most important tick species in Brazil, since intense infestations caused by this parasite can seriously harm livestock animals. Infested herd usually present reduced milk and meat production, leading to substantial economic losses. The control of tick infestations in animals is carried out mainly by using different chemical acaricides, which can result in selection of resistant tick populations and environmental contamination. Thus, alternative methods for tick control have been employed along the last decades. One of the most studied approaches has been anti-tick vaccines, which require the discovery of new immunogenic tick molecules to be used in the development of efficient formulations. OBJECTIVES: Then, the main objective of the present study is to identify immunogenic targets that could be useful in control of *R. microplus* using the phage display technique. MATERIALS AND METHODS: A phage display library was constructed from *R. microplus* salivary glands cDNA fragments and screened against IgG antibodies purified from the serum of a tick-susceptible cattle previously exposed to several infestations. After two biopannings, phages were isolated and had DNA extracted and sequenced in order to identify the tick antigenic peptides expressed on their surface. Relative mRNA levels from the screened targets in female salivary glands during blood feeding were accessed by qPCR. DISCUSSION AND RESULTS: The DNA sequencing revealed that the most frequent phages after the second biopanning expressed peptides correspondent to a Fukutin-like protein (52.5%) and a S18 ribosomal protein (20%). The gene expression levels from both targets were modulated along female engorgement. The perspectives will be the expression of the identified proteins and their evaluation as immunogenic targets. CONCLUSION: In the future, the selected salivary targets could be useful for the development of an effective vaccine against the *R. microplus*.

Keywords: Rhipicephalus microplus, Phage display, antigens /
Supported by: CAPES, CNPq, FAPESP and INCT-EM

F.55 - Antitumor effect of limonene and peraldehyde against murine melanoma cells

Jociele Gonçalves [1], Leonardo Machado Martins[1], Fernanda Fernandes Miranda da Cunha[1], Maria Carolina Mariano Cesar[1], Denise Costa Arruda[1]
[1]Núcleo Integrado de Biotecnologia, Universidade de Mogi das Cruzes (São Paulo, Brasil)

INTRODUCTION: Melanoma is the most aggressive form of skin cancer due to its high rate of metastasis. New therapies are being developed against this cancer, focusing in cell death and metastasis inhibition. Natural compounds are attractive therapies, and two terpenes, limonene and peraldehyde, showed promising results. OBJECTIVES: Determine the antitumor effect of limonene and peraldehyde in murine and human melanoma cells. MATERIALS AND METHODS: Trypan Blue assay was performed to evaluate the viability of B16F10-Nex2, SK-Mel-25 (murine and human melanomas, respectively), 3T3 (murine fibroblast), and Vero (epithelial-kidney) cells after treatment with limonene and peraldehyde. After that, a Trypan Blue assay with limonene and peraldehyde in presence of cell death (Z-VAD-FMK, 3-MA and Necrostatin) was applied. To confirm the cell death mechanism, fluorescence microscopies with DHE, TUNEL, and Propidium Iodide stained cells were performed. Statistical analysis were performed using Student's t-test. DISCUSSION AND RESULTS: Limonene and peraldehyde were cytotoxic for B16F10-Nex2 murine and SK-Mel-25 human melanoma cells after 24 hours, but did not affect the non-tumor cells lines 3T3 and Vero. The assays performed to evaluate and determine the cell death mechanism suggested that the compounds are inducing apoptosis and autophagy. CONCLUSION: Our results indicate that limonene and peraldehyde are inducing cell death through apoptosis and/or autophagy. Therefore, more studies are required to establish both compounds as novel therapies against melanoma.
Keywords: cell death, melanoma, terpenes / **Supported by:** FAPESP

F.56 - Envenomation resistance of snakes: a comparative study of the endogenous Phospholipases A2 Inhibitors content

Victor Koiti Kavazoi[2,1], **Caroline Serino Silva** [1], Caroline Fabri Bittencourt Rodrigues[1,2], Daniela Miki Hatakeyama[1,2], Kathleen Fernandes Grego[1], Karen de Morais-Zani[1], Anita Mitico Tanaka-Azevedo[1]
[1]Laboratório de Herpetologia, Instituto Butantan (São Paulo, Brasil), [2]Interunidades Em Biotecnologia, Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: Some snakes have natural resistance to envenomation, which could occur due to the presence of plasma proteins with inhibitory potential on certain venom toxins, such as metalloproteinases (SVMP) and phospholipases A2 (PLA2). These components, mainly phospholipase A2 inhibitors (PLIs), have attracted considerable attention on understanding the mechanisms of venom resistance, as well as a biotechnological resource to improve snakebite treatment. PLIs have been found in Elapidae, Viperidae, Colubridae, and Boidae snake families. Although numerous works have identified and characterized PLIs, there is a lack of comparative studies that could shed light on understanding the differences on PLIs content and assist the selection of potential targets to further inhibitory studies. OBJECTIVES: So, in this work, we compared the PLIs content of 19 snake species sera, maintained in the Laboratory of Herpetology at Instituto Butantan, Brazil. MATERIALS AND METHODS: The sera of *Bothrops pauloensis*, *B. leucurus*, *B. insularis*, *B. fonsecai*, *B. jararacussu*, *B. alternatus*, *B. atrox*, *B. marmoratus*, *B. erythromelas*, *B. mattogrossensis*, *B. marajoensis*, *B. neuwiedi*, *B. moojeni*, *B. jararaca*, *B. cotiara*, *Crotalus durissus terrificus*, *C. d. collilineatus*, *Naja kaouthia*, and *Boa constrictor* snakes were submitted to affinity chromatography and the purified PLIs were analyzed by SDS-PAGE, immunorecognition assay, and two-dimensional electrophoresis (2-DE). DISCUSSION AND RESULTS: In addition to the presence of PLIs in 11 species already described in the literature, we described for the first time the presence of PLIs in 8 snake species and our findings highlight the diversity of snakes with endogenous PLIs through the initial structural characterization and comparison among different PLIs. CONCLUSION: Thus, the discovery of new species containing PLIs in their serum may be the key to elucidate aspects of their structure and function, including how these proteins interact with PLA2 and other biological natural effects, which are still unknown. **Keywords:** Phospholipase A2, Phospholipase A2 inhibitors, Snake envenomation / **Supported by:** CAPES, FAPESP and CNPq

F.57 - Electrochemical Immunosensing Platform Based on Quantum Dots - Applications for Zika Detection

Jéssika Fernanda Ferreira Ribeiro [1], José Rodrigo[1], Caroline Santos[1], Claudilene Ribeiro[3], Paulo Euzebio[1], Goreti Pereira[1], Beate Santos[1], Giovannia Pereira[1], Daniela Santoro[5], Rogério Ribeiro[6], Adriana Fontes[1]
[1]Depto de Biofísica e Radiobiologia, [2]Departamento de Química Fundamental, Universidade Federal de Pernambuco (Pernambuco, Brasil), [3]Programa de Pós-Graduação em Biotecnologia, Rede Multidisciplinar de Pesquisa, Univ. Federal de Uberlândia (Minas Gerais, Brasil), [4]Departamento de Farmácia, Universidade Federal de Pernambuco (Pernambuco, Brasil), [5]Depto de Microbiologia, Imunologia e Parasitologia, Universidade Federal de São Paulo (São Paulo, Brasil), [6]Diretoria de Pesquisa e Inovação, Mint Consultoria em Materiais e Inovação Tecno, Diretoria de Pesquisa e Inovação, Mint Consultoria em Materiais e Inovação Tecnológica (Pernambuco, Brasil)

INTRODUCTION: Zika virus (ZIKV) infection has been declared a public health emergency due to its association with serious illness in newborns and adults. The detection of ZIKV and its associated biomolecules is still a challenge and has stimulated the search for complementary detection methods that can offer greater sensitivity, specificity, and practicality. The quantum dots (QDs) are semiconductor nanoparticles that present great potential to develop electrochemical biosensing platforms for Zika detection due to their excellent physicochemical properties. QDs have isotropic geometry and can enable efficient biomolecule immobilizations, in an orderly way, providing an increase in the electrode functional area and sensitive detections. OBJECTIVES: This study aimed to develop an electrochemical immunosensing platform based on screen-printed carbon electrodes (SPCEs), conductive polymer 2-(1H-pyrrol-1-yl)ethanamine (Pyam), and anti-EP ZIKV antibodies, for detection of protein E ZIKV (EP-ZIKV). MATERIALS AND METHODS: CdTe QDs were synthesized, characterized by optical and structural techniques, and covalently immobilized on the SPCE/PPyam surface. Then, anti-EP ZIKV was conjugated by covalent interaction with QDs, followed by detection of EP-ZIKV. The platform assembly was evaluated by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The detection was performed by differential pulse voltammetry (DPV). DISCUSSION AND RESULTS: The QDs were efficiently immobilized, and showed electrochemically stability, for at least 7 months. The anti-EP ZIKV antibodies were effectively immobilized on the PPyam/QDs surface, even after 2 months of electrode storage. The platform enabled the detection of EP-ZIKV with high sensitivity using minimal sample volumes (LOD = 0.1 ng mL-1). The system also showed specificity since it did not detect the EP of the dengue virus. CONCLUSION: The proposed combination favored the development of a sensitive and (electro)chemically stable platform for Zika detection in the viremic phase, which has the potential for transposition to other arboviruses.
Keywords: nanostructured platform, semiconductor nanoparticles, arboviruses

F.58 - Structural and Functional Validation of Chimera Defensins with Antimicrobial Potential

Ian Alejandro Fernandez Soliz [1]
[1]Pos-graduacao em Biotecnologia, Universidade Catolica Dom Bosco (Mato Grosso do Sul, Brasil), [2]Programa de Pós-Graduação em Genômica e Biotecnologia, Centro de Análises Bioquímica (CAPB), Programa de Pós-Graduação em Genômica e Biotecnologia da Universidade Católica de Brasília (DF, Brasil), [3] Montpellier SupAgro, 3BPMP, Univ Montpellier, CNRS, INRAE, Montpellier SupAgro, Montpellier, France (Montpellier, France)
INTRODUCTION: Introduction: Defensins are cationic peptides that belong to the antimicrobial peptides (AMPs) family. AMPs can be found in many living organisms, to the role of defense. In plants, AMPs display multifunctional potential in biotic and abiotic stress control. OBJECTIVES: Objectives: This work aim is to evaluate the multifunctionality of chimeric defensin (ChD1), obtained by in silico modeling from a previous study in our lab. MATERIALS AND METHODS: Methods and materials: The ChD1 gene was inserted into the vector pYX212 and was introduced into *Saccharomyces cerevisiae* genome by electroporation. The yeast carrying empty vector was the negative control. A PCR was performed with plasmid extracted, followed by a sequencing analysis. Clones expressing ChD1 gene were used to zinc tolerance assay. RNA extraction will be performed from clones, and cDNA production to detected transcript. Protein extraction will be performed and evaluated by SDS-PAGE and gel bands (< 10 kDa) will be cut for peptide extraction to evaluation by Matrix-assisted laser desorption/ionization (MALDI). Extracted proteins will be analyzed by High-Performance Liquid Chromatography (HPLC) to ChD1 protein isolation. Multifunctionality assay (antibacterial, and inhibition of insect digestive enzymes) will be performed ChD1 protein. DISCUSSION AND RESULTS: Results: The agarose gel 1% image showed the amplification of the interest gene by PCR using specific primers. The sequencing of PCR product, also with specific primers, confirmed the insertion of ChD1 gene into yeast. The zinc bioassay results show that yeast, producing Ch1D, keep the zinc tolerance ability, when compared with control. CONCLUSION: Conclusions: These results contribute to studies against abiotic stress caused by heavy metal, and suggest that ChD1 is a potential candidate to transgenic crops production, with metal tolerant characteristic. This characteristic is important to agronomy sector. The heterologous ChD1 production, will be possible verify the effect of the changes in the primary sequence and to study the functional promiscuity of AMPs.
Keywords: Antimicrobial peptides, Plant defensins, Chimera / **Supported by:** Fundect

F.59 - Sericin Biofilm for Treatment of Burns in a Rat Experimental Model

Edicleia de Freitas[1], Taciane Stein da Silva Leal[1], Ana Paula Sone[2], Marcelino Luiz Gimenes[3], Lucinéia de Fátima Chasko Ribeiro[1], Gladson Ricardo Flor Bertollini[1], **Maria Aparecida Fernandez [1]**, Rose Meire Costa[1]
[1]Centro de Ciências Biológicas e da Saúde, Universidade do Oeste do Paraná (Paraná, Brasil), [2]Depto de Engenharia Química, Universidade do Oeste do Paraná (Paraná, Brasil), [3]Departmento de Engenharia Química, Universidade Estadual de Maringá (Paraná, Brasil), [4]Departmento de Biotecnologia, Genética e Biologia Celular, Univ. Estadual de Maringá (Paraná, Brasil)

Introduction: Burn is a traumatic wound caused by external agents, and that can destroy from the skin to deeper tissues. Depending on the severity of the wound, it compromises the functional integrity of the organ, making it necessary to search for effective treatments. Scientific studies point to the potential use of the silk biopolymer sericin, extracted from the *Bombyx mori* cocoon, Objectives: We aimed to analyze the healing properties of a sericin biofilm endowed with silver sulfadiazine in the treatment of wounds caused by deep second-degree burns. Materials and Methods: The experimental study was approved by the Unioeste Animal Use Ethics Committee and was carried out with 40 male Wistar rats, aged 12 weeks, which were divided into four groups: treatment of skin burns injuries with sericin biofilm endowed with silver sulfadiazine; Aquacel Ag® curative and untreated. After 13 days the animals were euthanized, the skin segment being removed in the interscapular region, and histological microscopic evaluations. Results and Discussion: In the animals of the skin injury by burn and treated with sericin biofilm, there was no complete re-epithelialization of the epidermis, remaining areas without epithelial lining and with fibrinoleukocyte crust, inflammatory response and organizational disarrangement in the epidermis strata. Hyperemia was visualized in both the papillary and reticular dermis. In these regions a large amount of inflammatory infiltrate was evident. Similar results were obtained in the Aquacel Ag® treated group. In the untreated group, the pathological alterations in the skin were more accentuated, with no indication of recovery of the strata in the epidermis. Conclusions: Thus, the morphological data revealed that the sericin biofilm had a slight influence on the skin healing processes, being necessary to increase the time of the experiment in order to better evaluate the effect of sericin biofilm in the healing of burn wounds.
Keywords: experimental burn wound, wound healing, silk sericin.
Supported by: European Union (Proyecto SEDA, LA/2016/378-553)

F.60 - Fluorescent Magnesium(II) Complex Containing Hesperidin Free And Immobilized In Polymeric Films

Vitória Cristina Peres do Prado [1], Rose Maria Carlos[1], Rafael Cavalieri Marchi[1]
[1]Departamento de Química, Universidade Federal de São Carlos (São Paulo (SP), Brasil)

INTRODUCTION: The use of chemical compounds as pesticides and in the preservation of perishable foods is limited by toxicity as they can cause risks to human health. However, these compounds are still in use because there have no competitive alternatives in terms of efficiency, viability and fewer risks. Thus, there is a great interest in the development of new sustainable technologies to contribute to a sustainable future. OBJECTIVES: Develop a new compost that is technologically sustainable. MATERIALS AND METHODS: $Mg(hesp)_2$ was obtained by the reaction of hesperidin with $MgCl_2$ in MeOH under reflux for 2h. Thermoplastic films were prepared by casting methodology. Fluorescence microscopic images on a Zeiss-Axio Observer7 inverted microscope. DISCUSSION AND RESULTS: The $Mg(hesp)_2$ investigated in this work is a nontoxic and low-cost compound, easy to prepare with high yield and soluble in aqueous solution (pH 2-12), presenting the necessary requirements for application in food and agriculture areas. The Mg(II) ion is an essential element for life (dose:300mg/day) and the hesperidin ligand (hesp) play beneficial role on health. $Mg(hesp)_2$ is stable in the solid state, in aqueous medium, at different pH and immobilized on starch and gelatin films. It presents high antioxidant activity (IC50=12,5 $mol.L^{-1}$) superior to the commercial preservative BHT (IC50=25,6 $mol.L^{-1}$). The films containing the complex were homogeneous showing a controlled release in water (3h - 10% release). The complex presents intense absorption (ε_{370}= 4300 ± 190 $mol^{-1}.L.cm^{-1}$), emission (490 nm) and short fluorescence lifetime. The fluorescence of the complex allows mapping the growth and quality of plants. For example, the addition of $Mg(hesp)_2$ in a bean plantation, on a laboratory scale, showed an increase in rooting, stem and number of leaves, the distribution throughout the plant detected by its fluorescence. CONCLUSION: $Mg(hesp)_2$ complex is a safe and low-cost potential candidate to fluorescent sensor for real-time plant growth and health as well as in food packaging.
Keywords: Fluorescent , Complex , Magnesium(II)
Supported by: FAPESP (Processo 2021/05433-9), CNPq, CAPES

F.61 - Antimicrobial Activity of Lys-substituted and Ala- substituted analogues of the Balteatide

Katielle Albuquerque Freire [1], Katielle A Freire[1], Katielle Freire[1]
[1]Centro de ciências naturais e humanas, Universidade federal do ABC (São Paulo, Brasil), [2]Departments of Bioengineering and Chemical and Biomolecular Engineering, University of Pennsylvania (Pennsylvania, USA)

INTRODUCTION: Multidrug-resistant bacteria represent the an increasingly worrying health problem, even more so with the low effectiveness of our existing antibiotics. Therefore, it's essencial to identify new effective antimicrobial alternatives. Animal venoms represent an underexplored source of potential antibiotics. Here, we designed a peptide (Bateatide-NH2) derived from the secretion of frog skin, of the species Phyllomedusa baltea, consisting of 10 amino acid residues, which has application as an antimicrobial against bacteria and fung. OBJECTIVES: Synthesize new lysine-substituted and alanine-substituted peptide analogs. With this, evaluate the influence of net charge and side chains. MATERIALS AND METHODS: Peptides were synthesized by solid-phase, using Fmoc-strategy, purified by HPLC, characterized by mass spectrometry and tested against bacteria. Biological tests performed: antibacterial activity assays against group ESKAPE, membrane permeabilization assays, membrane depolarization assays, skin abrasion infection mouse model (*In vivo*). DISCUSSION AND RESULTS: Lys-substituted analogues showed an increase in antimicrobial action against bacteria, the most effective substitution observed was in [K]1-Balteatide-NH2, which showed moderate activity against the strains of the group ESKAPE, as well as the ability to reach the outer membrane and the cytoplasmic membrane of Pseudomanas aeruginosa. And Balteatide-NH2 and [K]1-Balteatide-NH2 also demonstrated considerable *In vivo* anti-infective activity. CONCLUSION: The analogues obtained with the charge increase more active and broader antimicrobials action. The results pointed new characteristics of this family of cationic antimicrobial peptides - derived from frog, amplifying the possibility of these compounds becoming promising drugs for the treatment of infections resistant to antibiotics.

Keywords: peptides, antimicrobial, balteatide

F.62 - Characterization of a bifunctional β-xylosidase from Geobacillus thermodenitrificans

Vandierly Sampaio de Melo[1], Brisa Moreira Gomes[1], Felipe Santiago Chambergo Alcalde[1]
[1]Escola de Artes, Ciências e Humanidades, Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: Due to the growing demand for energy and environmental problems caused by the consumption of fossil fuels, the need to produce renewable fuels has increased. Second generation ethanol (2G) has emerged as a promising alternative to fossil fuels due to the use of plant biomass as feedstock. Hemicellulose is the second most abundant polysaccharide in plant biomass, with xylan being the main constituent. In this sense, for the total degradation of xylan and obtaining fermentable sugars, the participation of several enzymes that act synergistically, especially β-xylosidases, is necessary. OBJECTIVES: To express and characterize the recombinant enzyme β-xylosidase from Geobacillus thermodenitrificans in E. coli. MATERIALS AND METHODS: The putative enzyme gene β-xylosidase (GeoXIL) was obtained from analyzes of the CAZy database and later synthesized and cloned for the expression of the recombinant protein in E. coli BL21. Bioinformatics analyzes were performed on the GeoXIL amino acid sequence. The enzyme was purified by affinity chromatography on a nickel-sepharose column and biochemical characterization and thermostability analysis by circular dichroism were performed. DISCUSSION AND RESULTS: The enzyme GeoXIL was grouped within the family of glycosyl hydrolases 43 (GH43). From the activity tests, it was noted that GeoXIL has greater activity at pH 5.0 and temperature of 60 °C. In tests with metal ions and EDTA, the enzyme remained stable. GeoXIL showed bifunctional activity of β-xylosidase and α-L-arabinofuranosidase, with higher activity on the substrate p-nitrophenyl-β-D-xylopyranoside. The specific activity on p-nitrophenyl-β-D-xylopyranoside was 18.33 U mg-1 and catalytic efficiency of 20.21 mM-1 s-1; comparable to other β-xylosidases. CONCLUSION: Activity at high temperatures, thermostability and stability to metal ions are desirable characteristics for industrial enzymes. In this sense, the GeoXIL enzyme presents interesting biochemical characteristics for application in the enzymatic hydrolysis of plant biomass.

Keywords: β-xylosidases, GH43, second generation etanol / **Supported by:** CAPES

F.63 - Evaluation of the Physicochemical Characteristics and Stability of the Iturin with Industrial Interest Obtained by Biotechnological Route

Diego Fernandes Livio [1], Hiure Gomes Ramos Meira[1], Maria Auxiliadora Oliveira[1], Daniel Bonoto Gonçalves[2], Adriano Guimarães Parreira[1], José Antônio da Silva[1], Ronaldo Adriano Ribeiro[3], Paulo Afonso Granjeiro[1]
[1]Laboratory of Biotechnological Processes and Purification of Macromolecules, Federal University of São João del-Rei (Minas Gerais, Brazil), [2]Department of Biosystems Engineering, Federal University of São João del-Rei (Minas Gerais, Brazil), [3]Pesquisa e desenvolvimento, Distribuidora Amaral Ltda (Minas Gerais, Brazil)

INTRODUCTION: Iturin is a biosurfactant produced from microorganism, an amphiphilic molecule with multiple applications in different industrial sectors. It is considered a green molecule and has biodegradability. OBJECTIVES: Evaluate the physicochemical characteristics and stability of the iturin obtained by microrganism. MATERIALS AND METHODS: The CMC was determined by the intersection of the extrapolated lines from the TS of the iturin, at 25 ˚ C, and their respective aqueous solutions (0.0005 to 3.5 mg.mL $^{-1}$). The surface tension of aqueous solutions of the irutin (1.0 and 0.1 mg.mL $^{-1}$) was analyzed under different conditions: pH 2, 4, 6, 8, 12 and 13, after 30 minutes at 25 ˚C ; Submitted to 100 ˚ C for periods of 20, 40, 60, 100 and 140 min, after cooling to room temperature; and at saline concentrations of 3.5; 10; 15 and 20% (w/v). The emulsification index (IE24) of iturin (1 mg.mL $^{-1}$) in oils (1:1) homogenized for 1 min (25 ˚ C), after 24 h, between the emulsified height (EC) and total (AT). DISCUSSION AND RESULTS: The iturin reduced the TS from 73.1 ± 0.1 to 39.5 ± 0.05 mN.m $^{-1}$ and the CMC was 0.083 mg.mL $^{-1}$. At pH 8, the iturin solutions (1.0 or 0.1 mg.mL $^{-1}$) showed a lower TS (35 ± 0.1 and 37.9 ± 1 mN.m $^{-1}$), respectively, despite demonstrated stability in large range of pH (2-12). In general, the BS solutions (1.0 or 0.1 mg.mL $^{-1}$) did not show significant changes in surface tension when exposed to a temperature of 100 °C (35.5 ± 0.1 and 39.9 ± 0.7 mN.m $^{-1}$), respectively, as well as at different saline concentrations (35 ± 0.5 mN.m $^{-1}$ and 42 ± 0.2 mN.m $^{-1}$). Motor oil, with the highest carbon chain, had the best IE24 (85%). CONCLUSION: Iturin demonstrated great performance as surfactant and high stability in pH, temperature and saline concentration to industrial application.

Keywords: iturin, CMC, stability
Supported by: CNPq

F.64 - Epirubicin Carrier Cubosomes Functionalized With Biopolymers: Development And *In vivo* Studies In Zebrafish

Bárbara Vilela Pimenta [1], Rafael Ricardo Madrid Martinez[1], Patrick Mathews Delgado[1], Karin do Amaral Riske[1], Omar Mertins[1]
[1]Departamento de Biofísica, Escola Paulista de Medicina - Universidade Federal de São Paulo (São Paulo, Brasil)

INTRODUCTION: The production of nanostructured systems as drug carriers are very important, especially in administration of anticancer drugs, since they can perform controlled release of bioactives through different mechanisms. In this proposal, nano cubosomes functionalized with biopolymers are being developed as a material to encapsulate and carry epirubicin for future applications via oral administration. OBJECTIVES: Development of pH-responsive biopolymer-cubosomes with optimized structural characteristics for oral administration, favoring intestinal mucus adhesion and controlled release of epirubicin. MATERIALS AND METHODS: Monoolein as lipid and chitosan-N-arginine and alginate as biopolymers were used.1 The production and characterization of the functionalized cubosome were studied by physicochemical techniques, such as isothermal titration calorimetry, differential scanning calorimetry and dynamic light scattering. Next, encapsulation and release studies will be carried out, as well as the interaction between cubosomes and bovine serum albumin. Then, *In vivo* tests will be performed in zebrafish, by oral administration. DISCUSSION AND RESULTS: The addition of biopolymers to the cubosomes changed the zeta potential of the particles in two studied pHs (2.5 and 7.4). The size of the nanocubosomes changed after the incorporation of the biopolymers, especially at pH 7.4. The interaction between the cubosomes with albumin unveiled that the biopolymers shell provides significant increase in the transition temperature of albumin. CONCLUSION: The physicochemical analysis evidenced a pH-responsive effect in the cubosomes modified with the biopolymers, which leads to changes in surface charge and structural modifications. The interaction of the cubosomes with albumin was provided in pH 2.5 and 7.4 thanks to the inherent charge profile of the biopolymers.

Keywords: cubosomes, epirubicin, zebrafish / **Supported by:** Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP - Processo 2021/00971-2)

F.65 - Mesenchymal stem cells in an *In vitro* three-dimensional human skin model

Jeniffer Farias dos Santos [1], Gustavo Roncoli Reigado[1], Bruna Letícia Freitas[1], Silvya Stuchi Maria Engler[2], Felipe Santiago Chambergo[1], Viviane Abreu Nunes[1]
[1]Biotechnology, School of Arts, Sciences and Humanities, University of Sao Paulo (SP, Brazil), [2]Department of Clinical Analysis, Faculty of Pharmaceutical Sciences, University of Sao Paulo (SP, Brazil)

INTRODUCTION: The epidermis, the most superficial layer of the skin, is composed of keratinocytes, which proliferate in the basal layer and differentiate while express stratum-specific proteins such as cytokeratin (CK) 5 in the basal layer, CK10 in the suprabasal layers, involucrin, filaggrin and tissue kallikreins (KLK) in the more superficial layers. In case of skin traumas, epidermal homeostasis is affected, requiring clinical approaches to restore it. Tissue engineering is an important strategy for skin regeneration, and the use of mesenchymal stem cells (MSC) from the umbilical cord is interesting in cell therapy, due to its renewal and differentiation potential. OBJECTIVES: To evaluate the transdifferentiation potential of umbilical cord MSC into keratinocytes in an *In vitro* three-dimensional (3D) skin model, using type I collagen and dermal fibroblasts as dermal equivalent. MATERIALS AND METHODS: The expression of epidermal proteins, such as CK5, CK10, involucrin and filaggrin was investigated by immunofluorescence and real-time PCR, in addition to the activity of KLK in the hydrolysis of fluorogenic substrates. Basal keratinocytes were used as controls. DISCUSSION AND RESULTS: The results showed a not-typically stratified epithelium, being verified the expression of the markers CK5, CK10, involucrin and filaggrin, as well as increase KLK activity in the MSC-constructed epidermal equivalents, compared to MSC grown in proliferation medium, indicating the commitment to epidermis transdifferentiation of these cells. CONCLUSION: As it presents important structural proteins for epidermal formation and homeostasis, the model presented may be useful to investigate the epidermal transdifferentiation of MSC, as well as its potential applicability in regenerative medicine.

Keywords: mesenchymal stem cells (MSC), 3D skin model, dermal equivalente / **Supported by:** CNPq, CAPES, FAPESP

F.66 - Ultrastructural Analysis and Viability of Biofilm Formed by Oral Streptococci Under the Action of N-acetylcysteine

Gisele da Silva Sarkis [1], Ana Angélica Macedo[2], Fernando Mendes[3], Daniel Saito[1], Cristiane Pereira Borges Saito[1]
[1]Programa Multicêntrico de Pós-Graduação em Bioquímica e Biologia Molecular, Universidade do Estado do Amazonas (Amazonas, Brasil), [2]Laboratório de Pesquisa (campus Imperatriz), Instituto Federal do Maranhão (Maranhão, Brasil), [3]Escola Superior de Tecnologia da Saúde de Coimbra, Instituto Politécnico de Coimbra (, Portugal)

INTRODUCTION: Biofilms are microbial aggregates pervaded by extracellular polymeric substance (EPS), presenting increased cell stability, mechanical protection and resistance to disinfecting agents. N-acetylcysteine (NAC) has been proposed for control of bacterial biofilm in human infections, as it disrupts the biofilm structure and exposes microorganisms to antimicrobials. OBJECTIVES: The aim of this study was to evaluate the influence of NAC on biofilm structure/ultrastructure formed by oral streptococci species (*Stretococcus gordonii*, *S. mitis*, *S. mutans*, *S. oralis*, *S. salivarius*, *S. sanguinis*). MATERIALS AND METHODS: To this end, biofilm formation was induced on hydroxyapatite (HA) discs and circular coverslips incubated in biofilm medium (BM). A total of 84 samples was analyzed, with the treatment group (NAC) compared against sodium phosphate (negative) and chlorhexidine (positive) controls groups. The susceptibility of biofilm to NAC at different concentrations (0.78 to 25 mg/mL) was evaluated by crystal violet colorimetric assay, and biofilm ultrastructure analyzed by SEM and CM using SYTO 9 at an excitation wavelength of 488 nm. After statistical analysis (one-way ANOVA and Dunnett's test), NAC at concentrations of 6.25 and 12.5 mg/mL were selected in view of the evident divergence in the inhibition effect. DISCUSSION AND RESULTS: Although similar studies have shown the efficiency of NAC at 0.78 mg/mL against *S. mutans* biofilm, our results showed that NAC's minimum inhibitory concentration (MIC) for all species was 12.5 mg/mL, with the exception of S. oralis, whose MIC was 25.0 mg/mL. However, microscopy analysis showed that NAC at 12.5 mg/mL was as effective as chlorhexidine at 0.12% for this species. Moreover, NAC at 3.12 mg/mL appeared to stimulate biofilm growth (data not shown). CONCLUSION: Based on the results, it can be concluded that NAC may inhibit the formation of oral streptococci biofilm at concentrations of 12.5 and 25 mg/mL in a species-specific manner. The results obtained should be further validated by *in situ* intraoral research.

Keywords: scanning electron microscopy, confocal microscopy, oral biofilm / **Supported by:** FAPEAM

F.67 - A Nontoxic Strontium Nanoparticle that Holds the Potential to Act Upon Bone Modelling Cells

Larwsk Hayann G. da Silva[1], Vitor Freire Rocha[3], Raphael Martini Vicente[4], Marina Ferreira Cândido[2], Edgard Eduard Engel[3], Pietro Ciancaglini[1], Ana Paula Ramos[1]
[1]Chemistry, Faculty of Philosophy, Sciences and Letters at Ribeirão Preto (, Brasil), [2]Molecular and Cell Biology, Faculty of Medicine at Ribeirão Preto (, Brasil), [3]Department of Biomechanics, Medicine and Rehabilitation of the Locomotor System, Faculty of Medicine at Ribeirão Preto (, Brasil), [4]Department of Orthopedics and Anesthesiology, Faculty of Medicine at Ribeirão Preto - USP (, Brasil)

INTRODUCTION: Homeostasis is essential for maintaining the integrity of the bone tissue, providing mechanical support and protection to soft tissues and regulates the delivery and storage of electrolytes such as calcium and phosphate. Pathology-associated causalities like estrogen deficiency, long-term immobilized bone, and/or aging are commonly related to the loss of bone mass, thus increasing the chances of fractures. The filling of bone lesions is currently performed with the use of biomaterials. Due to their inertness and lack of bioactivity many biomaterials fail within 10-25 years. One could overcome this problem by adding bioactive nanoparticles (NPs) that mimics the structure of bone-apatite. Thus, we substituted Ca2+ by Sr2+ ions in the HA lattice. Strontium ions are well known for their ability to control osteoblasts' activity and affect matrix mineralization OBJECTIVES: To test the potential of the nanoparticles containing strontium in regulating osteoblast differentiation and matrix secretion *In vitro* MATERIALS AND METHODS: MC3T3-E1 cell line was used to archive *In vitro* mineralization. Cell viability was assessed by MTT assay after 7, 14, and 21 days. For the analysis of matrix mineralization, after 21 days of culture, Alizarin Red S staining was applied. Furthermore, TNAP activity was accomplished by degradation of p-nitrophenylphosphate (pNPP), and its subproduct was analyzed. Finally, to determine the NPs capacity in activating osteoblastic-related genes we analyzed the mRNA by qRT-PCR DISCUSSION AND RESULTS: We showed herein that the NPs in which 90 % of Ca2+ ions were substituted by Sr2+ (NanoSr 90%) upregulated TNAP activity and increased matrix mineralization deposition. Moreover, NanoSr 90% positively increased *In vitro* the mRNA levels of Runx2, a marker of osteoblast differentiation. Yet, no cytotoxic effect was observed CONCLUSION: Herein, we prepared Sr-substituted apatite as potential platforms to increase the bioactivity of biomaterials. We showed that the presence of Sr2+ in osteoblasts culture administrated from NPs increased the formation of mineral nodules

Keywords: Biomaterials, Strontium-substituted Nanoparticles, Biological mineralization

F.68 - Evaluation of Exclusive CDSs as Potential Antifreeze Proteins from the Antarctic Yeast *Metschnikowia australis*

Jessica Caroline Duarte [1], Heron Oliveira Hilário[2], Frederico Gabriel de Carvalho Oliveira[1], Andrea Mara Macedo[1], Carlos Renato Machado[1], Erich Birelli Tahara[1], Glória Regina Franco[1]
[1]Bioquímica e Imunologia, Universidade Federal de Minas Gerais (Minas Gerais, Brasil), [2]Pós Graduação em Biologia de Vertebrados, Pontifícia Universidade Católica de Minas Gerais (Minas Gerais, Brasil)

INTRODUCTION: The harsh Antarctic environment led organisms to evolve interest features for their survival on the continent. One organism isolated from the Antarctic Seawater is the yeast *Metschnikowia australis*. Although the genus is present in different habitats all over the globe, *M. australis* is the only *Metschnikowia* species currently isolated from the Antarctic continent. OBJECTIVES: To evaluate the ability of *M. australis* to produce antifreeze proteins (AFPs), a type of protein able to modulate ice growth and protect cells from freezing. MATERIALS AND METHODS: Our group sequenced and analyzed the *M. australis* genome and compared it with 35 genomes from other *Metschnikowia* species. We found 16 exclusive coding sequences (CDSs) classified as potential AFPs, which had their expression analyzed in this work. We first grew *M. australis* in temperatures of 4°C, 12°C, 20°C, and 28°C, then obtained their RNA and compared the expression of these 16 CDSs through conventional PCR and qPCR. DISCUSSION AND RESULTS: *M. australis* presented a better growth at lower temperatures and no growth at 28°C. From PCRs results, we found one CDS with higher expression when the yeast was cultivated at 4°C, low expression at 12°C, and no expression at 20°C. The other 15 CDSs were amplified from all three temperatures analyzed, but the expression was higher at 12°C and 20°C. RT-qPCR, results showed the same expression pattern: one CDS with higher expression at 4°C (around a 100-fold increase when compared to 12°C or 20°C) and 15 CDSs with expression around two times higher at 12°C and 20°C than at 4°C. CONCLUSION: Our results indicate this CDS could represent a potential AFP, and further studies are in progress to elucidate its involvement in *M. australis* protection against freezing.

Keywords: Antifreeze protein, Antarctic yeast, *Metschnikowia australis* / **Supported by:** CAPES, FAPEMIG e CNPq

F.69 - Cytotoxic Effects of Chitosan Nanoparticles Containing S-Nitrosoglutathione in Triple-Negative Breast Cancer Cells

Camila de Almeida Salvego [1], Fernanda Viana Cabral[1], Amedea Barozzi Seabra[2], Bianca de Melo Santana[2], Martha Simões Ribeiro[1]
[1]Centro de Lasers e Aplicações, Instituto de Pesquisas Energéticas e Nucleares (São Paulo, Brasil), [2]Centro de Ciências Naturais e Humanas, Universidade Federal do ABC (São Paulo, Brasil)

INTRODUCTION: Breast cancer is the most common type of cancer affecting women worldwide. Among the treatments, radiation therapy (RT) is frequently chosen as a primary strategy; however, it demands high doses of ionizing radiation to achieve a curative dose. To enhance RT effectiveness, an external agent can be used to sensitize cells before the treatment, allowing a dose reduction. Nitric oxide (NO) is an essential molecule linked to several organic processes, besides being described as a potential radiosensitizer of tumor cells by different mechanisms, including oxidative stress. However, NO have a short half-life in biological conditions, making it difficult to achieve anticancer effects. To overcome this, NO donors can be encapsulated into polymer-based nanoparticles, ensuring a sustained NO releasing. OBJECTIVES: To evaluate the cytotoxicity induced by chitosan nanoparticles containing S-nitrosoglutathione (GSNO-CS NPs) in 4T1 cells (murine triple-negative breast cancer). MATERIALS AND METHODS: Cells were cultivated, seeded in 96-well plates (2 x 10^4 cells/well), and incubated at 37ºC with 5% of CO_2 for 24 h. Both CS NPs and CS NPs containing GSNO encapsulated were added to the plates at different concentrations (0-2.4 mg/ml CS NPs, 0-6 mM GSNO) and incubated for 24 h. Cytotoxic effects were evaluated through Resazurin fluorometric assay in both groups. DISCUSSION AND RESULTS: Our results showed a 65% reduction in cell viability for GSNO-CS NPs groups treated at 6 mM, while only 30% of cells were killed when treated by CS NPs group. CONCLUSION: Our data suggest that GSNO-CS NPs were able to promote cytotoxicity effects, thus inducing oxidative stress in triple-negative breast cancer cells. Next steps involve the use of these nanoparticles before RT to evaluate its radiosensitizer effect.

Keywords: 4T1 cells, nitric oxide, radiosensitization
Supported by: CNPq e CNEN

F.70 - Potential of Trichoderma spp. for the Promotion of Plant Growth and Biological Control of Soybean Phytopathogens

Samanta Monção Silva [1], Rodrigo Mattos Silva Galeano[1], Clarice Rossato Marchett[1], Douglas Chodi Masui[1], Giovana Cristina Giannesi[1], Fabiana Fonseca Zanoelo[1]
[1]INBIO, Universidade Federal de Mato Grosso do Sul (Mato Grosso do Sul, Brasil)

INTRODUCTION: The use of *Trichoderma* for the biological control of soybean diseases such as charcoal root rot (*Macrophomina phaseolina*) and white mold (*Sclerotinia sclerotiorum*) is the most sustainable alternative when compared to the use of pesticides. These fungi produce enzymes that degrade the cell wall of phytopathogens, promote plant growth through the production of phytohormones, such as indole-3-acetic acid (IAA), and phosphorus solubilization. OBJECTIVES: To evaluate the potential of two strains of *Trichoderma* sp. (T15 and T24) as biological control agents of *M. phaseolina* and *S. sclerotiorum*, and the characteristics for promoting plant growth. MATERIALS AND METHODS: The antagonistic activity was determined by dual culture technique for 7 days. The production of IAA was determined in liquid medium at 30 °C, recovered aliquots every 24h for 7 days. The solubilization of phosphorus was performed in a liquid medium at 30°C after 7 days. *Trichoderma* isolates (15 and T24) were inoculated in *Trichoderma* Liquid Enzyme medium (TLE) containing 0.5% of the cell wall of the phytopathogens (*M. phaseolina* and S. sclerotiorum) and the enzymes NAGase, phosphatase, protease, β-1,3-glucanase, β-glucosidase and chitinase were evaluated in the period of 24h, 96h and 144h. DISCUSSION AND RESULTS: The fungi *Trichoderma* sp. (T15 e T24) showed inhibition against the phytopathogens *M. phaseolina* e *S. sclerotiorum*. *Trichoderma* sp. (T24) obtained higher IAA production (74.21 µg mL^{-1}) and phosphorus solubilization (263.9 µg mL^{-1}) after 72 h and 168 h, respectively. In the presence of cell wall of *S. sclerotiorum*, *Trichoderma* sp. T24 produced NAGase (12,5 U/mL) and protease (12,6 U/mL) in 144h, and *Trichoderma* sp T15 produced phosphatase (2,44 U/mL) and β-1,3 glucanase (10,5 U/mL) in 48 hours. Both strains did not produce β-glucosidase and chitinase. CONCLUSION: The *Trichoderma* sp (T15 and T24) demonstrated biotechnological potential by producing compounds and enzymes that help plant growth and the biocontrol of phytopathogenic fungi.

Keywords: Trichoderma, phytopathogenic fungus, biological control / **Supported by:** CNPq

F.71 - PROJECT_Green Propolis: Bioactive Compounds, and Antioxidant, Antimicrobial, Cytotoxic and Neuroprotective Potential

Tiago Silva Borges[1,2], Patricia Campos Santos [2], Maria Cecília O. dos S. Silva[2], Ana Beatriz Moreas Bastos[2], Marta Bruno Loureiro[2], Daniele Takahashi Bernal[2], Lourdes Cardoso de Souza Neta[4], Renato Delmondez de Castro[2], Ravena Pereira Nascimento[1,5], Silvia Lima Costa[5], Victor Diogenes de Souza[1,5], Paulo Roberto Ribeiro de Jesus[1,2,3], Luzimar Gonzaga Fernandez[1,2,3]

[1]Departamento de Bioquímica E Biofísica, Programa Multicêntrico De Pós-Graduação Em Bioquímica E Biologia Molecular, [2]laboratório de Bioquímica, Biotecnologia E Bioprodutos (Lbbb/Ics/Ufba), [3]metabolomics Research Group, Instituto de Química, Universidade Federal Da Bahia (Bahia, Brasil), [4]fitoquímica E Bioprospecção de Plantas, Universidade do Estado da Bahia (Bahia, Brasil), [5]laboratório de Neuroquímica e Biologia Celular (Labnq), Universidade Federal Da Bahia (Bahia, Brasil)

INTRODUCTION: Different types of propolis are naturals in different regions of Brazil. It contains complex chemical components, mainly flavonoids, and polyphenols, varying in geographic location, plant species, and season of the year in which they are produced. Green Propolis is known for its color; and it is produced by *Apis mellifera* bees that use *Baccharis dracunculifolia*, a specie found in the Brazilian Cerrado, as the primary plant source. OBJECTIVES: The objective of this work is to use a multidisciplinary, integrated, and bio-guided approach to evaluate Brazilian green propolis for antioxidant, antimicrobial, cytotoxic, and neuroprotective potential, correlating them with the metabolomic profile of the ethanolic extract and fraction in ethyl acetate. MATERIALS AND METHODS: The crude ethanol extract will be obtained by maceration and the ethyl acetate fraction will be prepared from it. The metabolic profile will be evaluated by high-performance gas and liquid chromatography coupled with mass spectrometry. The antioxidant activity will be performed using 2,2'-azinobis(3-ethylbenzothiaziline-6-sulfonate) (ABTS) and ferric reducing antioxidant power (FRAP) methods. Flavonoid analysis will be determined by spectrophotometry using quercetin standard. Antimicrobial activity against ATCC and multidrug-resistant bacteria will be evaluated using broth microdilution. Cytotoxic antitumor activity will be performed using a colorimetric method, and neuroprotective capacity using PC12 and spinal cord cells from Wistar rats and immunocytochemical analyses. DISCUSSION AND RESULTS: The Brazilian green propolis is a promising source of antioxidants, has a variety of active principles responsible for the various pharmacological properties of its extracts, and further studies are needed to evaluate these properties, characterize their metabolites, and identify metabolites with potential for applications that justify its use by traditional medicine, and biotechnological potential. CONCLUSION: It is expected that this project to contribute to a better characterization of green propolis in terms of active principles, *In vitro* biopharmacological properties, and biotechnological potential,which isimportant for future projects aimed at the production of phytopharmaceuticals.

Keywords: Flavonoids, Metabolomic profile, Traditional medicine

F.72 - PROJECT: Nanostructured Compositions of Benzalkonium Chloride Complexed with Cyclodextrins: Synthesis, Physicochemical Characterization and Bactericidal Evaluation in Staphylococcus aureus

TIAGO MARCEL OLIVEIRA[1,2], Ângelo Márcio Leite Denadai[1]

[1]Laboratório de Fisico-Quimica & Fluidos do Instituto de Ciências da Vida, Universidade Federal de Juiz De Fora (Minas Gerais, Brasil), [2]Química, Faculdade Unica de Ipatinga-Mg (Minas Gerais, Brasil)

INTRODUCTION: Although antibiotics are indispensable drugs, their potency is threatened by bacterial resistance.Thus, there is a need for alternatives to the use of antibiotics in situations where they are irreplaceable, looking for new formulations with minimal adverse effects. Bezalkonium Chloride (CBZ) is a cationic ammonium surfactant that has bactericidal action. Thus, the evaluation by means of controlled release systems associated with Cyclodextrins and the investigation of its bactericidal pharmacological action in Staphylococcus aureus cultures is justified. OBJECTIVES: To carry out the synthesis and physicochemical characterization of the nanostructured composition of the antimicrobial CBZ complexed with β-Cyclodextrin and Hydroxypropyl-β-cyclodextrin and to evaluate its antimicrobial activity. MATERIALS AND METHODS: Initially, the preparation of the inclusion complex (IC) of CBZ with β-Cyclodextrin andHydroxypropyl-β-cyclodextrin. Subsequently, the structural characterization will be carried out by means of tests and analysis of infrared spectroscopy by Fourier transform, nuclear magnetic resonance and thermal (thermogravimetry, differential thermal analysis and temperature and thermal diffusivity). Analyzes of isothermal titration calorimetry titrations will be performed. Biological assays will be performed following the guidelines of the Clinical & Laboratory Standards Institute, using the determination of the minimum inhibitory concentration necessary to reduce the viability (mean lethal dose: LD50) of Staphylococcus aureus bacteria by microdilution method. In addition, the drug-membrane interaction will be investigated from a colloidal point of view by measuring the hydrodynamic diameter and the zeta potential of the cells DISCUSSION AND RESULTS: At the end of the project, the identification and characterization of the formation of the IC is expected. As for the antimicrobial activity, better results of LD50 obtained with the IC are expected compared with results of CBZ and Cyclodextrins alone. In the evaluation of the interaction with bacterial cell membranes, an improvement in the neutralization capacity of CBZ is expected with the presence of cyclodextrins allowing better interaction with the cell membrane. **Keywords:** Benzalkonium Chloride, β-cyclodextrin, antibiotics

F.73 - PROJECT- Selection of synthetic antibodies by phage display against peptides derived from beta-casein A1 and A2

Bruna Andersen P. de Jesus [1], Lina María S. Echeverri[1], Anderson Albino Gomes[1], Leonardo Antônio Fernandes[1], Anderson V. de Andrade[1], Anelize Felício Ramos[1], Maria de Lourdes B.Magalhães[1], Gustavo Felippe da Silva[1]
[1]Engenharia Florestal, Universidade Do Estado De Santa Catarina (Santa Catarina, Brasil)

INTRODUCTION: In countries such as New Zealand, the selective use of type A2 producing cows has been common for over a decade. This choice is due to the notorious problems caused by the beta-casomorphine-7 peptide (BCM7), which is produced from the intestinal digestion of type A1 beta-casein. Beta-Casomorphine-7 (BCM-7) causes inflammatory problems in some A1 milk drinkers. Diseases such as diabetes and even autism have been linked, through scientific studies, to the presence of BCM7 in A1 dairy products. Thus, researchers have shown that milk from cows that produce exclusively beta-casein A2 do not generate, through their digestion, the bioactive peptide BCM7. A limited number of producers in Brazil are already selecting herds, via genetic sequencing, with a focus on expanding this market. However, this cattle selection strategy, which occurs by genotyping, is of high cost and methodological complexity, facts that make the participation of more producers and cooperatives in this market unfeasible, generating a slow expansion of this promising market. OBJECTIVES: The objective of the project is the phenotypic detection by immunodiagnosis of beta-caseins A1 and A2 by monoclonal antibodies selected by phage display, an alternative considered faster and more economical that can replace the genotyping of dairy cows through direct analysis of milk. MATERIALS AND METHODS: Selection of an antibody library using M13 bacteriophages against A1 and A2 peptides will be performed, identification of specific binding monoclonal phages, and expression and purification of scFv monoclonal antibodies (single-chain variable fragment). DISCUSSION AND RESULTS: As expected results, after obtaining scFv monoclonal antibodies, ELISA tests against milk samples containing beta-casein A1 or A2 will be tested.CONCLUSION: The identification of A2A2 milk-producing mothers will be carried out to register animals detected as A2A2.

Keywords: A2 Milk, Antibory Library, Immunodiagnosis / **Supported by:** UDESC, FAPESC, CNPQ

F.74 - Characterization of the Enzyme Glycerol Kinase (Gk) from Ricinus communis L. Overexpressed in Arabidopsis thaliana underWater Stress

Maria Cecília Oliveira dos Santos Silva[1,2], Amanda Átila Alves Santos Reis[1,2], Neide da Hora Conceição [1,2], Cintia Bispo Souza das Virgens [1], Cristiane Dantas Brito [1], Paulo Roberto Ribeiro de Jesus [1,2,3], Renato Delmondez de Castro [1], Luzimar Gonzaga Fernandez [1,2,3]
[1]Departamento de Bioquímica e Biofísica, Laboratório de Bioquímica Biotecnologia e Bioprodutos Instituto Ciências Saúde, Universidade Federal Bahia (BA, Brasil), [2]Departamento de Bioquímica e Biofísica, Programa Multicêntrico de Pós Graduação em Bioquímica e Biologia Molecular, Instituto Ciências Saúde, Universidade Federal Bahia (BA, Brasil), [3]Departamento de química orgânica , Metabolomics Research Group, Instituto de química, Universidade Federal da Bahia (BA, Brasil)

INTRODUCTION: *Ricinus communis* is an oilseed of great socioeconomic importance; its oil is also used in many industries and medicine. *Arabidopsis thaliana* is a herbaceous, cruciferous plant used as a model organism; it has a short life cycle, size and small genome. Environmental factors such as water restriction can affect biochemical reactions that determine germination. OBJECTIVES: This study aimed to evaluate the response of *Arabidopsis* overexpressing glycerol kinase of *R.Communis* (GK156) during germination and under water stress and characterize its genes compared with other species. MATERIALS AND METHODS: The seeds of GK156 and the wild type (Col-0) were germinated and submitted to different concentrations of PEG8000 and NaCl. The germinator and SISVAR software were used to determine Gmax, T50, U8416 and AUC parameters in the data analysis. Gene's family members of GK were verified using Phytozome. Then, sequence alignment and analysis of phylogenetic relationships (MEGAX) and conserved motifs (MEME) were performed. Cell sublocalization, cis-regulatory elements, and physicochemical characteristics were verified in CELLO, PlantCARE, and Protparam, respectively. DISCUSSION AND RESULTS: Col-0 and Gk156 did not differ in the control condition, with Gmax close to 100%. However, GK156 at the potential of -0.2 Mpa showed a reduction in germination, which was also observed in the corresponding NaCl concentration and at concentrations of -0.04 Mpa and 100mM, where it was lower than Col-0, without germination. *Rc*GK is a stable protein with a small multigene family, with sublocalization, mainly in the cytoplasm and mitochondrial with highly conserved motifs. Analysis of cis regulatory elements suggested that the expression is light regulated; this is the most active. CONCLUSION: It was concluded that seeds overexpressing *Rc*GK genes exhibit less resistance to water stress. These studies are important to expand the knowledge of these species regarding their different responses, aiming at a better adaptation to abiotic stress.

Keywords: Castor bean, Abiotic stress, Osmotic potential

F.75 - Engineering Peptides Defensin-Based For SARS-CoV-2 Spike Protein Detection

Leonardo Antonio Fernandes [1], Anderson Albino Gomes[1], Lina Maria Salazar Echeverri[1], Bruna Andersen Pereira de Jesus[1], Anderson Varela de Andrade[1], Partha Ray[2], Maria de Lourdes B. Magalhães[1], Gustavo Felippe da Silva[1]
[1]Centro de Ciências Agroveterinárias , Universidade do Estado de Santa Catarina (Santa Catarina, Brasil), [2]Department of Surgery, University of California San Diego (, Estados Unidos)

INTRODUCTION: COVID19 is a respiratory disease caused by the SARS-CoV-2 virus and is transmitted by aerosols. The penetration of SARS-CoV-2 into respiratory tract cells is mediated by the Spike protein using the ACE-2 (angiotensin-converting enzyme 2) receptor as a gateway. The structure of ACE-2 responsible for the interaction with the viral Spike protein is constituted by an alpha-helix. Based on this, by protein engineering technique, three recombinant proteins denominated hdeface2, pdeface2 and pdeface2-mut were developed using an alpha-helix of a human defesin and plant defensin, respectively, containing the hot spots amino acids of ACE-2 to mimic a natural interaction with the protein Spike OBJECTIVES: The aim is to couple them to signaling complexes, thus making it possible to develop a technology for detecting SARS-CoV-2. MATERIALS AND METHODS: The proteins genes were cloned into pET32a and BL21 PLysS bacteria were transformed for expression. An ELISA was performed by sensitizing a 96-well plate with 75 ng ECD (Extracellular Domain) protein and detected with 5 µg/ml biotinylated proteins. A dot Blot was performed by immobilizing 100 ng of ECD on a nitrocellulose membrane and detected with increasing amounts of proteins DISCUSSION AND RESULTS: In both ELISA and Dot Blot the recombinant proteins were able to detect the immobilized ECD protein. CONCLUSION: Tests using cell lines overexpressing SARS-Cov-2 Spike protein (Wuhan and Delta variants) are been performed with the three peptides to verify the interaction by flow cytometry method.
Keywords: ACE2, COVID19, Immunoassays / **Supported by:** CAPES

F.76 - PROJECT_Medicinal and Biotechnological Potential of Abarema cochliacarpos: Antimicrobial, Antioxidant, Antitumor, Cytotoxic, and Neuroprotective Activities

Cássia Santos[1], Patrícia Santos[2], Maria Cecília Silva[2], Ana Beatriz Bastos[2], Marta Loureiro[2], Daniela Takahashi[1,2], Renato Castro[1,2], Victor Diogenes Silva[1,4], Ravena Nascimento[2,4], Silvia Costa[4], Lourdes Neta[5], Paulo Jesus[1,2,3], Luzimar Fernandez[1,2,3]
[1]Instituto de Ciências da Saúde, Programa Multicêntrico de Pós-Graduação em Bioquímica e Biologia Molecular – PMBqBM/SBBq, [2]Departamento de Bioquímica e Biofísica, Instituto de Ciências da Saúde, Laboratório de Bioquímica, Biotecnologia e Bioproduto, [3]Instituto de Química, Metabolomics Research Group, [4]Depto de Biofísica e Bioquímica, Instituto de Ciências da Saúde, Laboratório de Neuroquímica, Univ.e Federal da Bahia-UFBA, (Bahia, Brasil), [5]Departamento de Ciências Exatas e da Terra , Universidade Estadual da Bahia (Bahia, Brasil)

Abarema cochliacarpos is a species native to Brazil, popularly known as Barbatimão, and used as a traditional medicine to treat leucorrhoea, hemorrhages, diarrhea, hemorrhoids, conjunctivitis, cleaning, and wound healing. The objective of the present work is to use a multidisciplinary, integrated, and bio-guided approach to correlate the metabolomic profile and medicinal potential of *A. cochliacarpos* in terms of antioxidant, antimicrobial, antitumor, and neuroprotective activity, and active principles present in the plant. The ethanol extract will be obtained from the maceration of the botanical organs (leaf, bark, and stem), and fractionation will be realized, if necessary, with hexane, ethyl acetate, or dichloromethane. The antioxidant activity will be evaluated through the 2,2'-azinobis(3-ethylbenzothiaziline-6-sulfonate) (ABTS) sequestration methodology and the ferric reducing antioxidant power (FRAP) assay. The antimicrobial activity of extracts on multiresistant bacteria *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Elizabethkingia anophelis* will be evaluated by broth microdilution methodology. Cytotoxic antitumor activity will be performed using a colorimetric method with 3-4,5-dimethylthiazol-2-yl,2,5-diphenyltetrazolium bromide (MTT). Moreover, the neuroprotective activity by analyzing proliferation, morphology, and the phenotype of cells of the PC12 lineage, after induction of damage by aminochrome. The results will correlate with the already performed metabolomic analysis and subsequent statistical analysis. Studies have shown that *A. cochliacarpos* has several medicinal properties, for example, antioxidant, antimicrobial, and cytotoxic activity. However, the antimicrobial activity against multidrug-resistant strains, and neuroprotective activity, correlated with the metabolic profile of extracts from different botanical organs have not yet been evaluated. *A. cochliacarpos presents* active principles responsible for biological and pharmacological properties, characterizing it as a medicinal plant with great biotechnological potential.
Keywords: Barbatimão, Ethnopharmacology, Medicinal Plant

F.77 - Production and biotechnological application of holocellulose-degrading enzymes from filamentous fungi for the efficient saccharification of sugarcane straw

Aline Pereira de Almeida [1,2], Camila L. Marciano[1,2], Fabiane Cruz Bezera[1], Clarice Rossato Marchetti[1], Josiane A. Scarpassa[3], Fabiana Fonseca Zanoelo[1,2], Giovana Cristina Giannesi[1,2], Douglas Chodi Masui[1,2], Roberto Ruller[1,2,3]
[1]Laboratório de Bioquímica Geral e de Microrganismos-LBq, [2]Programa Multicêntrico de Pós-Graduação em Bioquímica e Biologia Molecular-PPGP, Universidade Federal de Mato Grosso do Sul (MS, Brazil), [3]Instituto de Biociências Letras e Ciências Exatas, Universidade Estadual Paulista (SP, Brazil)

Sugarcane straw is an attractive source of holocelluloses. Filamentous fungi produce essential enzyme cocktails to hydrolyze holocellulose fractions to fermentable sugars for use in bioprocesses that generate products of high economic value. In this context, the production and biotechnological potential of holocellulose-degrading enzyme cocktails from filamentous fungi in the saccharification of energy cane straw (ECS) were evaluated. For this purpose, seven filamentous fungi were cultivated in submerged fermentation using ECS as a carbon source. Subsequently, the crude enzymatic extracts were separated and evaluated for holocellulasic activities and saccharification of pre-treated energy cane straw 1% (w/v). The enzymatic cocktails with the highest sugar yield were combined in a 1:1 ratio and used for saccharification under the same conditions above. Cellic ® CTec2 was used as a standard control. The experimental results showed that the highest production of holocellulose-degrading enzymes occurred at 168 h. In the ECS 1% (w/v) saccharification tests, the enzyme cocktails of Rc and Tk1 showed the highest hydrolysis performance. The Rc cocktail released 13.11 ± 1.17 g L^{-1} of glucose and 24.7 ± 1.29 g L^{-1} of total reducing sugars after 72 h. The Tk1 cocktail released 14.44 ± 0.37 g L^{-1} of glucose and 19.96 ± 1.50 g L^{-1} of total reducing sugars. These results corresponded to a yield of 70-80% glucose and 98-122% total reducing sugars compared to the commercial cocktail Cellic ® CTec2. In saccharification tests with the cocktail formulated by the mix of Rc + Tk1 (1:1), no significant difference was observed in the release of glucose and total reducing sugars compared to the saccharification of the commercial cocktail Cellic ® CTec2. Thus, the results suggest that these microorganisms can be used as a promising biotechnological alternative as enzyme producers for ECS holocellulose hydrolysis in bioprocesses. **Keywords:** Holocellulose-Degrading Enzymes, Hydrolysis, Energy Cane Straw / **Supported by:** CAPES and CNPq

F.78 - Biochemical Study of Phytases, Proteases and Xylanases Produced by Aspergillus sp Using Agro-industrial Residues for Application in Animal Feed

Ana Lorena de Oliveira Simas [1], Jessica Schlosser de Sá Teles[1], Nathalia Nunes Glienke[1], Nelciele Cavalieri de Alencar Guimarães [1], Douglas Chodi Masui[1], Roberto Ruller[1], Fabiana Fonseca Zanoelo[1], Giovana Cristina Giannesi[1]
[1]INBIO, Laboratório de Bioquímica Geral e Microrganismos, Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, MS, Brazil; (Mato Grosso do Sul, Brasil)

INTRODUCTION: The manufacture of animal feed in industry began to replace the use of animal proteins for vegetable protein sources. However, many of these plant ingredients contain limiting factors for digestibility, such as non-starch polysaccharides in corn, pentosans in wheat, oligosaccharides in soy and phytates. The search for enzyme sources and the knowledge of their properties are an important factor in the industry, especially those produced by filamentous fungi. Enzymes produced by microorganisms have high production yields with lower cost when grown in wastes. OBJECTIVES: To produce and characterize the phytases, proteases and xylanases produced by fungi (*Aspergillus* sp.) using agro-industrial residues in solid state fermentation (SSF) and select potential enzyme producers for the application in alternative animal feed. MATERIALS AND METHODS: Seven fungi were used from the filamentous fungi culture collection of UFMS (Campo Grande – MS). Carbon sources such as corn bran, brown rice bran and rice straw were used for enzyme production. DISCUSSION AND RESULTS: The best phytase production was with *A. niveus* (AN) in brown rice bran (0,83 U/mg) and *A. japonicus* (AJ) in rice straw (0,41 U/mg). For protease production *A. flavus* (AF) in wheat bran (0,6 U/mg) and AJ in rice straw (0,53 U/mg). For xylanase AN in brown rice bran (37,84 U/mg) and *Aspergillus* sp (M2) (26,9 U/mg). The best enzyme producers (AN, AJ and M2) and the best carbon sources were selected and the peak of phytase by AN was after 144h of cultivation in rice straw. For protease 96 hours with AN followed by AJ in rice straw. For xylanase 72 hours with M2 in rice straw. CONCLUSION: Thus, the addition of these enzymes to animal feed can improve the uptake of nutrients by the animals and can lead to production savings.
Keywords: Aspergillus sp., agro-industrial residues, animal feed

F.79 - ACE2-peptidomimetics for detecting SARS-CoV2 neutralizing antibodies

Anderson Albino Gomes [1], Leonardo Antonio Fernandes[1], Lina Maria Salazar Echeverri[1], Bruna Andersen Pereira de Jesus [1], Anderson Varela de Andrade[1], Gustavo Felippe da Silva[1], Anelize Ramos Felicios[1], Partha Ray[2], Maria de Lourdes Borba Magalhães[1]
[1]Center of Agroveterinary Sciences, State University of Santa Catarina (Santa Catarina, Brasil), [2]San Diego , University of California (California, United States of America)

INTRODUCTION: COVID-19 is an infectious disease caused by the new coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The virus is highly contagious and can be transmitted through direct and indirect contact with infected people through respiratory droplets when sneezing, coughing, or even speaking. We recently developed a new molecule capable of binding to the virus that causes COVID-19. OBJECTIVES: The objective was to use the molecule developed in a diagnostic platform assay for detecting SARS-CoV2 neutralizing antibodies. MATERIALS AND METHODS: We used a Brazilian Health Regulatory Agency (ANVISA)-approved neutralizing antibody test. The assay is based on the principle of competitive ELISA. Briefly, the biotinylated-ACE2 protein is immobilized on the streptavidin-coated microplates that can bind HRP-coupled SARS-CoV-2 Spike protein and generate a signal in the presence of HRP-substrate. DISCUSSION AND RESULTS: However, in the presence of SARS-CoV2 anti-S-protein binding antibodies (neutralizing sera), the availability of HRP-coupled SARS-CoV-2 Spike protein to bind the ACE2 protein decreases, thus resulting in a decrease of the measured signal. We modified this assay and replaced the biotinylated-ACE2 with the biotinylated p-deface2 protein. This was followed by adding HRP-coupled SARS-CoV-2 Spike protein and the test (neutralizing) or control (non-neutralizing) human serum provided with the kit. CONCLUSION: The presence of neutralizing antibodies disrupted the interaction between p-deface2 and HRP-coupled SARS-CoV-2 Spike protein, thus decreasing the measured signal compared to the control non-neutralizing sera, indicating that biotinylated p-deface2 could be applied in a diagnostic assay platform to screen between positive and negative SARS-CoV-2 neutralizing serum.

Keywords: diagnosis, neutralizing-antibody, SARS-CoV-2
Supported by: CNPq and CAPES

F.80 - Project Green Synthesis, Characterization and Cytotoxicity of Silver Nanoparticles Obtained from Different Yeasts

Thais da Silva Correa [1], Mariana Campos da Paz[1]
[1]Bioquímica, Universidade Federal de São João del Rei (Minas Gerais, Brasil)

INTRODUCTION: Silver nanoparticles (SN) have been studied as a promising approach for cancer management. They may be explored to improve therapeutical selectiveness for tumor cells, aiming greater effectiveness and lower occurrence of adverse effects than conventional therapies. Using plants, bacteria and yeasts to greenly synthetize SN turns the process faster, cheaper, and more ecofriendly than the chemical synthesis. Furthermore, in the green synthesis, the molecules provided by living organisms – instead of toxic chemical reagents – act as stabilizing agents, which decreases the potential health hazard of SN and increase their biomedical application. OBJECTIVES: This study aims to evaluate and compare the anticancer potential of SN obtained by using different yeasts in a green synthesis approach. MATERIALS AND METHODS: For the green synthesis of SN, silver nitrate will be added to the culture supernatant or to the cell extract of different yeast strains – *Saccharomyces cerevisiae*, Rhodotorula glutinis and Kluyveromyces marxianus. Temperature, pH and silver nitrate concentration will be varied to improve the yield. The different SN obtained will be characterized in terms of diameter, hydrodynamic diameter, potencial zeta and morphology. In order to evaluate the potential anticancer activity, all SN obtained from different yeast cultures and extracts will be added to tumor and non tumor cell lines for 24 and 48h. MTT assays will be performed and IC50 for each SN will be determined. Besides, tridimensional cultures of malignant cells will be treated with the different SN obtained and cytotoxicity will be evaluated by lactate dehydrogenase assay. DISCUSSION AND RESULTS: Establishment of an efficient protocol to green synthesis of SN is expected. Since S. cerevisiae, R. glutinis and K. marxianus are described as producing bioactive molecules, SN are expected to present antiproliferative activity. However, the characteristics and effects of each SN obtained will likely be different. CONCLUSION: SN stabilized by yeast's molecules are expect to have greater anticancer potential.

Keywords: silver nanoparticles, green synthesis, cancer therapy / **Supported by:** CNPq and FAPEMIG

F.81 - Production, Optimization, and Biochemical Characterization of an Amylase from the Thermophilic Fungus Humicola brevis var. thermoidea.

Camila Langer Marciano [1,2], Aline Pereira de Almeida[1,2], Fabiane Cruz Bezerra[1], Fabiana Fonseca Zanoelo[1,2], Giovana Cristina Giannesi[1,2], Roberto Ruller[1], Douglas Chodi Masui[1,2]
[1]Laboratório de Bioquímica Geral e de Microrganismo-INBIO, Universidade Federal de Mato Grosso do Sul (MS, Brasil), [2]Programa Multicêntrico de Pós-Graduação em Bioquíma -INBIO, Universidade Federal de Mato Grosso do Sul (MS, Brasil)

INTRODUCTION: Among the enzymes used in bioprocesses, amylases represent a group of biocatalysts used by the industry to saccharify starch. They cleave the glycosidic bonds from starch and release maltose, glucose, and shortening oligosaccharide chains. Amylase represents 25% of the global enzyme market, but the existing commercial options have pH and temperature limitations for industrial conditions. Therefore, it is necessary to investigate new and robust amylases that can support these extreme conditions. Thermophilic filamentous fungi present sources of enzymes with favorable characteristics for biotechnological processes. OBJECTIVES: Thus, the objective was to obtain an amylase from the thermophilic fungus *Humicola brevis var Thermoidea* and biochemically characterized it in the crude extract. MATERIALS AND METHODS: The amylolytic extract was obtained from solid-state fermentation (FES) and optimized using the one-factor-at-time (OFAT) methodology. For this investigation, were performed the best carbon source as amylase inducers, the nitrogen supplementation, the time-course of production, and the temperature to evaluate the ideal growth of H. brevis and the best combination for OFAT. The 3,5-dinitrosalicylic acid (DNS) method and commercial starch hydrolysis determined the amylolytic activity. The Read & Northcote methodology established the total protein concentration. DISCUSSION AND RESULTS: The highest amylase production occurred in a medium composed of wheat bran at 50°C for five days (140 ± 5 U. mL $^{-1}$ and 29 ± 3 U. mg $^{-1}$). The optimal enzymatic activity occurred at pH 5, at 60°C, and remained stable in the pH range of 5-6, with high thermostability at 50°C. Furthermore, the Hg^{+2} and Cu^{+2} ions strongly inhibited the enzyme, while Mn^{+2} stimulated it up to 80% (266.5 ± 2 U. mL $^{-1}$). CONCLUSION: The biochemical results suggest the amylase produced by *H. brevis* has attractive characteristics for use in bioprocesses.
Keywords: amylase, starch, Humicola / **Supported by:** Capes CNPq

F.82 - Biodistribution, Antitumor Activity and Toxicity of Polymeric Nanocarriers of metothrexate and etoposide

Carolina Alves Petit Couto [1], Gisele Rodrigues da Silva[2], Mariana Campos da Paz[1]
[1]Laboratório de nanotecnologia , Universidade federal de São João del Rei (, Brasil), [2]Farmacotécnica da escola de farmácia da universidade federal de Ouro Preto , Universidade Federal de Ouro Preto (, Brasil)

INTRODUCTION: Nanoencapsulation of chemotherapeutics is a promising approach in cancer management. Nanocarriers improves drug solubility and the efficiency of drug combination. OBJECTIVES: To evaluate the antitumor activity as well as the toxicity and the biodistribution of a nanometric micellar system consisting of poloxamer incorporated into two antitumor drugs (methotrexate and etoposide). MATERIALS AND METHODS: Poloxamer nanocarriers of methotrexate and etoposide (NPME) was previously synthetized. For the evaluation of antitumor activity, 4T1luc tumor-bearing mice will be intravenously injected with NPME or methotrexate + etoposide (free form). The therapeutical efficiency will be evaluated by detection of tumor bioluminescence each five days. After 30 days, mice will be euthanized and organs (including the tumor) and blood will be removed. Immunohistochemistry of tumors will be performed to confirm tumor cell death after treatment. To assess NPME toxicity, the main organs will be histologically analyzed and the blood will be used for hematological and biochemistry analysis. A biodistribution study of NPME will also be held. Radiolabeled (technetium–99m) NPME will be intravenously injected in tumor bearing mice and scintigraph images will be acquired after 1, 5, and 20 hours. Organs and blood will be removed and their level of radioactivity will be determined with an automatic gamma counter. DISCUSSION AND RESULTS: Previous *In vitro* analysis showed that NPME promoted apoptosis in 4T1 cells and that its cytotoxicity was significantly higher for tumor than for non-tumor cells. Furthermore, the nanoencapsulation of two drugs in the same micellar system seems to improve the antiproliferative activity of this nanocarrier. Therefore, *In vivo* studies are expected to corroborate the therapeutic efficacy of NPME. A passive accumulation of NPME in tumor is also expected, which will be probably related to a lower toxicity than that caused by free drugs. CONCLUSION: NPME may be a promising nanomaterial for cancer management. - **Keywords:** Cancer, nanotechnology, drug delivery

F.83 - Effects of 2,3,5-triiodobenzoic acid (TIBA) and iohexol in chemotherapy resistant cell lines

Jessica Sodre Silva De Abreu [1], Janaína Fernandes[1]
[1]Campus Duque de Caxias, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: TIBA (an ionic monomer) and iohexol (a non-ionic monomer) are iodinated contrast agents used in X-ray diagnostic techniques. However, such agents cause contrast-induced nephropathy, inducing oxidative stress and apoptosis of renal cells. TIBA also induces an increase in oxidative stress in different tumors, which causes the death of these cells. However, its activity in tumor resistance pathways has not yet been elucidated. Studies evaluating the antitumor activity of iohexol are still rare, even as the subcellular mechanisms involved in its cytotoxicity in tumor cells. OBJECTIVES: To evaluate the antitumor activity of TIBA and iohexol in cell lines resistant to chemotherapy. MATERIALS AND METHODS: The glioblastoma cell line (A172), the cell line resistant to vincristine of chronic myeloid leukemia (Lucena), and the normal renal epithelial line (VERO) were used. After 48h of treatment with different concentrations of contrast media, cell viability and DNA fragmentation (apoptosis) assays were performed. In addition, the assessment of the increase in reactive oxygen species of cells treated after 5h with the drugs was performed. Finally, we evaluated whether TIBA and iohexol modulate drug efflux pump activity and cell migration. DISCUSSION AND RESULTS: TIBA and iohexol decrease the cell viability of the three lines studied in a dose-dependent manner, in which the cells were less sensitive to treatment with iohexol; TIBA and iohexol induce apoptosis in the three cells studied in a dose-dependent manner, where TIBA caused the highest percentage of DNA fragmentation; TIBA and iohexol induce increase in cellular oxidative stress, and antioxidant treatment decreases apoptosis caused by the drugs; TIBA and iohexol inhibit the activity of efflux pumps; TIBA decreased the migration of glioblastoma cells and iohexol did not affect this cell mobility. CONCLUSION: The contrast agents studied here are cytotoxic to tumor cells through different pathways, showing promise in the treatment of cancer.

Keywords: TIBA, Iohexol, antitumoral
Supported by: FAPERJ

F.84 - PROJECT: Development of a CRISPR/Cas9 System for Overexpression of XlnR and AraR Transcription Factors and Production of (Hemi-)cellulases in Filamentous Fungi

Felipe Ferreira Silva [1], Kledna Reis[1], Marina Rodrigues[2], Anderson Melo[1], Natana Rabelo[3], Débora Lopes[1], Paulo Granjeiro[1], José Silva[1], Wagner Souza[4], Daniel Gonçalves[2]
[1]Campus Centro-Oeste Dona Lindu, Federal University of São João del-Rei (Minas Gerais, Brazil), [2]Department of Biosystems Engineering, Federal University of São João del-Rei (Minas Gerais, Brazil), [3]Department of Microbiology, Federal University of Minas Gerais (Minas Gerais, Brazil), [4]Center for Natural and Human Sciences, Federal University of ABC (São Paulo, Brazil)

The increasing demand to produce renewable fuels (biofuels) to replace the use of fossil fuels is reflected in the global political scenario, and Brazil has a leading role in the participation of renewables in its energy matrix. Hemicellulose, the second most abundant fraction of plant biomass after cellulose, which until recently was treated only as waste, has been seen as a potential source to produce biofuels and other value-added materials. It is a heterogeneous polysaccharide complex, composed mainly of xylose and arabinose whose degradation requires the production of many different enzymes, classified as (hemi)-cellulases, which are induced by biopolymers or their derivatives and mainly regulated at the transcriptional level through transcription factors (TFs). Within these TFs, it is possible to highlight two, mainly produced by filamentous fungi. XlnR, the main regulator of this enzyme complex, and AraR, a secondary homologous factor and important regulator in joint action with XlnR. Filamentous fungi, organisms that naturally produce enzymes that degrade these polysaccharides, have very effective and biotechnologically attractive protein production and secretion systems. In this context, the present work aims to develop a gene editing system via CRISPR/Cas9 in the filamentous fungus *Penicillium chrysogenum* to overexpress the TFs XlnR and AraR, and evaluate the influence of this overexpression on the production of (hemi)-cellulases. *In silico* design of CRISPR/Cas9 components were performed with components commonly used in heterologous expression in filamentous fungi. Protoplasts of *P. chrysogenum* will be transformed and the evaluation of the system will be assessed through enzymatic content and activity, saccharification degree, and screening of the highest enzyme producer clones. It is expected that this work will contribute to the state of the art and development of a national technology to produce enzymes and bioenergy, increasing Brazil's competitiveness in this important biotechnological sector.

Keywords: CRISPR/Cas, (Hemi-)cellulases, Filamentous fungi / **Supported by:** FAPEMIG, CNPq, CAPES

F.85 - PROJECT_Antioxidant, antibacterial, antitumor, and neuroprotective activity of Laguncularia racemosa L. from the North Coast of Bahia

Dhielly H. De Oliveira Castro [1,2], Patricia Campos Santos[2], Maria Cecília Silva[2], Paulo Roberto R. de Jesus[1,], Marta Bruno Loureiro [2], Daniele Takahashi[2], Brenda Antunes[2], Renato de Castro[2], Lourdes C. de Souza Neta[4], Ravena Pereira Nascimento[1,5], Silvia Lima Costa[5], Victor Diogenes de Souza[1,5], Luzimar Gonzaga Fernandez[1,2,3]
[1]Programa Multicêntrico de Pós-Graduação em Bioquímica e Biologia Molecular, [2]Laboratório de Bioquímica, Biotecnologia e Bioprodutos, [3]Metabolomics Research Group, Instituto de Química, [4]Química, [5]Laboratório de Neuroquímica e Biologia Celular, Universidade Federal da Bahia (Bahia, Brasil)

The specie *Laguncularia racemosa* is part of the mangrove vegetation and can develop in different soil conditions. They are being used in traditional medicine as a treatment agent for some diseases. However, there are few ethnopharmacological studies and identification of the metabolites responsible for their biological and pharmacological properties. To evaluate the medicinal potential as for the antioxidant, antimicrobial, antitumor, and neuroprotective activity of *Laguncularia racemosa L.* extracts from the North Coast of Bahia. Ethanol extracts from different botanical structures (leaf, bark, and stem) will be prepared by maceration. The antioxidant activity will be evaluated by the 2,20-azino bisacid 3-ethylbenzthiazoline-6-sulfonic (ABTS) radical removal method and the ferric reducing antioxidant power (FRAP) assay, while the quantification of flavonoids will be by spectrophotometry using catechin as default. The antimicrobial activity will be through successive microdilution in broth, and multiresistant bacterial strains such as *Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Elizabethkingia anophelis* will be used. The test with a C6 cell line derived from a Wister rat glial tumor and 3-4,5- dimethylthiazol-2-yl,2,5-diphenyltetrazolium bromide (MTT) will be used for the evaluation of a cytotoxic antitumor activity. Neuroprotective activity will be evaluated using PC12 and spinal cord cells from Wistar rats and immunocytochemical analyses. There is a need for national studies to explain the pharmacological potential of *L. racemosa*, such as its antioxidant potential and antimicrobial activity on multidrug-resistant bacteria, such as those that will be analyzed, as well an antitumor and neuroprotective activity and correlate with biocompounds responsible for these properties and identified in the extracts. It is expected to contribute to the characterization of the species regarding the metabolic profile, determination of biological and pharmacological properties related to the active principles identified in *L. racemosa*, and traditional knowledge. **Keywords:** White mangrove, Ethnopharmacobotany, Active principles

F.86 - PROJECT Investigation of new substrates of the human enzyme thiopurine methyltransferase

Ana Carolina Silva dos Santos [1], Julianna Dias Zeidler[2], Marcelo Reis[1], Gisele C. Amorim[1], Luisa Andrea Ketzer[1,2]
[1]Núcleo Multidisciplinar de Pesquisa UFRJ - Xerém em Biologia, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil), [2]Laboratório de Bioquímica de Vírus, Instituto de Bioquímica Médica Leopoldo de Meis (Rio de Janeiro, Brasil)

INTRODUCTION: Thiopurine methyltransferase (TPMT) is a cytosolic enzyme that catalyzes the methylation of aromatic and heterocyclic sulfhydryl compounds, using S-adenosylmethionine as a methyl donor. Among its substrates are the thiopurine drugs 6-mercaptopurine, 6-thioguanine and azathioprine, which are used in the treatment of patients with neoplasms, autoimmune diseases and transplants. TPMT activity in humans is inherited as an autosomal trait with codominance, exhibiting genetic polymorphism. Some works suggest that TMPT would be able to methylate selenium compounds. Although selenium methylation produces less toxic forms, it is still unclear how this process of methylation of selenocompounds occurs in humans. OBJECTIVES: The present project intends to investigate whether the methylation of selenium compounds (ebselen, L-selenomethionine, diphenyl diselenide) is catalyzed by human thiopurine methyltransferase. MATERIALS AND METHODS: The experimental strategy used will be the expression of the human TPMT enzyme (wild and mutant clones) in two models: i) *Escherichia coli* for further purification and activity analyses; and ii) embryonic of human kidney cells (HEK293) and investigation of the tolerance of these cells in response to exposure to high concentrations of selenocompounds. The enzyme purification will allow the analysis of kinetic parameters with classic and new substrates. HEK293 cells will be incubated with different concentrations of selenocompounds and the following parameters will be evaluated: cell viability and proliferation, activity of antioxidant enzymes, lipid peroxidation, production of superoxide anion and expression of the TPMT enzyme. DISCUSSION AND RESULTS: The main expected results include elucidating the role of human TPMT in the methylation of selenocompounds and discovering the possible consequences of the treatment of selenium compounds in the antioxidant defense of HEK293 cells. CONCLUSION: The present project has great relevance for the understanding of the methylation process of selenocompounds.
Keywords: enzyme, selenocompounds, methylation / **Supported by:** FAPERJ

F.87 - Rapid detection of the New Viral Species that Infects Wheat in Brazil, the Wheat Stripe Mosaic Virus (WhSMV), Through Reverse Transcription Loop-Mediated Isothermal Amplification.

Anderson Varela De Andrade [1], Anderson Albino Gomes[1], Lina Maria Salazar Echeverri[1], Leonardo Antônio Fernandes[1], Anelize Felício Ramos[1], Bruna Andersen Pereira de Jesus[1], Gustavo da Silva Felippe[1], Maria De Lurdes Borba Magalhães[1], Fernando Sartori Pereira[1], Fabio Nascimento Da Silva[1]
[1]Produção Animal E Alimentos, Universidade Do Estado De Santa Catarina - Centro Agroveterinário (Santa Catarina, BRASIL)

INTRODUCTION: Wheat (Triticum spp.) is one of the most cultivated cereals in the world. According to the National Supply Company, Brazil produced about 7.9 tons in 2021. The southern region is responsible for growing 91.62% of Brazilian wheat, according to the Technical Office for Economic Studies of the Northeast. Due to this, wheat becomes a major contributor to the region's economy. Wheat stripe mosaic virus (WhSMV) was recently identified, a virus transmitted through the soil by the bacterium Polymyxa graminis, whose infection can lead to a loss of 50% in production. Molecular techniques for virus detection are already used at the laboratory level, but it is necessary to seek alternatives for detection in the field. The RT-LAMP technique (reverse transcription followed by loop-mediated isothermal amplification) can be used both for detection of pathogens through genetic material and presents high speed, specificity and efficiency under isothermal conditions. The process is simple, low cost and more resistant to inhibitor interference.OBJECTIVES: The aim of the project is to develop a RT-LAMP-based diagnostic test for the WhSMV virus. MATERIALS AND METHODS: After defining the conserved area of the WhSMV gene, the primers were designed using PrimerExprlorer V5 software. RT-LAMP was performed with Bst DNA polymerase 2.0 Turbo and M-MLV enzymes. The specificity of the primers was verified using the RNA of the healthy wheat leaf and the RNA of the RSNV virus (Rice stripe necrosis virus) as specifity control. The process was carried out in a thermocycler at 60°C for 35 min. The results were evaluated using 1.5% agarose gel electrophoresis. DISCUSSION AND RESULTS: WhSMV was detected by the RT-LAMP method using the designed primer set. The sensitivity test identified only WhSMV. CONCLUSION: The rapid identification and early treatment of such a pathogen can improve grain production, making wheat and its derivatives more economically profitable for producers and millions of consumers worldwide.
Keywords: RT-LAMP, WhSMV, WHEAT / **Supported by:** FUMDES,FAPESC, CAPES, CNPQ.

F.88 - Evaluation of the potential application in the environment and health of a biosurfactant produced from microorganisms

Maria Auxiliadora de Oliveira [1], Hiure Gomes Ramos Meira[1], Diego Fernandes Lívio[1], Adriano Guimarães Parreira[1], Daniel Bonoto Gonçalves[2], José Antônio da Silva[1], Paulo Afonso Granjeiro[1]
[1]Laboratory of Biotechnological Processes and Purification of Macromolecules, Campus Centro-Oeste, Federal University of São João del Rei, Divinópolis-MG, Brazil (MG, Brazil), [2]Department of Biosystems Engineering, Campus Department of Biosystems Engineering, Campus Dom Bosco, Federal University of São João del Rei, São João del Rei, Brazil. (MG, Brazil)

INTRODUCTION: Biosurfactants (BS) are secondary metabolites secreted by several microorganisms. These biomolecules have many functions that can be used in various industrial areas, such as health and environment. OBJECTIVES: The objective of this work was to evaluate the potentials of application of BS, in face of the bactericidal, fungicidal, effluent settler properties, in the spread of motor oil, in the bioremediation of motor oil in sand and its toxicity in *In vivo* tests with brine shrimp MATERIALS AND METHODS: The BS was tested for its antimicrobial activity against *Aeromonas hidroplyla*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Colletotrichum sp.* through antibiogram tests. The potential of BS to act in the bioremediation of oils and decantation of sewage effluents was evaluated. The toxicity of BS was evaluated in *In vivo* test using *Artemia salina* DISCUSSION AND RESULTS: At a concentration of 500 mg/mL, BS showed an inhibition halo of 9 mm for *Aeromonas hidroplyla*, while for *E. coli*, *P. aeruginosa*, *C. albicans*. *Colletotrichum sp.* there wereno inhibition. In oil dispersion, BS showed better performance, obtaining a scattering ratio of 75% against 48.7 and 59.7% for Triton X-100 and SDS, respectively. In the concentration of 0.1 and 0.5 g/L the BS showed a recovery rate of motor oil from contaminated sand equivalent to 80 and 23%, respectively.For treatment of effluents, BS demonstrated a decanting capacity in 24h, while with aluminum sulfate was 30 min. At high concentrations (0.5 and 1 mg/mL) the mortality rate for BS was only 10%, demonstrating not toxic to *Artemia salina*, while the mortality rate for SDS was 100%. CONCLUSION: We concluded that the BS produced from a microorganism had a high potential for application in various sectors of the industry, such as health and the environment.
Keywords: Biosurfactants, Applications - lipopeptides, Environment
Supported by: CAPES, CNPq, FAPEMIG and UFSJ.

G - Drug Discovery and Delivery

G.01 - Evaluation of lipid components and their proportions in the manufacture of stable nanostructured lipid carriers

Juliana Oliveira da Silva Lopes Pereira [1], Franscico Alexandrino Jr[2], Helvecio Vinícius Antunes Rocha[2], Beatriz Ferreira de Carvalho Patricio[1]
[1]Departamento de Ciências Fisiológicas, Universidade Federal do Estado do Rio de Janeiro (Rio de Janeiro, Brasil), [2]Laboratório de Micro e Nanotecnologia, Farmanguinhos, Fundação Oswaldo Cruz (Rio de Janeiro, Brasil)

INTRODUCTION: Nanostructured lipid carriers (NLC) have been growing as a solution to deliver drugs with low solubility and permeation through the gastrointestinal tract and skin. These nanoparticles are a combination of solid lipid (SL), liquid lipid (LL), and surfactant. Therefore, the selection of these components is important to guarantee a size smaller than 300 nm, low polydispersity, and good stability. OBJECTIVES: Study the impact of the type and concentration of the lipid component on the production of NCL. MATERIALS AND METHODS: A literature survey was conducted to select the most used SL, LL, and surfactants for the production of NCL. Then, a fractional factorial experimental design was used to determine the best combination between these components and their ratio to produce NCL formulations. The NCL formulations had their stability assessed for 30 days based on particle size, polydispersity index (PDI), and zeta potential. DISCUSSION AND RESULTS: After the literature survey, Glyceryl monostearate and glyceryl distearate were selected as SL, Phosal® 50 PG and Crodamol GMCC were selected as LL, and Tween™ 80 and Poloxamer 188 were selected as surfactants. The experimental design produced 24 formulations in duplicate. The formulations prepared with Poloxamer 188 showed a translucent blue color, while those with Tween 80 showed an opaque white color. NLC produced with glyceryl monostearate as LS formed lumps after a few days and a increase in the nanoparticle size. In opposition, formulations with glyceryl distearate as LS remained with a size close to 200 nm and were more stable during the experiment. CONCLUSION: The experimental design was interesting to observe the relationship between the components and the stability of the system. The choice of LS and surfactant significantly altered the parameters such as size and PDI over 30 days.
Keywords: Design experiment, Nanotechnology, Nanostructured lipid carriers

G.02 - NOVIRUSES2BRAIN: Developing Broad Spectrum Brain-Targeting Drugs Against Flaviviruses And Other Envelope Viruses

Miguel Augusto Rico Botas Castanho [1]
[1]Biochemistry, Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Portugal. (, Portugal)

INTRODUCTION: Co-infections with different kinds of viruses is likely, mainly when the same vector is considered. Aedes mosquitos, for instance, serves as vector for several human viruses, such as Zika virus, Dengue virus, or Chikungunya virus. Broad spectrum antivirals are needed.Moreover, ideally, these antivirals should penetrate the brain as some of the viruses cause neurological disorders. OBJECTIVES: We are developing drugs able to inactivate Zika virus, Dengue virus, and HIV, among others, while having the ability to traverse the blood-brain barrier (BBB). This is deemed important mainly for Zika virus because, so far, there is no effective remedy for infection with this virus due to the limited ability of antiviral drugs to cross blood–placental barrier (BPB) and/or BBB. MATERIALS AND METHODS: Chemically, the drugs consist on the conjugation of an antiviral porphyrin to a trans-BBB peptide. Proprietary trans-BBB peptides were obtained from templates based on domains of the capsid protein of Dengue virus. The activity, toxicology and brain-targeting efficacy of a panel of conjugates were evaluated both *In vitro* and *In vivo* . DISCUSSION AND RESULTS: One of the conjugates, named PP-P1, crossing both BPB and BBB, has shown to be effective against Zika Virus (IC50 1.08 µM) and has high serum stability (t1/2 ca. 22 h) without altering cell viability at all tested concentrations. CONCLUSION: Project-associated references: 1. Bioconjugate Chem. 2021, 32, 6, 1067–1077; https://doi.org/10.1021/acs.bioconjchem.1c00123 2. Pharmaceutics 2022, 14(4), 738; https://doi.org/10.3390/pharmaceutics14040738
Keywords: Drug, Peptide, Membrane
Supported by: Work supported by the European Union (H2020-FETOPEN-2018-2019-2020-01 grant no 828774).

G.03 - Study of the interaction between Coumarin 343 (C343) and human serum albumin (HSA)

Carmen Regina de Souza [3], Valdecir Farias Ximenes[3], Aguinaldo Robinson de Souza[3]
[1]Departamento de Química - Faculdade de Ciências - POSMAT, Universidade Estadual Paulista (São Paulo, Brasil)

INTRODUCTION: The human serum albumins (HSA) are proteins that have the property of carrying drugs[1-3] This is due to the fact that they have preferential binding regions, or sites, for drugs (DS1, DS2 and DS3), located in the Domains and Subdomains of the protein. The Coumarin 343 (C343) belongs to the class of coumarins, in which it has a wide variety of applications in the medical field: appetite suppressant, eczema treatment and others[5,6,7] OBJECTIVES: Determine the stoichiometry of the interaction using the Job's Plot technique, and also to determine the value of the binding constant (Ka) between HSA and C343. MATERIALS AND METHODS: To determine the Ka value, we performed the titration experiment, in which 5 µL of HSA solution (0.5 mM) was titrated under the C343 solution (1 mM) every 2 minutes. In the Job's Plot method, we used solutions with concentrations of up to 22.5 µMol, varying the initial concentrations of HSA and C343. Analyzes were performed on a Perkin Elmes L55 fluorescence spectrophotometer; a correction factor was used for the values used for Ka. DISCUSSION AND RESULTS: We verified that C343 interacts with the HSA protein, and in the formation of this complex we obtained a Ka value equal to 5.3 x 10^4 M^-1. Through the Job's Plot methodology, we determined that the complexed structure (protein-ligand) has a stoichiometry of 1:1. CONCLUSION: According to the value of the binding constant obtained in the analyses, we can conclude that C343 has affinity for a single site of the HSA protein, because according to the proportionality results, the formation of the complex is 1:1. From the methodologies used, we obtained important and essential results for the understanding of the interaction between HSA and C343.

Keywords: Coumarin 343, Human Serum Albumin, Job's plot

G.04 - In Silico Repurposing Of FDA Approved Drugs Against Zika Virus NS3 Protease

Alessandra Sbano[1], Manuela Leal da Silva[2]
[1]DIMAV, Programa de Pós-Graduação em Biotecnologia, Instituto Nacional de Metrologia, Qualidade e Tecnologia (RJ, Brasil), [2]Instituto de Biodiversidade e Sustentabilidade (NUPEM), Universidade Federal do Rio de Janeiro (RJ, Brasil)

INTRODUCTION: The Zika virus (ZIKV) causes major epidemics in Brazil. According to WHO, it is a global health problem and can cause microcelaphy in neonates of Zika virus-infected mothers. In this research, in silico repurposing of approved drugs is the strategy to reduce time and spent money on the search for antivirals. The NS3 protease is a good molecular target, being essential in viral replication. OBJECTIVES: Using computational repurposing of FDA-approved drugs intends to find a potential antiviral against NS3 protease by Zika virus. MATERIALS AND METHODS: We chose the FDA-approved drug database, totaling 1615 compounds. Open Babel generated 3D conformations and added polar hydrogens according to pH 7.4. Applied The same pH in NS3 structure protonation by the PDB2PQR server. Using Autodock Vina, we performed the virtual screening (VS) simulations. The exhaustiveness was 16, and 20 poses were generated for each drug. We performed parameterization in previous steps, in redock. Then, the grid-box was positioned around the more essential pocket residues (His 51, Asp 75, and Ser135). We made the ADMET predictions using pkCSM, SwissADME, and Osiris Property Explorer softwaews. The interactions at the pocket in lead molecules are being done, using Pymol and Discovery Studio's softwares. DISCUSSION AND RESULTS: Was selected the top100 drugs with lower docking energy by Autodock Vina for the other analysis. Although approved drugs are filtered according to elected drug-likeness parameters according to the system, such as the potential to cross the blood-brain barrier, toxicity, and mutagenic. After filtering, were defined as lead 28 drugs, and are being analyzed to other affinity binding scores. CONCLUSION: The structure-based virtual approach selected drugs with potential for new use, being promising for other steps in the search for potential Zika antivirals.

Keywords: Antiviral, drug repositioning, ZIKA vírus
Supported by: PRONAMETRO (INMETRO) and CAPES Biocomputacional

G.05 - Pegylation of Liposomal Amphotericin B Enhances Oral Effectiveness in Hamsters with Cutaneous Leishmaniasis

Helane Lúcia Oliveira de Morais [1], Guilherme Santos Ramos[1], Virgínia Mendes Russo Vallejos[1], Raquel Martins de Almeida[2], Mateus de Melo Lima[2], Ricardo Toshio Fujiwara[2], Frédéric Frézard[1]
[1]Departamento de Fisiologia e Biofísica, Universidade Federal de Minas Gerais (Minas Gerais, Brazil),
[2]Departamento de Parasitologia, Universidade Federal de Minas Gerais (Minas Gerais, Brazil)

INTRODUCTION: Leishmaniases are neglected diseases caused by the Leishmania parasite and found in tropical and subtropical regions and southern Europe. The few drugs available have toxicities. Liposomal amphotericin B (AmB) or Ambisome® is the most effective therapeutic agent for visceral leishmaniasis, but it has limited clinical efficacy in cutaneous leishmaniasis (CL) and HIV/VL co-infection. Another limitation is its use restricted to the parenteral route. OBJECTIVES: To evaluate the effect of PEGylation of liposomal AmB on its stability in simulated gastric fluid and on oral efficacy in hamsters with CL. MATERIALS AND METHODS: Formulations of AmB in conventional and PEGylated liposomes were prepared and characterized for particle size by DLS, drug encapsulation efficiency by UV/Vis spectrophotometry and AmB aggregation state by circular dichroism (CD). These parameters were also evaluated after incubation for 2h at 37ºC in acid solution (pH 1.5-2). Hamsters were infected in the paw with Leishmania amazonensis and treated with liposomal AmB formulations orally through daily doses of 5 mg/kg for 10 days. Treatment effectiveness was evaluated by recording the lesion size and compared to that with oral miltefosine (10 mg/kg). DISCUSSION AND RESULTS: Conventional and PEGylated formulations showed populations of monodisperse vesicles with mean diameters between 110 and 130 nm, drug encapsulation efficiencies greater than 98% and CD spectra consistent with a non-aggregated form. The PEGylated formulation showed greater stability than the conventional one when exposed to acidic medium. Treatment of infected hamsters with the PEGylated formulation promoted a significant reduction in lesion size compared to the untreated group, at a more pronounced level than treatment with the conventional formulation and an equivalent level to miltefosine treatment. CONCLUSION: : This work demonstrates that PEGylation of liposomal AmB enhances its stability in simulated gastric fluid and its oral efficacy in an hamster model of CL.
Keywords: amphotericin B, leishmaniasis, liposomes / **Supported by:** CAPES

G.06 - In Silico Investigation of Natural Products as Possible Inhibitors of SARS-CoV-2 Protease 3Clpro

Maria Eduarda Alves Esteves [1], Manuela Leal da Silva[2,1]
[1]Pós-graduação em Biologia Computacional e Sistemas, Instituto Oswaldo Cruz (Rio de Janeiro, Brazil), [2]Núcleo em Ecologia e Desenvolvimento Socioambiental de Macaé - NUPEM, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brazil)

INTRODUCTION: Covid-19 is a clinical condition of acute respiratory syndrome, caused by the SARS-CoV-2 virus. The pathogen, belonging to the Coronaviridae family, has positive single-stranded RNA, responsible to produce two main polyproteins. The protein material, hence, is cleaved by proteases, originating the mature viral proteins responsible for the assembly of new viruses. Most of the cleavage points are carried out by the $3Cl_{pro}$ enzyme, thus making it a promising target for the development of drugs that can act as inhibitors in the treatment of covid-19. OBJECTIVES: This work proposes an *in silico* search for natural products belonging to the Brazilian flora, which have inhibitory potential against $3Cl_{pro}$ of SARS-CoV-2. MATERIALS AND METHODS: The methodology used here was divided into: (I) System preparation and parameterization; (II) Virtual screening using the NuBBE database against $3Cl_{pro}$ (PDBid 6XQT), with: AutoDock Vina (a) and GOLD Suite - Score Function: ChemPLP (b) and GoldScore (c); (III) Prediction of ADMETOx parameters with SwissADME, ADMETlab 2.0 and Osiris. DISCUSSION AND RESULTS: It was established in step (I) with (a), affinity energy equal to -8.5 Kcal/mol and RMSD 1.46 Å between the first mode resulting from exhaustivity 100 and the ligand extracted from the A chain of $3Cl_{pro}$ (PDBid 6XQT). Using (c), the score was 98.90 and RMSD 0.67 Å, and, for (b): 80.88 and RMSD 1.99 Å. In step (II), out of a total of 2223 substances, 451 presented a final score, calculated by normalizing the scoring functions, below 0.3. Of this total, 22 substances contented the bRO5 pharmacokinetic rules and are negative for carcinogenicity, tumorigenicity, respiratory toxicity, AMES and PAINS (III). CONCLUSION: In conclusion, among the 22 natural products classified as possible inhibitors of $3Cl_{pro}$ of SARS-CoV-2, the flavonoids and alkaloids stood out once they already have antimicrobial properties described.
Keywords: natural products, in silico, SARS-CoV-2
Supported by: CAPES

G.07 - Biochemical and Pharmacological Characterization of Hyaluronidase (Sp-H) from the scorpionfish Scorpaena plumieri venom

Thamires Souza Vargas[1], Helena Bulhões Fiorotti[2], Fabiana Vasconcelos Campos [1], **Thamiles Nogueira Vilela[1]**, Luciana Souza de Oliveira[3], Márcia Helena Borges[3], Juliana B. Coitinho Gonçalves[1], Suely Gomes de Figueiredo[1]
[1]Ciências Fisiológicas, Universidade Federal do Espírito Santo (ES, Brasil), [2]Laboratório de Bioquímica, Instituto Butantan (SP, Brasil), [3]Diretoria de Pesquisa e Desenvolvimento, Fundação Ezequiel Dias (MG, Brasil)

INTRODUCTION: Hyaluronidases are ubiquitous enzymes in animal venoms. These glycosaminidases are invariant components of these venoms, being often called dispersion factors. Due to their hydrolytic effect on hyaluronic acid, these enzymes affect the integrity of the extracellular matrix, promoting the dissemination of toxins from venoms and thus potentiating the local and systemic effects of envenomation. OBJECTIVES: The aim of this study was to characterize a hyaluronidase (named Sp-H) present in the venom of the scorpionfish Scorpaena plumieri, considered the most venomous of the Brazilian coast. MATERIALS AND METHODS: Sp-H was obtained in a high degree of homogeneity through a protocol involving three fractionation steps: saline precipitation, molecular filtration and anion exchange chromatography. DISCUSSION AND RESULTS: Physicochemical analyses showed that Sp-H has an apparent molecular mass of 69,2 kDa (SDS-PAGE analysis), a large proportion of β-sheets (~ 40%) (circular dichroism analysis), optimal activity in the pH range between 5.4 to 6.4, and stability when stored in 0.1M phosphate buffer pH 5.9, at 4°C, -25°C and -80 °C for 30 days. In addition, an anti Sp-H serum produced in rabbits neutralized the activity of hyaluronidase *in vitro*. Therefore, it can be suggested that this specific serum can be used as a therapy for accidents with scorpionfish, as it minimizes the dispersing effect of Sp-H that leads to a decrease in the local and systemic effects of the envenomation. CONCLUSION: The knowledge gathered here comprise the first step in the study of a new hyaluronidase –Sp-H, what may contribute to a better understanding of the pathophysiology of the envenomation by S. plumieri, and also to the exploration of its biotechnological potential as a dispersing agent.
Keywords: Venom, Scorpaena plumieri fish, Hyaluronidase / **Supported by:** Capes, CNPq, INCTTox

G.08 - Application of Atomic Force Microscopy in the Design of Solid Pharmaceutical Forms

Wellington Silva Ferreira [1], Beatriz Ferreira Carvalho de Patricio[2], Paulo Mascarello Bisch[1], Gilberto Weissmüller[1]
[1]Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro (Rio de Janeiro, Brazil),
[2]Department of Physiological Sciences, State University of Rio de Janeiro (Rio de Janeiro, Brazil)

INTRODUCTION: Drug formulations basically consist of two types of inputs, active ingredients and excipients. The design of pharmaceutical forms depends on the interaction of these compounds, since such parameters directly impact their processability. To understand these interactions, the atomic force microscope comes as a tool since it allows the evaluation of the physicochemical parameters of the surface of the excipients, as well as the analysis of interaction between the components of the formulation through the functionalization of cantilevers, thus helping to choose the components of the formulation, optimizing development time and cost. OBJECTIVES: Use AFM in the design of solid pharmaceutical formulations, using efavirenz (EFV) as a drug model. MATERIALS AND METHODS: The raw material (IFA and excipients) were fixed on glass slides and topographically analyzed by the Peak Force Quantitative Mechanical Property Mapping (PFQNM) method. Adhesion analyzes between the EFV and excipients were performed using cantilevers functionalized with crystals of the active ingredient by the Force Volume method. DISCUSSION AND RESULTS: It was possible to observe the nanomechanical properties of the samples with the PFQNM topographic analyses, while the interaction analyzes allowed us to understand the adhesive and cohesive properties of the compounds when exposed to the same environment, making it possible to obtain an idea of the behavior materials during the formulation processability. CONCLUSION: The AFM is a very versatile tool, but not yet fully explored by the pharmaceutical industry, the results together with the range of articles in the literature, corroborate with the idea of the usefulness of such techniques within the industry during the design of solid pharmaceutical formulations.
Keywords: AFM, Solid Pharmaceutical Formulations, Design of Pharmaceutical Forms

G.09 - Designing Sulforaphane-loaded Thermosensitive Hydrogels for Inflammatory Diseases Treatment Applications

Lorena Doretto da Silva [1], Daniele Ribeiro de Araujo[1], Vinicius de Andrade Oliveira[1]
[1]Human and Natural Sciences Center, ABC Federal University (São Paulo, Brazil)

INTRODUCTION: Phytochemicals are a potential alternative therapy due to their healthy and economical approach. They can be used alone, or combined with available therapeutic regimens for the treatment of inflammatory diseases. Sulforaphane (SFN), an isothiocyanate derivative with anti-inflammatory and immunoregulatory pharmacological properties, has been shown improved therapeutic efficacy against inflammatory diseases, such as autoimmune, transplant, and dermatitis. OBJECTIVES: to develop sulforaphane-loaded thermosensitive hydrogels and evaluate whether treatment can prevent inflammatory diseases, through modulation of the immune system. MATERIALS AND METHODS: sulforaphane-loaded thermosensitive hydrogel (0,1%) composed of hyaluronic acid (HA) (0,5%) in poloxamer PL407 (at 20% or 25% w/v) were synthesized and characterized. DISCUSSION AND RESULTS: Rheological analysis showed that the incorporation of HA and SFN in PL407 decreased the G'/G" ratio, but elastic modulus (G') values were greater than viscous modulus (G"), favoring the structure of the hydrogel, being liquid-viscous. PL407 25% had a sol-gel transition temperature of 4ºC lower than 20%. Micellization temperatures were similar between the PL407 hydrogel formulations, being ~20 °C (20%) and 18 °C (25%). PL407 (20) systems showed lower enthalpy (ΔH) values (-2.60 J/g) compared to PL407 (25) (-2.96 J/g). The *In vitro* drug release kinetics (in phosphate buffer 5 mM, pH 7.4) was linear up to 12 h, reaching maximum values after 24 h. 20% and 25% PL407, with HA, sustained the SFN release profile with drug concentrations values of 271.8 ± 6.23 µg/mL (24 h) and 234 ± 4.31 µg/mL (36 h). CONCLUSION: Considering the physicochemical and biopharmaceutical properties described, the PL407 25%-HA-SFN system was selected to perform *In vitro* and *In vivo* assays in inflammatory disease models.
Keywords: hydrogels, inflammatory diseases, sulforaphane / **Supported by:** FAPESP; CAPES

G.10 - Characterization of fluorescence in graphene oxide-based nanomaterials

Beatriz Ribeiro [1], Devaney Do Carmo[2], Glaucia Machado-Santelli[1]
[1]Department of Cell and Developmental Biology, Institute of Biomedical Sciences (São Paulo, Brazil), [2]Department of Physics and Chemistry, Paulista State University (São Paulo, Brazil)

INTRODUCTION: Graphene oxide (GOX), due to the presence of several hybrid layers of carbon atoms (sp2), presents a large surface and a large number of free and oxygen-rich groups, being able to carry several materials, including drugs and dendrimers, which are synthetic macromolecules of multibranched polymers composed of repeated monomeric units, whose structure also allows the association with different molecules. OBJECTIVES: The objective of this work was to obtain fluorescent labeling of graphene oxide-based materials to analyze the interaction with cells. MATERIALS AND METHODS: For this, three materials were used: GOX, GOX associated with DAB-AM-16 dendrimer (GOXD) and GOX associated with PAMAM dendrimer (GOXP). We evaluated the fluorescence labeling with three fluorophores: rhodamine-B, lofins NO2 and OH and propidium iodide in the spectrophotometer and in the fluorescence microscope. The graphene oxides and the fluorophores were shaken at 700 rpm for 12 hours for adsorption to occur. Subsequently, the MCF-7 breast tumor cell line received these associated materials for 24 hours. After this time, the cells were observed under a fluorescence microscope and, later, the fluorescence was measured in the spectrophotometer. In addition, we evaluated cell viability by the sulforhodamine-B assay with different concentrations of fluorophores. DISCUSSION AND RESULTS: Our results showed that fluorescent labeling of graphene oxide-based materials was successful with lofins and propidium iodide, showing a higher fluorescence profile for the GOXP material associated with propidium iodide, which can be explained due to the ability of PAMAM to be an excellent candidate for application in image detection. In addition, cell viability was not altered at low concentrations of fluorophores. CONCLUSION: In conclusion, our work showed that the fluorescent labeling of graphene oxide-based materials was successful and did not impair the viability of cells. Our efforts are important for future investigations on the internalization of these materials and the association with drugs, aiming to potentiate future treatments.
Keywords: graphene oxide, dendrimers , tumor cell
Supported by: FAPESP and CNPq

G.11 - Development and Characterization of Polymeric Nanoparticles Containing Amphotericin B

Gabriel Barcellos[1], Franscisco Alexandrino-Junior[1], Flavia Silva[1], Kercia Cruz[2], Valdeene Santos[3], Patricia Veras[2], Francine Azeredo[3], Helvécio Rocha[1], **Beatriz Ferreira de Carvalho Patricio [4]**

[1]VDEPI, Instituto de Tecnolologia em Fármacos (Rio de Janeiro, Brasil), [2]Fiocruz, Instituto Gonçalo Moniz (Bahia, Brasil), [3]Departamento do Medicamento, Universidade Federal da Bahia (Bahia, Brasil), [4]Departamento de Ciências Fisiológicas, Universidade Federal do Estado do Rio de Janeiro (RJ, Brasil)

INTRODUCTION: Amphotericin B (AmB) is the most potent drug for the treatment of leishmaniasis and it is only available in intravenously formulation because its low permeability and solubility in gastrointestinal tract. However, those are associated with nephrotoxicity, low adhesion to the treatment and high costs, OBJECTIVES: Therefore, the objective of this work was to develop and characterize polymeric nanoparticles (PNs) containing AmB for the use in the oral route. MATERIALS AND METHODS: For this, polycaprolactone (PCL) and polylactic acid (PLA) PNs were obtained by nanoprecipitation, with the addition of an organic phase — comprised of the polymer dissolved in acetone and AmB dissolved in a mixture of methanol and dimethyl sulfoxide — to an aqueous phase with 0.3% (w/w) polysorbate 80. The PNs were characterized by size, polydispersity index (PdI), drug concentration ([AmB]), *In vitro* cytotoxicity and *In vivo* pharmacokinetics in rats. DISCUSSION AND RESULTS: PCL and PLA NPs displayed size inferior to 200 nm, PdI < 0,2 and [AmB] > 180 µg/mL. In the *In vitro* cytotoxicity studies, the PCL PNs presented inhibition of cell viability (EC50) of 43.71 µg/mL in bone marrow macrophages (BMMΦ) and 66.57 µg/mL in HepG2 cells, while PLA PNs presented EC50 of 13.15 µg/mL in BMMΦ and 17.24 µg/mL in HepG2 cells. The *In vivo* pharmacokinetics assay indicated the use of the PCL and PLA formulations resulted in an estimated bioavailability of 9.2% and 11.8%, respectively, displaying a 12-fold increase in relation to the free drug. CONCLUSION: This study demonstrating that the use of nanotechnology is promising for the development of oral formulations for drugs with low bioavailability.

Keywords: amphotericin B, polymeric nanoparticles, drug delivery system / **Supported by:** CNPq; Unirio, Inova-Fiocruz; Farmanguinhos

G.12 - Development of lipid nanocapsules for the topical delivery of Benzocaine

Maria Fernanda Pacheco Da Silva [1], Fernando Freitas de Lima [1], Eneida de Paula[1]

[1] Bioquímica e Biologia Tecidual, Universidade Estadual de Campinas (São Paulo, Brazil)

INTRODUCTION: The topical anesthetic benzocaine (BZC) is often used for the process of local antinociception in cutaneous or mucous tissues. Despite of this, there are reports of toxic effects associated with BZC, such as functional anemia (methemoglobinemia induced by the p-aminobenzoate metabolite) and tissue hypoxia (Khanal et al, 2018; doi:10.1097/MJT.0000000000000782; Nascimento et al, 2008, doi: 10.1590/S0034-70942008000600011). One pharmaceutical strategy to reduce side effects of anesthetics is the use of Drug Delivery System (DDS), since encapsulation in such carriers can prevent drug degradation, prolong the release and anesthesia times, thus reducing toxicity (Araújo et al., 2019, doi: 10.1080/17425247.2019.1629415). OBJECTIVES: To incorporate BZC into a lipid based DDS: lipid nanocapsules (LNC), in an attempt to attenuate undesirable effects of the anesthetic. MATERIALS AND METHODS: Control and BZC-containing LNC were prepared at 2.0-3.0% benzocaine, by the phase inversion method. The proportion of surfactants (Kolliphor HS 15) and triglyceride (Mygliol) were modified to produce stable and low size particles, as followed by Dynamic Light Scattering and Nanoparticle Tracking Analysis. For the quantification of BZC HPLC was used. DISCUSSION AND RESULTS: The developed LNCBZC formulation (average size = 98 nm, polydispersity index, PDI = 0.056, Zeta potential, ZP = -22.10 mV and 9.7 x 10^8 nanoparticles/mL) remained stable for 2 months of storage at room temperature. After that period some phase separation was observed, accompanied with changes in the size, PDI, ZP and nanoparticles concentration. CONCLUSION: The analyses of the formulation parameters made it possible to determine the influence of excipients on the stability of the prepared LNC. In face of that we intend to modify the proportion of Mygliol, water and drug in the formulation to increase the stability time and to proceed with the following steps of the study, towards a novel DDS for topical pain treatment.

Keywords: Benzocaine, Drug Delivery Systems, Lipid nanocapsules

G.13 - Characterization of Cruzain Inhibitors as Potential Trypanocidal Agents

Lucas Abreu Diniz [1], Viviane Corrêa Santos[1], Marina Mol Sena Andrade[2], Renata Barbosa de Oliveira[2], Rafaela Salgado Ferreira[1]

[1]Bioquímica e Imunologia, Universidade Federal de Minas Gerais (Minas Gerais, Brasil), [2]Departamento de Produtos Farmacêuticos, Universidade Federal de Minas Gerais (Minas Gerais, Brasil)

INTRODUCTION: Cruzain is the major cysteine protease from *Trypanosoma cruzi*, and it is a validated target for the development of new drugs. This enzyme is associated with relevant processes such as host cell invasion and metacyclogenesis. OBJECTIVES: To characterize analogs of compound VS17 - a cruzain competitive inhibitor previously described by our group. MATERIALS AND METHODS: Proteolytic activity was measured by monitoring the cleavage of a fluorogenic substrate. Compounds were initially screened at 100 µM, and the enzyme velocity in their presence was compared to a DMSO control. IC_{50} curves were determined for the most potent inhibitors, based on at least seven compound concentrations. To verify whether the compounds are potential aggregators, we evaluated if their potency was affected by pre-incubation with BSA or different Triton X-100 concentrations. To determine the mechanism of inhibition, cruzain activity was measured at eight different substrate concentrations (0.1-20.0 µM) and five inhibitor concentrations (12-200 µM). DISCUSSION AND RESULTS: Among the 17 compounds screened, 4 were hits against cruzain, causing over 70% inhibition at 100 µM. We determined IC_{50} values for compounds IZN, IN-8, and HI-2, which were 43, 46, and 45 µM, respectively. To evaluate time-dependent inhibition, we compared IC_{50} values with and without pre-incubation. Only HI-2 displayed time dependence, as its IC_{50} was reduced to 23 µM upon pre-incubation. For IN-8, we observed reduced inhibition after pre-incubation with BSA. Kinetic assays were performed to determine the mechanism of inhibition by IZN. A non-competitive behavior was observed, as K_{Mapp} was unaltered and maximum velocity decreased with the increase of inhibitor concentration. CONCLUSION: The results suggest that IZN is a non-competitive inhibitor against cruzain, while HI-2 might be a time-dependent inhibitor, and IN-8 a promiscuous inhibitor by aggregation.

Keywords: Chagas disease, Cruzain, Cysteine protease inhibitors

G.14 - Design and evaluation of covalent inhibitors for trypanosomatid dihydroorotate dehydrogenases

Thamires Froes[1,2], Jyoti Chauhan[3], Mario Rodriguez[1,2], Iohan Sacocchi[1,2], Joe Eyermann[3], Lori Ferrins[3], Flávio Emery[1,2], Maria Cristina Nonato[1,2]

[1]Biomolecular, School of Pharmaceutical Sciences of Ribeirão Preto (SP, Brazil), [2]Biomolecular, Center for the Research and Advancement of Fragments and molecular Targets (SP, Brazil), [3]Dept. of Chemistry & Chemical Biology, Northeastern University (MA, EUA)

INTRODUCTION: Chagas Disease (CD) and Leishmaniasis are neglected tropical diseases caused by *Trypanosoma cruzi* and Leishmania spp., respectively. CD affects approximately 6-8 million people, and an estimated 700.000 to 1 million new cases of Leishmaniasis occur annually. The available drugs display high toxicity and low efficacy, highlighting the need for novel therapeutic alternatives to fight these diseases. DHODH catalyzes the fourth and rate-limiting step in de novo pyrimidine biosynthesis of T. cruzi and Leishmania spp. Most trypanosomatids DHODH (TrypDHODH) inhibitors are orotate analogs that follow a reversible mechanism of action. The presence of a catalytic cysteine in TrypDHODH makes it possible to design covalent inhibitors for this target. OBJECTIVES: Identify covalent inhibitors of TrypDHODH. MATERIALS AND METHODS: Docking experiments were performed to design an initial set of 20 putative inhibitors incorporating different warheads. Thermal shift (ThermoFMN and Thermofluor- TSA) and enzymatic assays were carried out in a single concentration (500µM and 125 µM, respectively) of each putative inhibitor, with (1h) and without incubation. The most promising inhibitors had their Kinact/KI constant calculated. DISCUSSION AND RESULTS: TcDHODH was employed as a model for TSA, in which a +ΔTm implies that ligand binding results in a more stable protein conformation, while a -ΔTm means that a less stable conformation was formed. NEU-7222 (1-phenylprop-2-yn-1-one) (500µM), bearing an alkyne electrophile, produced the highest shifts in ThermoFMN (ΔTm: -1.0) and Thermofluor (ΔTm: -4.0). Its inhibition profile is time- and concentration-dependent. NEU-7222 (125 µM) decreased the enzymatic activity of TcDHODH to 40% at 0h and 65% after one hour of incubation. The covalent profile was confirmed by Kinact/KI constant calculation (86.49 µM). Similar results were obtained for L. braziliensis and L. major DHODHs. CONCLUSION: The innovative strategies used to design TrypDHODH covalent inhibitors were successful. Next, X-ray crystallography data will guide fragment-to-lead optimization to improve compounds' potency and selectivity.

Keywords: Chagas diseases, TcDHODH, covalent inhibitors / **Supported by:** FAPESP, CNPq, and NIH

G.15 - Hyaluronic Acid Incorporation Modulates Rheological and Drug Release Properties in Poloxamer-Based Hydrogels

Anderson Ferreira Sepulveda [1], Mont Vollrath-Kumpgdee[2], Margareth K. K. D. Franco[3], Fabiano Yokaichiya[4], Daniele R. de Araujo[1]
[1]Human and Natural Sciences Center, Federal University of ABC (SP, Brazil), [2]Pharmaceutic Technology, University of Applied Sciences (, Germany), [3]CNEN, Nuclear and Energy Research Institute (SP, Brazil), [4]Physics Department, Federal University of Parana (SP, Brazil)

INTRODUCTION: Synthetic polymer Poloxamer (PL) 407 (15% and 30% w/w) and binary formulation PL 407 15% + PL 338 15% (BF), with natural polymer hyaluronic acid 0.5% w/w, were designed as bupivacaine or ropivacaine thermosensitive release systems. OBJECTIVES: The aim of this work is to characterize structure and stability of drug delivery systems. MATERIALS AND METHODS: These systems were characterized by calorimetry, rheology, SANS, and release profile. DISCUSSION AND RESULTS: Calorimetry results demonstrated all formulations are stable at storage and physiological temperatures. PL 407 30% and BF systems are structurally more organized and with higher consistency (G'/G'' ~ 50) at 37 °C and with lower gelation temperature (Tg ~ 14 °C) than PL 407 15% ones (G'/G'' ~ 0.30 and Tg ~ 45 °C, respectively), however BFs have increased viscosity and slightly higher stiffness (G'/G'' ~ 60) when compared to PL 407 30% formulations, due to more hydrophilicity of PL 338 chains than PL 407. Adding HA, it is observed enhanced viscosity but diminished consistency (G'/G'' ~ 0.40). When a drug is incorporated, it is seen that it promotes increased interaction between chains. Although material alteration when incorporating HA or drug is observed, SANS results showed that the type of supramolecular structure is dependent on the concentration of Poloxamer. Systems with low concentration of Poloxamer have lamellar type, while formulations with 30% of Poloxamer have both cubic and hexagonal structures. In addition, PL 407 30% formulations undergo greater compression when bupivacaine is added (~ 29.7 nm at 25 °C and 37 °C). As drug release profiles showed, BFs release drugs in a more controlled way than other formulations. Moreover, HA hinders the release of both drugs. CONCLUSION: Thus, it is clear that the incorporation of more hydrophilic polymers is able to modulate the drug release rate according to the hydrogels rheological parameters.

Keywords: Supramolecular structures, Physico-chemical analysis, Drug delivery / **Supported by:** CNPq

G.16 - Lunatin-1: A Scorpion Venom Peptide That Induces Cellular Death In LNCap And MDA-MB-231 Cancer Cell Lines

Brenda Raíssa de Oliveira [1,2], Maria José González Armijos[2], Pedro Pires Goulart Guimarães[1], Adriano Monteiro de Castro Pimenta[2], Dawidson Assis Gomes[2], Marcella Nunes de Melo-Braga[2], Thiago Verano-Braga[1]
[1]Physiology and Biophysics, Universidade Federal de Minas Gerais (MG, BRASIL), [2]Biochemistry and Immunology, Universidade Federal de Minas Gerais (MG, BRASIL)

INTRODUCTION: Previous studies demonstrated that Lunatin-1, a peptide isolated from *Hadruroides lunatus* scorpion venom, regulates apoptosis in human promyelocytic leukemia HL-60 cell line as well as induces death in MCF-7 and MDA-231 human metastatic cancer cells lines by unknown mechanisms. OBJECTIVES: Evaluate the possible Lunatin-1 anti-tumor activity in metastatic human cancer cells, focusing on its mode of action MATERIALS AND METHODS: Synthetic Lunatin-1 was purified by high-performance liquid chromatography (HPLC). Purified Lunatin-1 was analyzed by mass spectrometry (MALDI-TOF/TOF) for synthesis and purification quality control. Lunatin-1 similarity search against human proteins was performed by Blast online tool. Gene ontology was done by GhostKOALA and David online tools. LNCap and MDA-231 cells were treated with different concentrations of Lunatin-1, and cell viability was evaluated by Resazurin assay. IC50 was determined by GraphPad Prism. DISCUSSION AND RESULTS: Lunatin-1 was successfully purified, as confirmed by mass spectrometry. Lunatin-1 induced cytotoxicity in LNCap cells even in the lowest concentration used (1.5 μM, $p < 0.001$). In the MDA-MB-231 cell line, Lunatin-1 presented cytotoxic activity with IC 50 of 20.1 μM. On the other hand, Lunatin-1 was cytotoxic for non-tumoral cells HEK-293 with concentrations higher than 25 μM. Therefore, Lunatin-1 presents different cytotoxic activity when comparing tumoral and non-tumoral cell lines (LNCAP x HEK293, $p < 0.001$). The bioinformatic analysis demonstrated that Lunatin-1 has similarities with proteins related to alternative splicing, phosphoproteins, transporter proteins, breast cancer proteins and prostate neoplasm proteins. Due to Lunatin-1 similarity to breast cancer proteins and prostate neoplasm proteins, its mechanism of action may be related to these proteins. CONCLUSION: Lunatin-1 may represent a potential prototype for developing anti-tumor drugs.

Keywords: Lunatin-1, scorpion peptides, anticancer peptides / **Supported by:** FAPEMIG, CAPES, CNPq

G.17 - Multivariate Analysis of Process Variables in the Preparation of Nanostructured Lipid Carriers

Gabriela Geronimo [1], Gustavo Henrique Rodrigues da Silva[1], Marcia Cristina Breitkreitz[1], Fabíola Viera Carvalho[1], Talita Cesarim Mendonça[1], Eneida de Paula[1]

[1]Biochemistry and Tissue Biology, University of Campinas (SP, Brazil), [2]Department of Analytical Chemistry, University of Campinas (SP, Brazil)

INTRODUCTION: An approach to improve the activity of already established drugs is the use of Drug Delivery Systems (DDS), capable of increasing the pharmacological activity and reduce the toxicity of active molecules. Nanostructured lipid carriers (NLC) are DDS commonly prepared by the emulsification-ultrasonication technique. Additionally, Design of Experiments (DoE) is a multivariate tool for process optimization. OBJECTIVES: To analyze the effects of emulsification and sonication processes variables on the optimization of pharmaceutical nanoformulations, using DoE. MATERIALS AND METHODS: A face-centered (α=1) central composite design was used to evaluate the preparation method of NLC formulations containing an oily (triglyceride) phytopharmaceutical excipient with anesthetic, anti-inflammatory, and promising antitumor activity. The input variables were the strength (3500–15500 rpm) and time (2–4 minutes) of ultra-Turrax agitation, besides the amplitude (20–80%) and time (2–15 minutes) of 50 W, 20 kHz tip sonication. The desirable outputs were smaller particle sizes and polydispersity (PDI), and larger Zeta Potentials (PZ), in module. Such parameters, plus particle concentration were followed during 90 days of stability at 25oC, in 29 formulations. DISCUSSION AND RESULTS: Particles with average diameters 167-305 nm, PDI = 0.130-0.232 and ZP = |22–38| mV were obtained. The size of the particles and their polydispersity were inversely related to the time and amplitude of tip sonication but did not change with time or strength of the emulsification process. The model was not suitable for the PZ response (disregarded). CONCLUSION: Multivariate analyses revealed sonication as the determinant process for the obtention of homogenous, small sized and stable NLC. So, although emulsification assists in the preparation, is the high energy of tip sonication that assures the smallest particles diameter and lipid matrix organization for achieving stable NLC. Moreover, such process optimization will be helpful, saving time in the development of future formulations. **Keywords:** Drug Delivery Systems, Nanostructured Lipid Carriers, Design of Experiments / **Supported by:** FAPESP, CNPq/Brazil

G.18 - In Silico Optimization of Fragments Bound to S. mansoni Thioredoxin Glutathione Reductase to Propose New Schistosomicidal Molecules

Rocío Lucía Beatriz Riveros Maidana[1,2], Lauro R. de Souza Neto[2], Lucas de Almeida Machado[3], Nicholas Furnham[4], Raymond J. Owens[5], José Brandão Neto[6], Frank von Delft[6], Ana Carolina R. Guimarães[1], Floriano Paes Silva Junior[2]

[1]Laboratório de Genômica Funcional e Bioinformática, Instituto Oswaldo Cruz/Fiocruz (Rio de Janeiro, Brazil), [2]Laboratório de Bioquímica Experimental e Computacional de Fármacos, Instituto Oswaldo Cruz/Fiocruz (Rio de Janeiro, Brazil), [3]Faculty of Exact and Natural Sciences, University of Buenos Aires (Buenos Aires, Argentina), [4]Department of Infection Biology, London School of Hygiene and Tropical Medicine (London WC1E 7HT, United Kingdom), [5]Research Complex at Harwell, R92 Rutherford Appleton Laboratory (Didcot, United Kingdom), [6]Beamline I04-1, Diamond Light Source (Didcot, United Kingdom)

INTRODUCTION: Schistosomiasis is a neglected tropical disease caused by *Schistosoma* spp. The treatment is based on a single drug, praziquantel. Despite its success, the lack of effectiveness against juvenile forms and the possibility of drug resistance justify the need to develop new drugs. Thioredoxin Glutathione Reductase of *Schistosoma mansoni* (*Sm*TGR) plays a crucial role in the redox homeostasis of the parasite. Fragment-Based Lead Design (FBLD) consists of the screening of small fragments to a protein target. Fragments can bind to more than one site in the target and act as starting points to the development of new leads. Through a previous FBLD screening campaign, 32 fragments were identified in 8 different binding sites. OBJECTIVES: This work has the goal of optimize the fragments bound to *Sm*TGR using an *in silico* FBLD optimization approach. MATERIALS AND METHODS: 3 fragments ligated to an allosteric site called the *"doorstop pocket"* and 3 fragments ligated to the NADPH binding site were selected for the optimization process. Druggability prediction of the selected binding pockets and description of fragment-protein interactions were performed. Next, fragments were optimized using a growing approach using the AutoGrow4 software. The resulting molecules were filtered according to their druglike properties and synthetic accessibility scores and then were submitted to an ensemble docking experiment. Finally, those molecules were ranked according to its druglike properties and their binding affinity to the *SmTGR* conformations. DISCUSSION AND RESULTS: 683 molecules were obtained after the growing process. After filtering, 123 potential inhibitors were obtained and ranked through the ensemble docking. CONCLUSION: Using a FBLD optimization approach for the ligated fragments produced 123 potential inhibitors. All molecules follow the Lipinski 5 Rule and present good scores of synthetic accessibility. **Keywords:** FBLD, Schistosomiasis, SmTGR. **Supported by:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001

G.19 - Design of inhibitors for the catalytic and allosteric sites of the papain-like cysteine proteases vivapains of plasmodium vivax by in silico methods

Mariana Simões Ferreira [1], Diego Enry Barreto Gomes[2], Pedro Pascutti[1]
[1]Instituto de Biofísica Carlos Chagas Filho, Univ. Federal do Rio de Janeiro (RJ, Brazil), [2]Physics, University of Auburn (, USA)

INTRODUCTION: Malaria is an infectious disease which contaminates at least 200 million people, killing 600 thousand of them in previous years. Infection in humans is caused by parasites Plasmodium spp., especially the species P. falciparum and P. vivax. Increasing resistance to treatment of their infections has hampered the combat of this disease. In Brazil, the most important agent is P. vivax motivating the finding of new biological targets and potential inhibitors. An important step for maintenance of the cycle of malaria in humans is the hydrolysis of haemoglobin. Enzymes involved in such process are papain-like cysteine proteases vivapains (VPs) of P. vivax representing interesting therapeutic targets. OBJECTIVES: Here, we have accessed the protein conformations by Molecular Dynamics (MD), exploring their catalytic and allosteric sites to find potential inhibitors through Virtual Screening (VS) assays. MATERIALS AND METHODS: Comparative modelling of VPs was done with Modeller based on the experimentally solved structure of FP3 from P. falciparum, which presents 60% of identity. Both apo and protein-ligand structures were simulated in replicates up to microseconds using Amber18. Mapping of key residues for ligand interaction was done combining docking of molecular fragments on protein surface and mixed solvent (Water-70%/Ethanol- 30%) simulations. DISCUSSION AND RESULTS: We found correlation of volume variation between catalytic and allosteric sites of VP4 from MD simulations, indicating a possible mechanical allosteric mechanism. VS assays were done against molecules dataset at catalytic and allosteric sites using a clustered structure from the simulations. For VP4, the ranking of ligands was reevaluated with machine-learning algorithms, finding several potential inhibitors from the LASSBio-UFRJ dataset. CONCLUSION: Analysis of the complexes pointed as key residues Phe83, Tyr159 and Lys36, Glu54 from the catalytic and allosteric sites, respectively, for inhibitor specific chemical groups interactions, important for further ligand optimisation.

Keywords: drug design, molecular dynamics, malária / **Supported by:** CAPES

G.20 - The influence of cationic amphiphilic on the inner structure of cubosomes: ionic liquid versus cationic surfactants

Amanda Santos Palma [1], Raphael Castro Dias [1,2], Barbara Malheiros [2], Mayra Cristina Gomes Lotierzo[2], Bruna Casadei[1], Leandro Ramos Souza Barbosa[1,2,3]
[1]Department of General Physics , University of São Paulo (, Brazil), [2]Department of Biochemical and Pharmaceutical Technology, University of São Paulo (, Brazil), [3]Brazilian Synchrotron Light Laboratory (LNLS), Centro Nacional de pesquisas em energia e materiais (, Brazil)

INTRODUCTION: Cubosomes are nanoparticles that can be used as a drug delivery system. They are able to encapsulate hydrophilic, hydrophobic and amphiphilic drugs for targeted delivery. Moreover, cubosomes can reduce the side effects and enhance the drug quantity delivered. Cubosomes are composed of a lipid (Phytantriol) and a polymer (F127) to act as a stabilizer. In this study, we used these nanoparticles to encapsulate lysozyme, a protein capable of destroying gram-negative bacteria as it destabilizes the cell membrane. Above all, ionic liquid and a cationic surfactant will also be added to produce a cationic nanoparticle. This combination can be suitable for gene therapy as well. OBJECTIVES: In this study, we aim to verify the interference of ionic liquid and a cationic surfactant in the cubosome inner structure and see how it interferes with the size and polydispersity in the presence of lysozyme. MATERIALS AND METHODS: Cubosomes will be produced with Phytantriol (lipid) and F127 (stabilizer) using the bottom-up approach. When the samples are ready, [C14min][CL] (ionic liquid) and TTAB (cationic surfactant) will be added separately. In these samples, we will vary the concentration of lysozyme. The samples will be characterized by: DLS, ζ-Potential and SAXS. DISCUSSION AND RESULTS: This project is still under development and the data will be shown in the meeting. Observing the results analyzed by mr. Raphael Dias Castro, one can conclude that the presence of both TTAB and IL interfere in the cubosome size, making them larger (twice its original size). One can also say that TTAB and IL give the cubosomes a cationic profile. CONCLUSION: Both TTAB and IL interfere in size and ζ-potential, altering its size and charge in the shear plane. We expect that the lysozyme does not interfere as much as the mentioned components. The results obtained by Mr. Raphael Dias Castro are expected, given the structure of the added molecules.

Keywords: cubosomes, lysozyme, DLS

G.21 - Dual-Responsive Polymersomes as Chemotherapeutic Drug Nanocarriers for the Co-Delivery of Vemurafenib and Doxorubicin

Natália Aimée D'Angelo[1], Carlota Rangel-Yagui[2], **André Moreni Lopes [1]**
[1]*Não possui Depto, Universidade Estadual de Campinas - UNICAMP (SP, Brasil), [2]Departamento de Tecnologia Biquímico-Farmacêutica, Universidade de São Paulo - USP (SP, Brasil)

INTRODUCTION: Nanocarriers for co-encapsulation of chemotherapeutic drugs have been widely studied for the last years due to their important advantages, e.g. improved bioavailability and cellular uptake, increased solubility of drugs, and lower chances of multidrug resistance (MDR). OBJECTIVES: In this context, this work describes the co-encapsulation of two chemotherapeutic drugs [vemurafenib (VEM) and doxorubicin (DOX)] in poly(ethylene glycol)-b-poly(ε-caprolactone) (PEG-PCL)-based polymersomes (Ps), as well as the drug release behavior from the PEG-PCL-based Ps under tumor and physiological conditions. MATERIALS AND METHODS: The chemotherapeutic drugs were co-encapsulated in three PEG-PCL copolymers, namely PEG45-PCL44, PEG114-PCL98, and PEG114-PCL114. In parallel, we also evaluated the stability of the Ps nanoformulations in different temperatures (4, 25, and 37 °C) versus different period of time (0 to 30 d). DISCUSSION AND RESULTS: According to our results, co-encapsulation of DOX and VEM resulted in drug loading (DL) ranges of 12 to 18% and 16 to 26% and encapsulation efficiency (EE) of 35 to 39% and 43 to 55%, for DOX and VEM, respectively. The Ps nanoformulations were stable at 4, 25, and 37 °C and the release of drugs was faster with the smaller PEG-PCL blocks (i.e., PEG45-PCL44 > PEG114-PCL98 > PEG114-PCL114). The PEG-PCL-based Ps also demonstrated higher drugs release in an acidic environment (i.e., pH 5.0 at 37 °C, found in tumor cells) compared to physiological conditions (pH 7.4 at 37 °C). CONCLUSION: In conclusion, the VEM+DOX-PEG-PCL-based Ps may be a promising approach to cancer therapy with potential synergic effect, lower dosage, and lower risk of causing MDR.
Keywords: drug delivery systems (DDS), polymersomes (Ps), self-assembly / **Supported by:** FAPESP/Brazil

G.22 - Development and Characterization of Nanostructured Lipid Carriers Loaded with Docetaxel for the Treatment of Breast Cancer

Fabíola Vieira de Carvalho [1], Lígia Nunes de Morais Ribeiro[1,2], Ludmilla David de Moura[1], Gustavo Henrique Rodrigues da Silva[1], Gabriela Geronimo[1], Talita Cesarim Mendonça[1], Hery Mitsutake[3], Marcia Cristina Breitkreitz[3], Eneida de Paula[1]
[1]Department of Biochemistry and Tissue Biology, Institute of Biology (São Paulo, Brazil), [2]Institute of Biotechnology (MInas Gerais, Brazil), [3]-, Institute of Chemistry (São Paulo, Brazil)

INTRODUCTION: Breast cancer is the cancer with the highest incidence in women worldwide. Docetaxel (DTX) is a taxoid antineoplastic agent which mechanism of action involves inhibition of microtubule formation and angiogenesis plus induction of apoptosis. Technological strategies can improve the effectiveness of chemotherapy by promoting sustained release or directing the drug to tumor cells, thus reducing its systemic toxicity. Nanostructured lipid carriers (NLC) are capable of encapsulating hydrophobic drugs in their blend-of-lipids matrix, which imperfections prevent early drug expulsion and increase the colloidal stability of the nanoparticles. OBJECTIVES: To develop/characterize NLCDTX using factorial design and physicochemical analyses. MATERIALS AND METHODS: A formulation of NLC composed of myristyl myristate, copaiba oil, Miglyol®, Pluronic F-68 and 0.5% DTX was developed. The stability of the formulation at room temperature (25º C) was evaluated for 8 months regarding size, polydispersity (PDI), zeta potential (ZP), nanoparticle concentration, pH and DTX encapsulation efficiency (%EE). The sustained release of encapsulated DTX was compared to that of the reference drug using Franz-type cells. DISCUSSION AND RESULTS: The optimized formulation had particles with homogenous distribution (PDI< 0.2) of sizes (190-230 nm) negative ZP (|25 – 35| mV), ca. 4.1013 particles/mL, pH ~4.9, and 100 %EE. TEM images showed spherical nanoparticles in which DSC and XRD tests demonstrated a decrease in lipid matrix structural organization, after drug encapsulation. Kinetic experiments revealed the sustained release of encapsulated DTX for 54 h, in comparison to the reference (10h). CONCLUSION: In the search for a less toxic and more effective treatment for breast cancer we optimized a novel NLCDTX formulation with the natural excipient copaiba oil - with intrinsic anticancer and analgesic properties. *In vitro* the formulation promoted sustained release of DTX, as desirable to diminish the serum levels/systemic toxicity. These pharmacological properties are now being tested over normal and breast cancer cell lines.
Keywords: Breast cancer, Docetaxel, Drug delivery
Supported by: Capes, FAPESP

G.23 - Synthesis and characterization of peptide bioconjugates with antitumor activities

Letícia Oliveira Catarin Nunes[1], Geovana Navegante[1], Maria Carolina Oliveira Arruda Brasil[2], Valeria Valente[1], Eduardo Maffud Cilli[2], **Norival Alves Santos-Filho [1,2]**
[1]São Paulo State University (UNESP), School of Pharmaceutical Sciences (Sao Paulo, Brazil), [2]Department of Biochemistry and Organic Chemistry, Chemistry Institute (Sao Paulo, Brazil)

INTRODUCTION: Anticancer peptides emerge as tools of interest in anticancer therapy, considering the toxicity and limited success of traditional treatments. However, the low specificity and high toxicity of peptides on normal cells, lead to the search to work with anticancer peptides bioconjugated with aptamer peptides. Aptamers are small molecules that show high selectivity to cell membrane markers, such as CD44 membrane glycoprotein. OBJECTIVES: The objective of this work is synthesize peptide bioconjugates between Melittin, a recognized anticancer and cytotoxic peptide, and an aptamer peptide that binds to the tumor marker CD44, as well as to evaluate their binding potential in cell lines, such as breast adenocarcinoma (MCF-7, ATCC HTB-22), lung adenocarcinoma (A549, ATCC CCL-185) and non-tumor lung cells (MRC-5, ATCC CCL-171). MATERIALS AND METHODS: Peptides were synthesized by solid phase peptide synthesis and purified by high performance liquid chromatography. To analyze peptide binding, cell cultures were submitted to automated microscopy using the In-Cell Analyzer. Cell viability assay was performed using the MTT technique (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium) for the three tested strains. DISCUSSION AND RESULTS: The bioconjugates were synthesized with three different strategies, being the aptamer linked to the N-terminus or C-terminus of Melittin and the aptamer linked to the N-terminus of Melittin, however separated by a spacer, to analyze whether this spacing could give better mobility to the molecules. The analysis by In Cell Analyzer, showed evident binding at different concentrations. In the cell viability assay, it was possible to conclude that the bioconjugates present better specificity than Melittin. Despite the conjugation of Melittin with the aptamers, the molecules did not show changes in their secondary structures. CONCLUSION: Considering the search for alternative therapies for the treatment of cancer, aptamers appear as an innovative strategy for the localization of specific molecular targets in tumor cells.
Keywords: CD44, Melittin, Peptides

G.24 - Immobilization of SARS-CoV-2 Spike receptor-binding domain on Polyhydroxybutyrate Nanoparticles

Maritza Araujo Todo Bom [1], Maria Luisa T. Budel[1], Emanuel Maltempi de Souza[1], Marcelo Müller dos Santos[1]
[1]Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Paraná (PR, Brasil)

INTRODUCTION: The production of vaccines from proteins or viral protein fragments, such as SARS-CoV-2 Spike protein or its receptor-binding domain (RBD) has attracted worldwide attention due to the COVID-19 pandemic. However, the use of proteins as antigens in vaccines generally requires an adjuvant that provides stability and reduces protein degradation. PHB nanoparticles are a viable alternative for protein immobilization because they are biocompatible and biodegradable. PHB is also economical when compared to other polymeric materials, as it can be industrially produced by microbial fermentation using low-cost agro-industrial inputs. OBJECTIVES: Over-express recombinant SARS-CoV-2 Spike RBD anchored to the PHB-binding SBD tag of Alcaligenes faecalis PHA depolymerase for immobilization on PHB nanoparticles. MATERIALS AND METHODS: E. coli BL21(λDE3) carrying pET6HisRBD-SBD was cultivated in LB and protein expression was auto-induced at 30°C for 24 hours. PHB nanoparticles were produced by nanoprecipitation and concentrated by centrifugation. For the immobilization assay, 50 µL of RBD-SBD (0.3 µg mL-1) was mixed with 50 µL of nanoparticles for 5 minutes at room temperature. After the incubation, the nanoparticles were washed and ressuspended in SDS sample buffer. Nanoparticles and supernatants were assessed by SDS-PAGE and Bradford quantification. Recognition of RBD-SBD by anti-RBD IgG was tested by modified ELISA. DISCUSSION AND RESULTS: The SBD tag prove to be efficient in the immobilization of the recombinant RBD in PHB nanoparticles. Over-expressed RBD-SBD was recognized by anti-RBD IgG antibodies in modified ELISAs using immobilized nanoparticles, which suggests that the protein-antibody interaction was not impaired by the immobilization of RBD. CONCLUSION: These results show the potential of PHB nanoparticles as an antigen carrier in immunological tests as well as vehicles for vaccines against COVID-19 or other infections.
Keywords: SARS-CoV-2, polyhydroxybutyrate, nanoparticles
Supported by: CNPq, CAPES and Fundação Araucária

G.25 - Designing SARS-CoV-2 Virus Major Protease (Mpro) Allosteric Inhibitors as Potential Treatments for COVID-19

Luiza Valença Barreto [1], Mariana Laureano Souza[1], Rafaela Salgado Ferreira[1]
[1]Departamento de Bioquímica e Imunologia, Universidade Federal de Minas Gerais (Minas Gerais, Brasil)

INTRODUCTION: Infection by the SARS-CoV-2 virus can result in acute respiratory distress syndrome, leading to a long-term reduction in lung function, arrhythmia, or death. To devise therapeutic strategies to neutralize SARS-CoV-2 infection and the associated COVID-19 pathology, it is crucial to develop new drugs. Thus, we propose the development of SARS-CoV-2 major protease (Mpro) allosteric inhibitors. Mpro is a conserved cysteine protease in the coronavirus family, that cleaves the pp1A and pp1B polyproteins in at least 11 conserved sites, playing a key role in viral replication. OBJECTIVES: Two allosteric sites have already been described for this protease, based on crystallographic structures in complex with inhibitors. In this study, we propose the identification of allosteric Mpro inhibitors through virtual screening by molecular docking. MATERIALS AND METHODS: To select a docking protocol, we performed redocking and crossdocking studies with AutoDock Vina and DockThor. Then, we virtually screened two libraries from collaborating groups, each composed of about a thousand compounds, by docking the molecules at the two allosteric sites. DISCUSSION AND RESULTS: Considering the better performance of DockThor to reproduce known binding modes of allosteric inhibitors, the screening was performed through the DockThor portal. The results were evaluated using the UCSF Chimera software, searching for compounds predicted to perform interactions observed in crystallographic complexes and considering the overall complementarity between the ligands and the binding site. CONCLUSION: The compounds prioritized will be validated through enzymatic assays with Mpro.
Keywords: COVID-19, Drug Design, Mpro protease / **Supported by:** CAPES, FAPEMIG, CNPq

G.26 - Synthesis of new copper complexes using natural products as ligands with anticancer activity.

Rafael Nunes[1], Giselle Cerchiaro[1]
[1]Centro de ciências naturais e humanas, Universidade Federal do ABC (São Paulo, Brazil)

INTRODUCTION: Metal complexes are interesting for the treatment of cancer, due to the unique properties of metals, including the redox potential, several possible coordination structures with different types of ligands, the most diverse geometries, in addition to different reactivity with biomolecules. The discovery of cisplatin decades ago represented a significant advance in the search for cancer therapies. This made efforts to develop compounds with other structures and use other metallic centers. In this context, the present work seeks to synthesize new copper complexes with imine ligands from natural aldehydes, such as vanillin, cinnamaldehyde, and ethylvanillin. OBJECTIVES: The project aims at the synthesis and characterization of new copper imines using natural aldehydes. Investigate the anticancer action of these complexes *In vitro* tests. MATERIALS AND METHODS: The ligands were obtained by reacting these aldehydes with the amines 1,3-Diaminopropane, 2,2-dimethyl-1,3-propanediamine, and 1,3-diamino-2-propanol. The complexation reaction was conducted with copper in perchlorate salt, and six imine copper complexes were obtained. The complexes were characterized using infrared and UV-vis spectroscopy, elemental analysis, mass spectrometry, and nuclear paramagnetic resonance. The cytotoxicity of the complexes was evaluated using the MTT assay on neuroblastoma cell lines SH-SY5Y, CHP 212 and LN-18. ISCUSSION AND RESULTS: The complexes presented a structure with a proportion of a ligand to one metallic center. The *In vitro* assays showed the cytotoxic potential of the complexes for the neuroblastoma cell lines, presenting IC50 values between 90 and 300μM. On the other hand, the isolated ligands showed lower toxicity. CONCLUSION: The newly synthesized copper imine complexes showed toxicity against neuroblastoma tumor cells, and the presence of copper in the structure increased the *In vitro* activity of these imine compounds.
Keywords: metal complexes, cancer, copper
Supported by: FAPESP, CAPES

G.27 - The Mesoionic Compound MI-D Impairs Tumor Cell-induced Angiogenesis *in vitro*

Ronaldo Figueira De Oliveira [1], Luiz Fernando Pereira[1], Selene Elifio-Esposito[1]
[1]School of Life Sciences, Pontifícia Universidade Católica do Paraná (Paraná, Brazil)

INTRODUCTION: Angiogenesis is the formation of new vessels from the pre-existing vasculature and is one of the essential processes for tumor progression. The mesoionic compound MI-D acts as a mitochondrial uncoupler and has presented antitumor and antiangiogenic effects when used to treat colon carcinoma cells grafted onto the chicken chorioallantoic membrane. OBJECTIVES: The present study aims to evaluate the effects of MI-D (3.12 and 6.25 μM) on the angiogenic capacity of human endothelial cells (EA.hy926) and its ability to inhibit angiogenesis induced by colorectal carcinoma cells (HT29) *in vitro*. MATERIALS AND METHODS: The induction of apoptosis in endothelial cells by MI-D was measured with Annexin-V/ 7-AAD staining by flow cytometry, while the migratory capacity was assessed by scratch wound healing assay for 24 and 30 hours. *In vitro* angiogenesis was analyzed by the ability of endothelial cells to form capillary-like tubules in a gelatinous matrix after MI-D incubation alone or in a conditioned media generated from HT29 cells culture. DISCUSSION AND RESULTS: MI-D induced apoptosis in 85% of the endothelial cells at the highest concentration tested and inhibited cell migration in both the 24 and 30-hour periods. For *In vitro* angiogenesis tests, when applied only with the culture medium, the drug concentrations of 3.12 and 6.25 μM reduced the formation of capillary-like structures, by 25% and 34% respectively. However, only the highest concentration of the compound (6.25 μM) maintained the antiangiogenic action, by reducing the number of tubule like structures, when the tumor cell-conditioned medium was used. CONCLUSION: In this study, MI-D interfered in essential aspects of forming new vessels by endothelial cells, such as maintenance of viability, migration, and formation of tubular structures, which reinforces the suggestion that the compound acts as an inhibitor of angiogenesis.
Keywords: Angiogenesis, Mesoionic compounds, Cytotoxicity / **Supported by:** PUCPR

G.28 - Identification of Zika virus NS2B-NS3 protease inhibitors through a high-throughput fluorescence-based assay

Isabela Dolci [1], Gabriela Noske[1], Ellen Silva[1], Andre de Godoy[1], Glaucius Oliva[1], Rafaela Fernandes[1]
[1]Physics Institute of Sao Carlos, University of São paulo (São Paulo, Brazil)

INTRODUCTION: Zika virus (ZIKV) is the causing agent of zika fever, a mosquito-borne disease that became a public health problem in 2015/2016 in the Americas and mainly in Brazil, where it was related to the cases of microcephaly and Guillain-Barré syndrome. The potential of the virus to re-emerge causing new epidemics is always latent and, therefore, there is an urgent need for the discovery and development of specific treatments against viral infection, since there are still no vaccines or antivirals available. Viral enzyme-based assays are useful strategies to test large compound libraries for ZIKV inhibitors. NS3 protease domain, which requires NS2B as its cofactor, is responsible for the cleavage of the immature viral polyprotein into the mature NS proteins, thus, representing an interesting target for drug development OBJECTIVES: The objective of this work was to evaluate 6000 small-molecule compounds as inhibitors of the recombinantly expressed NS3 protease using the fluorogenic peptide substrate, Bz-nKRR-AMC. MATERIALS AND METHODS: ZIKV protease containing the 45-96 residues of NS2B cofactor linked to residues 1-177 of NS3 protease domain by a glycine rich linker [G4SG4], was expressed in E. coli, purified by nickel-affinity and molecular exclusion chromatography and had its enzymatic activity validated using aprotinin, a protein already described as an inhibitor of flavivirus proteases. Next, we used the purified enzyme in the fluorescence-based HTS in 384 well-plate-format. DISCUSSION AND RESULTS: We identified 7 compounds capable of inhibiting NS2B-NS3 activity in more than 80% at 10 μM, that will be further evaluated in a concentration-dependent manner to determine their half-maximal inhibitory concentrations (IC50). CONCLUSION: The HTS fluorescence-based assay used in this work has shown to be an effective approach for the rapid identification of potential inhibitors of ZIKV protease.
Keywords: ZIKV, NS2B-NS3, drug Discovery / **Supported by:** FAPESP

G.29 - Development of a Liposomal Remdesivir Formulation, through Evaluation of Different Methods and Lipid Compositions

Sabrina Mendes Silva Araujo [1], Guilherme Santos Ramos[1], Virgínia Mendes Russo Vallejos[1], Anderson Kenedy Santos[1], Pedro Pires Goulart Guimarães[1], Frédéric Jean Georges Frézard[1]
[1]Department of Physiology and Biophysics, Universidade Federal de Minas Gerais (Minas Gerais, Brazil)

INTRODUCTION: Remdesivir (RDV), used as drug / cyclodextrin (CD) complex given intravenously, has been presented as a promising antiviral drug against COVID-19, but it showed toxicity and low efficacy in clinical studies. The encapsulation of drugs into liposomes is an effective means to improve their therapeutic index. However, achieving sustained drug release properties from liposomes is challenging for amphiphilic drugs such as RDV. OBJECTIVES: To develop and compare different liposomal formulations of RDV and select the most promising formulation for comparison of cytotoxicity with conventional RDV/CD formulation. MATERIALS AND METHODS: RDV was encapsulated into conventional or PEGylated liposomes, using either the Dehydration/Rehydration (DR-CONV and DR-PEG formulations) or the Ethanol Injection (EI-CONV and EI-PEG formulations) method. DR-CONV and DR-PEG formulations consisted of the RDV/CD complex incorporated into the aqueous compartment of the liposomes, whereas EI-CONV and EI-PEG formulations incorporated RDV in the membrane. Particle size distribution was analyzed by DLS. The drug encapsulation efficiency (EE) was determined by UV spectrophotometry. The kinetic of drug release was assessed in conditions of dialysis at 37oC. Cytotoxicity on Vero-E6 and Calu-3 cell lines was evaluated using the resazurin assay. DISCUSSION AND RESULTS: All liposome formulations showed monodisperse vesicle populations with mean diameters in the range of 100-150 nm. EE was lower in DR-CONV and DR-PEG (35%) than in EI-CONV and EI-PEG (55%). EI-CONV showed a longer half-time of drug release (5.3 h), when compared to EI-PEG (2.5 h), DR-CONV (3.6 h) and DR-PEG (2.4 h). The selected EI-CONV formulation showed lower cytotoxicities on Calu-3 and Vero-E6 cells (IC50 = 1.038; 934 µM), in comparison to the conventional RDV/CD formulation (IC50 = 239; 268 µM). CONCLUSION: A novel liposomal formulation of RDV has been developed with the drug incorporated into the membrane, sustained drug release properties and lower cytotoxicity, compared to the conventional drug formulation.
Keywords: cytotoxicity, liposomes, remdesivir / **Supported by:** CAPES and CNPq

G.30 - Leishmanicidal effect of eugenol derivatives on Leishmania braziliensis *In vitro* macrophage infection

Larissa Coelho Pereira [1], Luciana Ângelo de Souza[1], Róbson Ricardo Teixeira[2], Roberta da Silva Evangelista[2], Gustavo Costa Bressan [1], Juliana Lopes Rangel Fietto[1]
[1]Bioquímica e Biologia Molecular , Universidade Federal de Viçosa (Minas Gerais , Brasil), [2]Química, Universidade Federal de Viçosa (Minas Gerais , Brasil)

INTRODUCTION: Leishmaniases are vector-borne parasitic diseases caused by pathogenic species of *Leishmania*. *Leishmania braziliensis* is the main causative agent of American Tegumentar Leishmaniasis (LTA). LTA chemotherapy is compromised by the high cost, high toxicity, and development of parasite resistance, which has led to the need to develop new therapies. OBJECTIVES: The main goal of this work is to evaluate the action of eugenol-derived compounds associated with a triazole ring as possible leishmanicidal agents against *L. braziliensis* as possible new therapeutic targets for the treatment of Cutaneous Leishmaniasis. MATERIALS AND METHODS: Toxicity to model host cells was done by resazurin method and phenotypic assays were done by plate fluorescence method using green fluorescent *L. braziliensis* – M2904-GFP and macrophages Raw 264.7 as host cells. Amphotericin B was used as a positive control of Leishmanicidal action and a total of 22 eugenol-derived compounds containing 1,2,3-triazole nuclei were screened at 10µM. The treatments were done for 48h. The Cytotoxic Concentration (CC50), Effective concentration (EC50), and Selectivity Index (SI) were determined as the best two compounds. DISCUSSION AND RESULTS: None of the compounds were toxic to macrophages. Compounds RE21 and RE27 showed a significant leishmanicidal effect, EC50 of 28.09 µM and 52.03 µM, respectively. The SI was 9.9 for RE21 and > 5.7 for RE27. CONCLUSION: Compounds derived from eugenol associated with a triazole ring have potential as possible new leishmanicidal agents against *L. braziliensis* and could be useful to the development of new treatments for Cutaneous Leishmaniasis.
Keywords: Eugenol, Leishmania, Leishmaniasis

G.31 - Fluorescent labeled liposomes decorated with iron-nanoparticles for theragnostic application

Norma Lucía Buriticá Zuluaga [1], Gustavo Penteado Battesini Carretero[1], Koiti Araki[2], Alcindo Aparecido Dos Santos[2], Robson Raphael Guimarães[2], Beatriz dos Santos Cugnasca[2], Iolanda Midea Cuccovia[1]
[1]Depto de Bioquímica, Univ. de São Paulo (São Paulo, Brazil), [2]Depto de Química, Unive. de São Paulo (São Paulo, Brazil)

INTRODUCTION: Nanomedicine is a critical tool in therapeutic targeting. Several nanomedicines, such as monoclonal antibodies, liposomes, micelles, and dendrimers, are widely used in treatment and diagnosis. Recently, nanomedicine has gained attention owing to its imaging potential. OBJECTIVES: Obtain a conjugated nanostructure constituted of a lipid liposome scaffold, iron oxide nanoparticles to allow image tracking, and loaded with a fluorescent marker as a sensor. This nanostructure could provide a high sensitivity and precision theragnostic tool. MATERIALS AND METHODS: Large unilamellar vesicles (LUV) with an average size of 100 nm, composed of dioleyl phosphatidylcholine (POPC) and the positively charged lipid 1,2-Dioleoyl-Glycero-3-Phosphocholine (DOTAP), were prepared by extrusion through polycarbonate filters. LUVs diameter and zeta potential were measured by Dynamic Light Scattering, in a Zetasizer Nano 317. 4,4-Difluoro-4-bora-3a, 4a-diaza-s-indacene (BODIPY) and the iron oxide nanoparticles were prepared using standard procedures. Fluorescence was measured in a Hitachi F 7000 fluorimeter. DISCUSSION AND RESULTS: Extruded LUV presented an average diameter of 123,7 nm, PdI of 0,121 and ZP of + 56,5 mV; sequential addition of the NP changed the measured properties to 256,7 nm, 0,321, and -35,1 mV. This confirmed LUV and NP interaction and indicated that at a 0,4 NP/LUV ratio (m/m), LUV surface is saturated with NP. BODIPY fluorescence spectra (exc: 440nm) presents a maximum at 515 nm; addition of LUV increased emission without shifting the maximum. This confirmed LUV and BODIPY interaction and allowed us to obtain binding isotherms of this interaction. Interaction with LUV, NP, and LUV-NP aggregates was studied by varying BODIPY concentration in presence of these aggregates and confirmed the interaction of BODIPY with LUV and LUV-NP and no BODIPY and NP interaction. CONCLUSION: We obtained a stable Liposome composite decorated with iron nanoparticles and a fluorescent probe, a promising nanocomposite for theragnostic applications.

Keywords: Nanoparticles, Liposomes, Theragnostic / **Supported by:** FAPESP, CNPq

G.32 - Gold Nanoparticles Ameliorate Renal Function and Tubule-Interstitial Injury Induced by Subclinical Acute Kidney Injury in Mice

Rodrigo A. da Silva Peres [1], Rodrigo P.da Silva Aguiar[1], Douglas Esteves Teixeira[1], Diogo de Barros Peruchetti[1], Guilherme Ferreira de Castro[1], Ana Beatriz Chasco Leal [1], Natalia Barreto da Silva Ribeiro[2], Yago Amigo Pinho Jannini de Sá[2], Marco Aurélio Martins[2,3], Patricia Machado Rodrigues e Silva Martins[2,3], Ana Acácia de Sá Pinheiro[1,3], Celso Caruso Neves[1,3,4]
[1]Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro (RJ, Brazil), [2]Oswaldo Cruz Institute, Oswaldo Cruz Foundation (FIOCRUZ) (Rio de Janeiro, Brazil), [3], Rio de Janeiro Innovation Network in Nanosystems for Health-NanoSAÚDE/FAPERJ (RJ, Brazil), [4], National Institute of Science and Technology for Regenerative Medicine (RJ, Brazil)

INTRODUCTION: Subclinical acute kidney injury (subAKI) is a syndrome characterized by tubular-interstitial injury (TII) associated with a pro-inflammatory phenotype in the absence of apparent changes in glomerular function. subAKI is a risk factor for the development of acute and chronic kidney disease. Therefore, the identification and the precocious treatment could help to halt the development of more severe renal disease. Pharmacological applications of gold nanoparticles (AuNPs) have been studied for the treatment of different inflammatory diseases, but its effect on the renal disease still unknown. OBJECTIVES: We aimed to investigate the effects of AuNP in renal function of subAKI animal model. MATERIALS AND METHODS: subAKI model was developed in 8-10 weeks-old C57BL/6 male mice by daily injection of BSA (i.p., 10g/kg/day) for 7 days (CEUA-045/17). When indicated mice received concomitantly an intraperitoneal injection of AuNP suspension (10µ/Kg). At day 6, mice (n=5/group) were kept in metabolic cages for 24 h. Blood and urine were collected for functional analysis. DISCUSSION AND RESULTS: Glomerular function, measured by plasma creatinine, blood urea nitrogen, and creatinine clearance, was not changed in subAKI, AuNP and subAKI+AuNP groups. However, markers of tubular injury such as urinary LDH, proteinuria (24h), protein-to-creatinine ratio (UPCr) were significantly increased in subAKI (51.72±19.40 AU, 3.66±1.35 mg/24h, 9.65±4.57, respectively) when compared to control (21.45±9.75 AU, 1.38±1.02 mg/24h, 4.22±1.07, respectively). These effects were partially avoided in subAKI+AuNP group (29.65±19.40, 1.86±0.87 mg/24h, 4.77±1.69, respectively). Additionally, subAKI group presented albuminuria, measured by urine immunoblotting, what was partially avoided in subAKI+AuNP group. subAKI animals showed reduced expression of megalin associated to a reduced reabsorption of FITC-albumin. AuNP treatment improved megalin expression and FITC-albumin uptake. CONCLUSION: These data indicate that AuNP treatment ameliorates TII observed in subAKI opening new possibilities to the precocious treatment of this syndrome.

Keywords: Kidney function, tubule-interstitial injury, gold nanoparticles / **Supported by:** CAPES, CNPq, FAPERJ

G.33 - Discovery and Characterization of Anti-*Trypanosoma cruzi* Compounds by Docking-Based Virtual Screening

Viviane Corrêa Santos [1], Paulo Gaio[1], Fabiana Simões Machado[1], Rafaela Salgado Ferreira[1]
[1]Departamento de Bioquímica e Imunologia, Universidade Federal de Minas Gerais (Minas Gerais, Brasil)

INTRODUCTION: Chagas disease affects millions of people on the Americas. The only two approved anti-*Trypanosoma cruzi* medicines lack efficacy in the chronic phase of the disease and have several side effects. Thus, it is important the development of medicines with a better pharmaceutical profile. The cysteine protease cruzipain is a widely studied target in *T. cruzi*; it is associated with host cell invasion, parasite differentiation, and macrophage response modulation. Cruzipain is divided into two subfamilies: Family I, more expressed in the epimastigote form and Family II, more expressed in the amastigote and trypomastigote forms. OBJECTIVES: Discovery and characterization of cruzain inhibitors as anti-*Trypanosoma cruzi* compounds. MATERIALS AND METHODS: We screened 372,632 molecules from the Leads Now subset of ZINC12 database with docking and assayed 17 against cruzain, the recombinant form of a Family I cruzipain. DISCUSSION AND RESULTS: We identified a competitive cruzain inhibitor named VS17 (potency measured as IC_{50} = 3.0 ± 2 μM and K*i* = 4.6 μM). Molecular dynamics simulations of VS17 indicate that the S-isomer is the most stable in its interaction with the enzyme's active site. Based on these results, we designed VS17 analogs that were docked and evaluated in biochemical assays; they revealed that the furan ring is essential for enzymatic activity and that oxygen cannot replace the nitrogen in the tetrahydroisoquinoline ring. Fluorine insertion into the tetrahydroisoquinoline ring (analog VS31) was tolerated, but its activity against cruzain decreased (IC_{50} = 106 ± 30 μM). VS17 and VS31 inhibited the release of trypomastigotes from macrophages infected with *T. cruzi*. However, VS31 (IC_{50} = 10 μM) was more potent than VS17 (potency could not be determined). They also inhibit epimastigote growth in culture, but VS17 (IC_{50} = 8 μM) had higher potency in this assay. CONCLUSION: These data might indicate that VS17 targets primarily Family I cruzipains and VS31, Family II.

Keywords: Chagas disease, cruzain, docking /**Supported by:** CAPES, CNPq, FAPEMIG, DAAD, L'oreal-UNESCO

G.34 - SEARCHING FOR NEW CRYSTALLIZATION CONDITIONS IN X-RAY CRYSTALLOGRAPHY FRAGMENT SCREENING AGAINST HUMAN PRION PROTEIN

Festus Adebayo Atiba [1], Maria Cristina Nonato[1]
[1]Laboratory of Protein Crystallography, Department of Biomolecular Sciences, School of Pharmaceutical Sciences, Universidade de São Paulo, Ribeirao Preto (Sao Paulo, Brazil)

INTRODUCTION: Prion diseases are progressive neurodegenerative diseases that affect both humans and animals. They are rare and affect only a small fraction of populations around the world with no adequate drug therapies to combat the diseases. Prion diseases are caused by the misfolding of the cellular prion protein PrP, into a self-templating conformer called scrapie prion protein, PrPsc. PrP has no validated small molecule binders, no known binding pockets related to its native function, and does not belong to any class of proteins that have previously been successfully drugged. OBJECTIVES: This project aims to exploit fragment screening by X-ray crystallography (FSXC) as a technique to identify potential druggable spots in human PrP. As a first step, our aim was to identify PrP crystallization conditions that can be adequate for FSXC. MATERIALS AND METHODS: We search for conditions with minimum concentrations of salt and additives (CdCl2) to avoid undesirable interactions with the protein that could prevent fragment binding. DISCUSSION AND RESULTS: We were able to reproducibly replace NaCl with PEG 6K and decrease the concentration of CdCl2 to 1mM. Preliminary data sets were collected at Soleil Synchrotron Source and crystals diffracted at high resolution (1.89A and 2.0A). Data was phased and partially refined and structural analysis revealed that our model is homologous to PDBIDs: 3HAF and 3HER. CONCLUSION: Fragment screening testing a library of 602 fragments provided by Prof. Marcio Diaz from ICB-USP is currently underway. We hope to unprecedentedly identify novel ligands and pockets for ligand interaction on human PrP as an innovative strategy to treat prion diseases.

Keywords: Fragment screening, Human Prion Protein (PrP), X-ray Crystallography.
Supported by: CAPES

G.35 - Identification of Immunomodulatory drugs that Inhibit Multiple Inflammasomes and impair SARS-CoV-2 infection

Letícia de Almeida [1], Tamara S Rodrigues[1], Adriene Y Ish imoto[1], Amanda A Seribelli[1], Amanda Becerra[1], Camila C S Caetano[1], Keyla S G de Sá[1], Samuel Oliveira[1], Marco A A Silva[1], Dario S Zamboni[1]
[1]Depto de Biologia Celular e Molecular e Bioagentes Patogênicos, Faculdade de Medicina de Ribeirao Preto (São Paulo, Brazil)

INTRODUCTION: Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) caused the COVID-19, an infectious and inflammatory pandemic disease that has affected more than 360 million people worldwide, leading to more than 5.6 million deaths. Although the disease is mild or asymptomatic in most cases, some patients develop severe inflammation mediated by inflammasomes. As the NLRP3 inflammasome and additional inflammasomes are implicated in disease aggravation, drug repositioning to target inflammasomes emerges as an important strategy to treat COVID-19. OBJECTIVES: The aim of this research was to perform a screening compound library to identify inflammasome inhibitors. MATERIALS AND METHODS: We developed a high throughput screening using pore formation as a read-out to evaluate the inflammasome inhibition. DISCUSSION AND RESULTS: In this high-throughput screening we identified 3 FDA-approved drugs that function as pan-inflammasome inhibitors. Our best hit, **c618**, effectively inhibits both inflammasome activation and SARS-CoV-2 replication. **c618** potently inhibited inflammasome activation in human monocytes infected *In vitro* and PBMCs from COVID-19 patients. CONCLUSION: This study provides relevant information regarding the immunomodulatory mechanisms of function of this highly promising drug for the treatment of inflammatory viral diseases including COVID-19.
Keywords: Drug screening, SARS-CoV-2, Inflammasomes
Supported by: CNPq, FAPESP

G.36 - Search of Selective Inhibitors for Human Dihydroorotate Dehydrogenase as a Strategy in Fighting Against COVID-19

Maria Cristina Nonato [1]
[1]Ciências Biomoleculares, Faculdade de Ciências Farmacêuticas de Ribeirão Preto - USP (SP, Brasil)

INTRODUCTION: This proposal aims at contributing to the development of an innovative therapy against COVID-19, either through drug repurposing or by identifying new chemical identities, all based on the selective inhibition of the human enzyme dihydroorotate dehydrogenase (HsDHODH). HsDHODH takes part of the de novo pyrimidine biosynthetic pathway and has been considered an attractive target for the development of antivirais. The fact that the virus relies on the host cell biochemical machinery to provide nucleosides for its viral replication cycle makes enzymes involved in the biosynthesis of nucleosides, such as HsDHODH, potential targets for the development of broad spectrum antivirals. OBJECTIVES: The specific objectives involve: cytotoxicity and *In vitro* antiviral assays ls in presence of selected inhibitors of human DHODH; Determination of the structure of HsDHODH-ligand complexes by X-ray crystallography; Synthesis of new analogues; Enzymatic and biophysical assays with the new analogues to identify ligands. MATERIALS AND METHODS: The cytotoxicity and antiviral assays were performed using using Calu-3 cells following the MTT assays. HsDHODH was expressed in E.coli and purified using affinity chromatography. Inhibiton assays were carried out using 2,6-dichloroindophenol (DCIP) as the final electron acceptor. Co-crystallization assays for HsDHODH were performed using vapour diffusion methods in sitting drops. Data collection was perfomed at Manaca beamline from Sirius. DISCUSSION AND RESULTS: HsDHODH activity was evaluated in presence of a atovaquone-based library of more than a hundred compounds. Inhibitors in nanomolar range were identified and evaluated regarding their citotoxicity and antiviral properties. Two compounds were selected for crystallographic studies and for lipid-based nanoparticle formulations. Brequinar, a known DHODH inhibitor, was used as a control in all experiments. CONCLUSION: Lapachol was identifed to inhibit HsDHOH with IC50 of 123 nM and CC50 of 29,23 uM against Calu-3 cells. At 1uM it inhibits 50% of viral replication. Crystallographic studies and structural optimization of HsDHODH-lapachol interaction is in progress.
Keywords: drug discovery, covid-19, Dihydroorotate dehydrogenase
Supported by: FAPESP

G.37 - PROJECT: Evaluation of the Antimicrobial and Cytotoxic Potential of Novel Cu(II) and Rh(III) Complexes of Thiosemicarbazones

Alice Rodrigues de Matos [1], Sandra Bertelli R. de Castro[2], Gabriella Freitas Ferreira[1], Jeferson Gomes da Silva[1]
[1]Departamento de Farmácia, Universidade Federal de Juiz de Fora / Campus Governador Valadares (Minas Gerais, Brazil), [2]Faculdade de Medicina do Mucuri, Universidade Federal dos Vales do Jequitinhonha e Mucuri (Minas Gerais, Brazil)

INTRODUCTION: Resistance to pre-existing antimicrobials and the increase in the number of cancer cases have motivated the search for new molecules with antimicrobial and cytotoxic activities. Among the several classes of promising compounds from a pharmacological point of view, the thiosemicarbazones have been highlighted. These compounds contain the R_1R_2C=N-NH-C(=S)-NR_3R_4 group and have demonstrated the antimicrobial, antiviral and cytotoxic potential. Furthermore, the possibility of modifications in its chemical structure and its chelating capacity make this class very promising for new investigations. OBJECTIVES: The present work aims to synthesize, characterize and evaluate the antimicrobial, cytotoxic and antitumor potential of new metal complexes of Cu(II) and Rh(III) obtained from 2-hydroxyphenyl-derived thiosemicarbazones. MATERIALS AND METHODS: Thiosemicarbazones will be obtained from the direct reaction of aldehydes and ketones with thiosemicarbazones catalyzed by acid. The metal complexes will be prepared from the reaction of thiosemicarbazones with metal salts in methanol or ethanol. They will be characterized mainly by elemental analysis and spectroscopic techniques (infrared and nuclear magnetic resonance spectra). The antimicrobial activity of the compounds will be evaluated against gram-negative and gram-positive bacteria and fungi using the broth microdilution method. The death curve for the all compounds will be obtained. Promising compounds will be tested with first-choice drugs via fractional inhibitory concentration tests and interaction with the surface of microorganisms. In addition, cytotoxicity of all compounds against tumor and non-tumor strains will be evaluated using the colorimetric assay with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Promising compounds will be tested for potential apoptosis induction and evaluation of NF-κB expression in tumor cell lines. DISCUSSION AND RESULTS: It is expected that the complexes are more active or selective than the free ligands in tumor cell lines and/or against tested microorganisms.

Keywords: antimicrobial activity, cytotoxic activity, thiosemicarbazones

H - Science Education

H.01 - interdisciplinary proposal for teaching chemistry in high school: biological control of insect pests
Igor Oliveira de Almeida [1], Luís Felipe Costa Ramos[1], Cristiane AnoBom[1], Danielle Oliveira[1]
[1]Departamento de Bioquímica, Universidade Federal do Rio de Janeiro (, Brasil)

INTRODUCTION: Natural Sciences and its Technologies teaching in High School needs to be contextualized with the students' environment, promoting curiosity and scientific investigation, for a more meaningful learning. OBJECTIVES: The aim of this work was the construction of a workshop that offers the study of Chemical Solutions in a contextualized, interdisciplinary, and experimental format involving Chemistry, Biology, and the environment, using the theme pesticides. MATERIALS AND METHODS: The methodology was to develop a workshop divided into five meetings for second graders of a private school in the city of Duque de Caxias-RJ. In the bioassay, conducted during the workshop, students exposed the Anticarsia gemmatalis caterpillar to increasing concentrations of Bt (0.2, 0.5 and 2 mg/mL) and deltamethrin (0.025, 0.0625 and 0.25 mg/mL). This project was approved by the UFRJ Research Ethics Committee (CAAE 42358421.0.0000.5257). DISCUSSION AND RESULTS: In the first meeting, students receive a survey questionnaire followed by construction of a conceptual map on pesticides. Additionally, they were instructed to watch the documentary "O veneno está na mesa 2". In the second meeting, a debate was held on the topic, based on provocative questions, with an active participation of the students. In the next meeting, there was an interactive class focused on solutions and pesticides, followed by the fourth meeting where a bioassay was performed on the survival of A. gemmatalis species, a specific soy caterpillar-plague, against different concentrations of pesticides: Bacillus thuringiensis (Bt), commercial Bt (DimyPel®) and Deltamethrin (commercial chemical insecticide, Forth® brand). In a fifth meeting, the results obtained were discussed with the students and a follow-up conceptual map with the same focal question was designed. In addition, students produced several forms of virtual scientific divulgation (social networks, videos, and blogs), sharing their results. CONCLUSION: Through the analyses of students' performance, reports and descriptions we observed that chemical solutions learning was facilitated after the workshop. **Keywords:** High School, Scientific Education, Pesticides

H.02 - Insertion of Collaborative Learning in the Biochemistry Discipline of the Undergraduate Nutrition Course: A Case Study
Lívia Marini Palma [1], Beatriz Della Libera Silva[1], Graciely Nunes Rosa Borges[1], Tatiana de Seixas Tavares Silva[1], Vagner Rocha Souza Simonin[1]
[1]UniRedentor, Centro Universitário Redentor, Itaperuna-RJ, Brazil (Rio de Janeiro, Brazil)

INTRODUCTION: Collaborative Learning has often been defended in academic environment as a recognized methodology with potential to promote a more active learning through the stimulus. Biochemistry teaching has become the subject of intense discussions, due to the difficulty in understanding the fundamental metabolic pathways. OBJECTIVES: The aim of this work was to use collaborative learning methodology in biochemistry teaching. MATERIALS AND METHODS: The collaborative learning methodology adopted was based on activities carried out in discussion groups in the classroom, using the team-based learning method. The material to biochemistry's study used in collaborative activities was obtained from São Paulo's University website. At the end of the school term, the students were submitted to the same approval criteria as a traditional methodology class. After that the performance of this students were compared to students who attended the classes were only expositive method was used. The observations of the professor responsible for the discipline were noted and described in the results of this work. DISCUSSION AND RESULTS: Collaborative Learning activities reflected an increase in interaction among students, an increase in the frequency of questioning was done in the class and a better performance during the course. The performance and quality of students responses in summative evaluations were superior when the collaborative activities were developed, which suggests a better understanding of Biochemistry contents worked. Comparatively, approval rate in Biochemistry after collaborative activity's execution was 54.17%, while the same rate presented 32% when the classes were only expositive. CONCLUSION: According to data presented, a formative assessment during the learning process is fundamental for all the appreciation and engagement of the student in the construction of knowledge. Learning about Biochemistry, exploring new teaching strategies and, consequently, increasing disciplinary evasion are points that need to be discussed at conferences by multidisciplinary teams. **Keywords:** Biochemistry teaching, collaborative learning methodology, teaching and learning / **Supported by:** Sociedade Universitária Redentor

H.03 - The Flipped Classroom: Enabling Strategy Teaching-Learning Process in Biochemistry Classes during the Pandemic at UFRRJ

André Marques dos Santos [1], Késia Nogueira da Silva[2], Ana Carolina Callegario Pereira[3]
[1]Departamento de Bioquímica, Instituto de Química, Universidade Federal Rural do Rio de Janeiro (Rio de Janeiro, Brasil), [2]Discente do curso de Ciências Biológicas, bolsista PIBIC-UFRRJ, Universidade Federal Rural do Rio de Janeiro (Rio de Janeiro, Brasil), [3]Professora, Centro Universitário de Volta Redonda (Rio de Janeiro, Brasil)

INTRODUCTION: The COVID-19 pandemic has profoundly impacted the teaching-learning process, migrating this process to the remote environment. In this context, active methodologies supported by information and communication technologies emerge as valuable tools for maintaining teaching in a virtual environment. Among the active methodologies, the Flipped Classroom (FC) is promising in the context of remote teaching, as it combines synchronous and asynchronous moments in the learning process. OBJECTIVES: Evaluate the contribution of FC as a methodological strategy for the teaching of biochemistry during the pandemic at UFRRJ. MATERIALS AND METHODS: The Research had a quali-quantitative nature and was developed in classes of the discipline *"IC383 – Bioquímica para Áreas Agrárias"* taught during remote teaching (2020-1, 2020-2, 2021-1). The content was made available in the form of video lessons in the virtual institutional learning environment ("*Sistema Integrado de Gestão de Atividades Acadêmicas" – SIGAA*). For the weekly meetings, held synchronously using Google Meet, various activities and discussions were prepared about the content addressed asynchronously. Questionnaires were used as a data collection instrument to assess acceptance of the methodology used. Formal assessments were maintained for the purpose of comparing data regarding classes from six previous semesters (2017-1, 2017-2, 2018-1, 2018-2, 2019-1, 2019-2), taught outside the pandemic context. The research was approved by the Ethics Committee for Research with Human at UFRRJ N. 1,211/18. DISCUSSION AND RESULTS: The students considered that the methodology favored their motivation, autonomy, and learning. They also pointed out that the FC could be used face-to-face. Compared to previous classes, there was a significant increase in the overall final average of the classes (5,95 ± 2,02 / 8,06 ± 1,08). CONCLUSION: The use of this methodological strategy made it possible to teach the discipline during the pandemic.
Keywords: online instruction, active learning, biochemistry education / **Supported by:** PIBIC/UFRRJ-CNPq

H.04 - Protocol to study the effect of polarization and fake news on vaccination against Covid-19.

Giselda Crsitina Ferreira [1], Ana Lígia Scott[1], Andre Ricardo Raucci[1], Carlos Kamienski[1]
[1]Observa, Universidade Federal do ABC (São Paulo, Brazil)

INTRODUCTION: Covid-19 pandemic is being accompanied by a spread of an intense volume of information, much of it misleading or false, in a phenomenon known as "infodemic" . The overabundance of information and excess of fake news makes it difficult to identify reliable sources and can affect adherence to disease containment measures such as vaccines. OBJECTIVES: We proposed a protocol to study the polarization on Twitter about the vaccine for the Covd-19 pandemic and how it can affect the vaccination rate of the country. This protocol can help us understand the problem of fake News about scientific topics. MATERIALS AND METHODS: We define polarizers as users of the Twitter platform who receive a large amount of replies, quotes and replies in a given period of time. A methodology is composed by 5 steps: i) set of data collected from Twitter (Brazil) of the narratives on the subject of the vaccine is obtained; ii) modeling the datas using graphs theory; iii) Metrics calculation as: Pagerank, centrality, in and out degree;iv) analysis of the graphs and identification of the polarizers; v) correlating the polarization with vaccination rate and politics events. DISCUSSION AND RESULTS: We observed that the number of users in favor of vaccination represents the vast majority of the profiles analyzed, regardless of political choice. A detailed analysis about the political profile, gender and profession of Polarizers was performed. CONCLUSION: We bring a sample in which the majority of the population is in favor of vaccination for Covid-19. All classified as left-wing profiles are in favor of vaccination, but not all those classified as right-wing are against it. It can also be concluded that there were spikes in the flow of tweets on the subject of vaccination against Covid-19 in scientific events such as beginning of vaccination in the United Kingdom; and announcement by Butanvac.
Keywords: Covid-19, Vaccine, Polarization / **Supported by:** Fapesp

H.05 - Interactive Study of Pentose Phosphate Pathway

Vera Maria Treis Trindade [1], Gabriel Cunha[2], Alisson Rocha[2], Marlise Bock Santos[2], Gabriela Trindade Perry[3], Christianne Gazzana Salbego[1]

[1]Dep.Bioquímica, Universidade Federal do Rio Grande do Sul (RS, Brasil), [2]Núcleo de Apoio Pedagógico à Educação a Distância, Secretaria de Educação a Distância - UFRGS (RS, Brasil), [3]Programa de Pós Graduação em Informática na Educação, Programa de Pós Graduação em Informática na Educação-UFRGS (RS, Brasil)

INTRODUCTION: This Digital Education Resource (DER) involves the study of glucose oxidation without the production of energy (ATP), but with the generation electron donor (NADPH) and pentose phosphates (ribulose phosphate, ribose phosphate and xylulose phosphate). In certain cells NADPH is used in reductive biosynthesis such as cholesterol, sex hormones, nitric oxide synthesis, oxidized glutathione/reduced glutathione redox system, etc. On the other hand, ribose phosphate (pentose phosphate) participates in the synthesis of nucleic acids, coenzymes, etc. This metabolic route occurs in the cytosol of different cells such as erythrocytes, hepatocytes, lactating mammary gland cells, sex glands and tumor cells. The pentose phosphate pathway is also known as the pentose phosphate cycle, the 6-phosphogluconate pathway, and the hexose mono phosphate pathway. OBJECTIVES: The purposes of this material are to facilitate the pentose phosphate pathway study and to associate it to other virtual resources in the interactive understanding of glucose metabolism. MATERIALS AND METHODS: The scientific content was developed by the Creation Group of Educational Objects in Biochemistry (GCOEB-UFRGS). The graphic art and programming were carried out by the team of the Pedagogical Support Core for Distance Education (NAPEAD-UFRGS) using the Adobe Illustrator software and HTLM editor. DISCUSSION AND RESULTS: The DER corresponds to a linear interactive study of the pentose phosphate pathway, in which the user finds a descriptive text associated with links to the animations of chemical reactions and/or illustrative figures, permeated with objective questions about the presented content, whose answer alternatives are commented. The descriptive text is organized into 8 items with specific links. Each item has several sub-items indicated with corresponding links (https://www.ufrgs.br/napead/projetos/pentoses/). CONCLUSION: The evaluations of interactivity, navigation characteristics and scientific contents of “Interactive Study of Pentose Phosphate Pathway” were considered excellent by undergraduate students from Biochemistry Department and graduate students from Biochemistry-PGP at UFRGS.

Keywords: digital educational resource, pentose phosphate cycle, 6-phosphogluconate pathway

Supported by: CAPES

H.06 - Adaptations For Emergency Remote Teaching Of Biochemistry

Gabriel Gerber Hornink [1]

[1]Biochemistry, Federal University of Alfenas (MG, Brazil)

INTRODUCTION: The COVID-19 pandemic surprised the world and demanded disruptive transformations at all levels of education. The difficulties in teaching and learning Biochemistry were potentiated in emergency remote teaching (ERT), however, generated the opportunity to rethink and improve the strategies and use of educational tools. OBJECTIVES: The objective is to present the structuring of a Biochemistry course during the ERT. MATERIALS AND METHODS: A preliminary survey was carried out in two classes in 2021 (71 students). Overall, the discipline was planned based on the flipped classroom, development of concept maps in the cloud, problem solving and virtual practices. DISCUSSION AND RESULTS: The students indicated concern about ERT, in general, sufficient or totally sufficient conditions to carry out activities, Moodle familiarity, and most felt resigned with ERT and few were motivated and confident. A sequence of asynchronous activities was structured per unit (tutorials, ebooks, video lectures, direct studies, educational software) that supported the synchronous moment (problems, challenges etc). Then they performed the virtual practice, which could have interactive videos, animations, simulations. Groups concept maps were developed in the CMap cloud (colors identifying each participant), incorporated into the forum. Individually, each student should interact with the other groups to improve the maps. Classes were supported synchronously and asynchronously by the monitor and teacher. The evaluation was based on concept maps, virtual problems, exercises and seminars (attendance was recorded by active participation). The maps proved to be an important tool for collaborative learning, as well as peer review made it possible to improve the maps. The construction of an individual conceptual map was used in the final test as a form of assessment of greater weight. CONCLUSION: The strategy used resulted in the active participation of the class and presented itself as a good option for emergency remote teaching, as well as being readapted for face-to-face activities.

Keywords: Biochemistry education, Emergency remote teaching, COVID-19

H.07 - ATP bioluminescent detection with recombinant firefly luciferase in practical bioenergetics classes

Vanessa Rezende Bevilaqua [1], Jaqueline Rodrigues Silva[2], Vadim Viviani[2]
[1]Physiology Department, Pontifical Catholic University (, Brazil), [2]Department of Physics, Chemistry and Mathematics, Federal University of São Carlos (São Paulo, Brazil)

INTRODUCTION: ATP is the universal molecule that stores and carries energy in living cells; therefore its detection can be of great value in practical classes. Among the reactions that use the energy of ATP is the firefly bioluminescence. In this reaction catalyzed by luciferase, the susbtrate luciferin is oxidized in the presence of ATP producing light. Because of ATP dependence in the bioluminescence reaction, the firefly luciferin and luciferase have been used for ATP quantification. OBJECTIVES: In this practical class, we used a recombinant luciferase cloned from Amydetes viviani firefly, found in campus of UFSCar at Sorocaba, to estimate visually the presence of ATP in biological samples through bioluminescence. MATERIALS AND METHODS: A. viviani firefly luciferase was expressed in E coli bacteria and purified by nickel affinity chromatography. Known ATP concentrations samples were used as standards. The biological samples that were used to visually quantify the intracellular ATP concentration were 2 Zophobas morio mealworms and 1mL of bacteria at OD600=1.0. To obtain a bioluminescence image, a common cell phone and a CCD LightCaptureII photodetection camera (ATTO, Japan) were used. DISCUSSION AND RESULTS: According to the visual estimation, the mealworm fat body extract sample had an estimated ATP concentration between 10 and 25 μM, while the bacterial extract sample had an ATP concentration lower than 2.5 μM. We also estimated the average amount of ATP moles per E.coli cell, obtaining a value of 1.56 mM, a value which is very close to the values reported in the literature (see the Nelson & Cox, 2009). CONCLUSION: By mixing luciferin and Amydetes firefly luciferase to fresh samples of lysed bacteria and fat body of mealworms, the bioluminescence produced is bright enough to be visually detected in dark environments and photographed with common cell phone cameras. This bioluminescent reagent system can be effectively used to estimate the ATP concentrations in different biological samples.
Keywords: Luciferase, ATP quantification, biological samples
Supported by: Fapesp/ 2020/07649-6

H.08 - Experimental Biochemistry Classes During the Covid-19 Quarantine: An Alternative for the Emergency Remote Teaching period

Rodrigo Vassoler Serrato [1]
[1]Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Paraná (Curitiba, PR, Brasil)

INTRODUCTION: The pandemic caused by Coronavirus (SARS-CoV-2) has brought challenges to all sectors, including academia. Biochemistry classes for undergraduate students have been significantly affected, especially those that rely on laboratories to perform experimental demonstrations and training. In 2020, universities adopted Emergency Remote Teaching (ERT) as a response to the spread of the pandemic, and online teaching became the main modality due to campus closure in favor of social distancing. OBJECTIVES: This work presents a series of 15 videos containing experimental protocols covering the full programs normally provided to undergraduate biochemistry courses and describes the planning, shooting and editing processes of the material with the objective of mitigating the difficulties imposed by the ERT period. MATERIALS AND METHODS: Each video class has been scripted to be presented in 5 steps: 1) Theme presentation; 2) Opening animation; 3) Theoretical introduction; 4) Experimental development; and 5) Results discussion and conclusion. The recording was performed at the teaching laboratories of the Biochemistry and Molecular Biology Department of UFPR, and all reagents and equipments used are of everyday use in undergraduate biochemistry laboratories. Editing and post-production were made using the software Hitfilm Express and a computer equipped with a 2 GB-dedicated GPU. DISCUSSION AND RESULTS: The use of asynchronous video classes has shown to be reasonably adequate, given the situation imposed by ERT during the Covid-19 pandemic. Students and professors were pleased to have the possibility to access the experimental content even if virtually. In general, the full content of biochemistry classes was covered due to the material presented herein. CONCLUSION: The content created during this work has been made available on the internet as a means to contribute to scientific dissemination to the general public, as well as to be used by students and professors as supplementary material to the teaching-learning process in the field of biochemistry.
Keywords: Experimental Biochemistry, Emergency Remote Teaching, Online Classes

H.09 - *Diabetes mellitus*: an interdisciplinary topic for the teaching of biology and chemistry

Fernanda de Azevedo Ribeiro[1], Amanda Mattos Santana[1], Rodrigo Maciel Lima[1]
[1]Biologia , Instituto Federal Fluminense (Rio de Janeiro, Brasil)

INTRODUCTION: Diabetes Mellitus (DM) is a metabolic disease that affects thousands of people around the world, ever earlier. It is known as a chronic noncommunicable disease (NCD), and presents a slow and severe evolution requiring treatment intensive care and adequate medical guidance. It mainly affects low-income countries where health care is restricted, thus allowing for an increase in number of deaths as with infectious diseases. OBJECTIVES: The objective of this work was to use the diabetes mellitus theme to break the fragmented view of teaching, which often prevents students from having the ability to recognize the relationship between the disciplines of Chemistry and Biology. MATERIALS AND METHODS: The research was based on the Three Pedagogical Moments, during four classes, in which the development of the class was initially guided by the initial problematization of the theme with the help of a questionnaire and three sets of images for textual production, followed by the organization knowledge related to the subject from the class taught with the help of the molecular model in a PET bottle; and finished with the use of the game, called Bio association, and again the application of questionnaires and set of images for the application of knowledge. DISCUSSION AND RESULTS: The results achieved showed that the playful approach developed contributed to the association and understanding of the disease mechanism, since, good part of the students presented results that showed advances in learning. The use of tools in order to facilitate the appropriation of the knowledge, assists in the assimilation of mechanisms as they involve visual memory and materialization of structures. CONCLUSION: It is possible to consider that the methodology applied was efficient to the proposed objective, since the students participated in the development of the class as expected.

Keywords: Diabetes Mellitus, Interdisciplinarity, Biochemistry
Supported by: IFF

I - Photobiology, Optogenetics and Neural Systems

I.01 - Photodynamic Therapy Towards Inactivation of Miltefosine-Resistant Leishmania amazonensis

Fernanda Cabral[1], **Martha Simões Ribeiro** [1]
[1]Centro de Lasers e Aplicações, Instituto de Pesquisas Energérticas e Nucleares (, Brazil)

INTRODUCTION: Cutaneous leishmaniasis (CL) is a chronic disease developed by Leishmania parasites that promotes destructive lesions. The emergence of drug-resistant parasites has been related to the misuse of drugs, being a major threat to global health. Although antimicrobial photodynamic therapy (APDT) has been reported as an attractive treatment against a broad spectrum of drug-resistant pathogens, the use of APDT against drug-resistant *Leishmania* parasites has never been explored. OBJECTIVES: This study aimed to explore the effects of methylene blue-mediated APDT (MB-APDT) on promastigotes and intracellular amastigotes of two different strains of *Leishmania amazonensis*, a wild-type (WT) and a miltefosine-resistant cell line (MFR). MATERIALS AND METHODS: Promastigotes and intracellular amastigotes were treated at different concentrations of miltefosine. Regarding APDT, we used a red LED (λ= 660±22 nm) at 20 mW/cm ^{2} and two MB concentrations. Parasites were exposed to radiant exposures of 0 to 25 J/cm ^{2} .DISCUSSION AND RESULTS: The miltefosine concentration necessary to reduce 50% (EC50) MFR promastigotes was found to be 5.6-fold higher than that of the WT strain. Amastigotes were even more resistant, and the concentration needed to effectively kill MFR was not able to be calculated once it was toxic to health macrophages. Differently, both promastigotes and intracellular amastigotes were susceptible to MB-APDT. Indeed, promastigotes were equally susceptible to treatment regardless of the MB concentration. EC50 calculated for the light dose delivered was nearly 3 J/cm2, which corresponds to an exposure time of 150 s. Surprisingly, amastigotes of MFR were more susceptible to MB-APDT at 50 µM MB concentration, and the light dose necessary to reduce 50% of resistant parasites was half of that of the WT strain (2.3 J/cm ^{2} and 4.7 J/cm ^{2} , respectively). CONCLUSION: These results indicate that MB-APDT could be a promising treatment to overcome the global issue of antileishmanial drug resistance in CL.

Keywords: methylene blue, miltefosine-resistant parasites, red light

I.02 - Biophotonic Strategy Associated with Hexyl Zinc Porphyrin for Inactivation of Candida spp.

Bruno Luis Raposo [1], Sueden Oliveira Souza[1], Gleyciane S. de Santana[1], José Ferreira Sarmento-Neto[2], Beate Saegesser Santos[3], Martha Simões Ribeiro[4], Júlio Santos Rebouças[2], Paulo E. Cabral Filho[1], Adriana Fontes[1]
[1]Departamento de Biofísica e Radiobiologia, Universidade Federal de Pernambuco (Pernambuco, Brasil), [2]Departamento de Química, Universidade Federal da Paraíba (Paraíba, Brasil), [3]Departamento de Ciências Farmacêuticas, Universidade Federal de Pernambuco (PE, Brasil), [4]Centro de Lasers e Aplicações, Instituto de Pesquisas Energéticas e Nucleares (SP, Brasil)

INTRODUCTION: The genus Candida is among the most frequent fungal pathogens worldwide. The indiscriminate use of antifungals enables the spread of resistant strains, which have been associated with high morbidity and mortality. Photodynamic inactivation (PDI) is a promising technology to treat resistant Candida spp. infections. PDI occurs when light excites a photosensitizer (PS) leading to the production of reactive oxygen species (ROS). Zn(II) porphyrins (ZnPs) present high efficiency for intracellular ROS generation and structural versatility for tailored lipophilicity and ionic character, modulating the bioavailability and interaction with cellular structures. OBJECTIVES: This study aimed to investigate the potential of ZnTnHex-2-PyP4+-mediated PDI to inactivate C. albicans and C. glabrata yeasts. MATERIALS AND METHODS: Candida yeasts (1×10^7 CFU/mL) were evaluated according to the groups: (i) control (without treatment); (ii) only ZnTnHex-2-PyP4+ (dark); (iii) only light (blue LED); and (iv) PDI (ZnP + light) using 10 min of pre-incubation. Different ZnP concentrations (0.15 to 1.25 µM) and light doses were firstly tested with C. albicans. Treated samples were diluted and seeded on Sabouraud agar for colony enumeration after incubation at 37 ºC for 24 h. DISCUSSION AND RESULTS: C. albicans viability decreased with increasing ZnP concentration, achieving complete eradication at 0.8 µM and 3 min of irradiation (24.1 mW/cm²). PDI with 1.25 µM and 1 min of irradiation resulted in a 2 log10 reduction only, demonstrating the importance of light dose in microbial photoinactivation. PDI parameters were subsequently adjusted for inactivation of C. glabrata. Complete C. glabrata eradication was achieved with ZnP at 0.8 µM, and 3 min of irradiation, however, at a higher irradiance (38.4 mW/cm²). Groups treated with either light or ZnP alone did not affect Candida spp. viability. CONCLUSION: These results suggest that the protocols used in this study were efficient for inactivating Candida spp. yeasts at sub-micromolar concentration ZnP and short irradiation times.

Keywords: Candida albicans, Candida glabrata, Photodynamic inactivation

I.03 - LIPIDOMICS ANALYSIS OF LEISHMANIA AMAZONENSIS FOLLOWING PHOTOXIDATIVE STRESS

Fernanda Viana Cabral [1], Terry K Smith[2], Martha Simões Ribeiro[1]
[1]Centro de Lasers e Aplicações CLA, Instituto de Pesquisas Energéticas e Nucleares (Sao Paulo, Brazil), [2]Schools of Biology & Chemistry, BSRC, University of St. Andrews (Scotland, United Kingdom), [3]Centro de Lasers e Aplicações CLA, Instituto de Pesquisas Energéticas e Nucleares (Sao Paulo, Brazil)

INTRODUCTION: Antimicrobial photodynamic therapy (APDT) is a well-known light-based technology that has been widely studied as an alternative approach to fight cutaneous leishmaniasis (CL). APDT induces lipid peroxidation in cellular membranes due to the generation of oxidative stress OBJECTIVES: In this study, we evaluated the role of 1,9-dimethylmethylene blue (DMMB)-mediated APDT on a wild-type (WT) and a miltefosine-resistant (MF) strain of Leishmania amazonesis and analyzed several cellular processes to get insights into the underlying mechanisms of APDT. MATERIALS AND METHODS: For this, APDT was carried out using red light (λ= 670±12 nm) and promastigotes were exposed to different concentrations of DMMB at 8 J/cm2. Then, we measured mitochondrial potential and intracellular levels of reactive oxygen species (ROS) and analyzed quantitative lipidomics of the main phospholipid classes using electrospray-mass spectrometry. DISCUSSION AND RESULTS: As a result, we observed overproduction of ROS, mitochondrial membrane depolarization, and a rapid lipid remodeling immediately after APDT. Of note, MF showed a higher content in levels of phosphatidylcholine (PC) as compared to the WT line before treatment, which suggests it could be also involved in the MF resistance mechanism. In addition, results showed that after APDT, PC levels were substantially decreased, while new phospholipid species of phosphatidylethanolamine (PE) were increased. CONCLUSION: In conclusion, our data suggest DMMB-mediated APDT promoted a significant lipid peroxidation in the parasite's membrane of both strains, which failed to manage redox imbalance, thus resulting in cellular malfunction and death.
Keywords: Lipidomics, cutaneous leishmaniasis, photodynamic therapy / **Supported by:** CNPq, CAPES, CNEN, FAPESP

I.04 - Photochemical Characterization of Methylglyoxal

Lohanna de Faria Lopes [1], Georg Thomas Wondrak[2], Maurício da Silva Baptista[1]
[1]Departament of Biochemistry, University of São Paulo (São Paulo, Brazil), [2]Department of Pharmacology and Toxicology, University of Arizona (Arizona, United States of America)

INTRODUCTION: Methylglyoxal (MG) is a reactive electrophile α-dicarbonyl compound that is kept at low concentration in conditions of abiotic stress. Under extreme environmental conditions, the concentration of MG increases substantially, being able to react with several nucleophiles, including biomolecules with amines and cysteine and severely decreasing the antioxidant defenses. The chemical reactivity of MG could be substantially increased by the absorption of light in the UV and visible ranges; however, this was never investigated before. OBJECTIVES: Light absorption can increase the MG-related oxidative stress and we have characterized its main photophysical properties such as, quantum yields and lifetimes of singlet and triplet excited as well as singlet oxygen quantum yield (ΦΔ). MATERIALS AND METHODS: Absorption and fluorescence spectra were obtained using a Shimadzu spectrometer (UV-2400-PC) and a Varian Cary Eclipse fluorimeter, respectively. ΦΔ was determined using a NIR fluorometer (SHB) equipped with a Hamamatsu PMT, with a 400 nm LED as the excitation source. Phosphorescence was performed at 77K (liquid nitrogen temperature) in a Hitachi F-4500 fluorimeter equipped with quartz Dewar. Φf (quantum yield of fluorescence emission) and ΦΔ were obtained by measuring and comparing with a known standard and the phosphorescence lifetime by the emission decay time. DISCUSSION AND RESULTS: Absorption spectra of MG solutions indicate absorbance extending to the visible range of the spectra (~490nm). ΦΔ and Φf were 0.13 and 0.006. The presence of triplet excited state was obtained by the low temperature phosphorescence of MG, whose lifetime was 224.2 milliseconds. CONCLUSION: It was possible to confirm that MG forms long-lived excited states that can substantially increase the cytotoxicity of MG, upon exposure to UV radiation and visible light.
Keywords: Methylglyoxal, Photochemistry, Oxidative stress
Supported by: CAPES; CNPq; FAPESP

I.05 - Photobiomodulation Therapy as a Radiosensitizer for Triple-Negative Breast Cancer

Camila Ramos Silva [1], Saulo de Toledo Pereira[1], Lucas Ramos de Pretto[1], Anderson Zanardi de Freitas[1], Martha Simões Ribeiro[1]
[1]Centro de Lasers e Aplicações, Instituto de Pesquisas Energéticas e Nucleares (São Paulo, Brazil)

INTRODUCTION: Radiotherapy (RT) is an essential cancer treatment and is estimated that approximately 52% of oncological patients will be submitted to this technique once. However, some tumors, such as triple-negative breast cancer (TNBC), present radioresistance, demanding high doses of ionizing radiation (IR) and a prolonged period of treatment, which contributes to secondary malignancies due to deposition of dose in organs at risk and several side effects. Moreover, this subtype of cancer shows a high incidence of metastasis and decreases the survival expectancy of the patient. Thus, the search for new agents that can act as a radiosensitizer to improve the RT effects has been growing. Conversely, photobiomodulation therapy (PBM), which is a promising therapy with increasing adhesion in clinical practice, has been used to mitigate the adverse effects of RT. Indeed, recent studies have associated PBM with RT to combat cancer. OBJECTIVES: In this study, we used TNBC-bearing mice as a radioresistant cancer model to verify if PBM could act as a radiosensitizer MATERIALS AND METHODS: PBM was applied in two different protocols before the RT with a high dose (60 Gy fractioned in 4 sessions). We evaluated the tumor volume progression, animal clinical evolution, lung metastases by optical coherence tomography, and animal survival DISCUSSION AND RESULTS: Our data indicate that PBM before each RT session arrested the tumor volume, improved the clinical signals of the animals, reduced the nodules in the lung, and extended animal survival. CONCLUSION: In the light of the knowledge gained, our data indicate that PBM could act as a radiosensitizer.
Keywords: phototherapy, radiomodifier, combined therapy

I.06 - Multimodal nanosystem with magneto-optical properties functionalized with Cramoll lectin for biosensing

Weslley Felix de Oliveira [1,2], Mariana Cabrera[3], João Victor Lima[2], Luana Coelho[1], Giovannia Pereira[3], Beate Santos[4], Paulo Cabral Filho[2], Adriana Fontes[2], Maria Tereza Correia[1]
[1]Departamento de Bioquímica, Universidade Federal de Pernambuco (Pernambuco, Brasil), [2]Departamento De Biofísica E Radiobiologia, Universidade Federal De Pernambuco (Pernambuco, Brasil), [3]Departamento de Química Fundamental, Universidade Federal de Pernambuco (Pernambuco, Brasil), [4]Departamento de Farmácia, Universidade Federal de Pernambuco (Pernambuco, Brasil)

INTRODUCTION: Magneto-optical nanomaterials are gaining prominence for biological applications. These nanosystems can be prepared through the conjugation of quantum dots (QDs) with superparamagnetic iron oxide nanoparticles (SPIONs), enabling to combine advantages of the superparamagnetic behavior of SPIONs and the unique fluorescent characteristics of QDs. Furthermore, these bimodal nanosystems can become biospecific when conjugated to biomolecules, such as Cramoll, a glucose/mannose-binding lectin extracted from *Cratylia mollis* seeds. OBJECTIVES: The study aimed to conjugate the Cramoll with the nanosystem composed of QDs and SPIONs and apply it as a biosensing platform using the glycoprotein fetuin as an analyte model. MATERIALS AND METHODS: Carboxylated QDs were covalently conjugated to amine-coated SPIONs, and then this bimodal nanosystem was conjugated with Cramoll. The optical properties and the zeta potential (ζ) of nanosystems were evaluated. Candida albicans yeasts were incubated with the nanosystem and analyzed through fluorescence microscopy and flow cytometry to evaluate the specificity/efficiency of the nanoprobe. Fetuin detection was performed by fluorimetry. DISCUSSION AND RESULTS: The QD absorption band was absent in the supernatant of the bimodal nanosystem, indicating effective conjugation with SPIONs. There was a redshift in the maximum emission of the bimodal nanosystem with respect to bare QDs; lectin conjugation did not cause a spectral shift. The multimodal nanoprobe had a lower ζ than the bimodal nanosystem. Approximately 90% of yeast cells were homogeneously labeled by the multimodal nanoprobe and after inhibition with methyl-α-D-mannopyranoside there was a considerable labeling reduction, indicating specificity. When incubated with different concentrations of fetuin (0.675-10.8 mg/mL), a linear decay in the fluorescence intensity was identified. Incubation with bovine serum albumin (control) did not significantly decrease the fluorescence intensity, and the new nanoprobe also detect fetuin previously diluted in serum. CONCLUSION: The obtained multimodal system showed specificity and effectiveness as well potential for use as a biosensing platform.
Keywords: iron oxide nanoparticle, lectin, quantum dot
Supported by: CAPES, CNPq, FACEPE, INCT-INFo, and LARNANO-UFPE.

I.07 - One-electron Oxidation of Biomolecules: Antioxidant Action of DOPA

J.R. Neyra Recky[1], M.L. Dántola[1], **Carolina Lorente [1]**
[1]Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA), Fac. de Ciencias Exactas, Universidad Nacional de La Plata (UNLP), CONICET. (La Plata, Argentina)

L-3,4-Dihydroxyphenyl-alanine, known as L-DOPA, is a natural amino acid and the precursor of the neurotransmitters dopamine, norepinephrine, and epinephrine, which are known as catecholamines. It is widely used in the treatment of Parkinson's disease, and it has been reported that L-DOPA may prevent H_2O_2-induced oxidative damage to cellular DNA and LDL oxidation [1,2,3]. Therefore, the objective of our work is to study the antioxidant properties of L-DOPA in photosensitized processes. Photosensitized oxidation of biomolecules occurs due to the absorption of radiation by chromophores that can be endogenous or exogenous. Pterin (Ptr) absorbs UV-A radiation and generates excited states that are harmful to living systems, causing damage to proteins, DNA, and lipids. Although Ptr may photoinduced damage by both type I (electron transfer) and type II (singlet oxygen) mechanisms, type I is the predominant mechanism at physiological pH. Therefore, Ptr triplet excited state and a given biomolecule undergo electron transfer reactions, and the corresponding radicals (Ptr radical anion and a radical cation of the biomolecule, B•+) are formed. In the presence of O2, Ptr is recovered, and the biomolecule is degraded [4]. In our laboratory we have obtained experimental evidence that clearly demonstrate that the presence of L-DOPA prevents Ptr-photosensitized oxidation of relevant biomolecules. Aqueous solutions containing Ptr and a given amino acid (tyrosine, tryptophan and histidine) or deoxynucleotide (2'deoxyguanosine 5'-monophosphate (dGMP) and 2'deoxyadenosine 5'-monophosphate (dAMP)) were exposed to UV-A radiaton (365 nm), in the absence and in the presence of L-DOPA. For all biomolecules tested was observed a decrease in the rate of consumption proportional to L-DOPA concentration. A mechanistic analysis indicates that after one-electron transfer with 3Ptr*, all biomolecules studied are recovered in a second one-electron transfer reaction from L-DOPA to B•+. The radical of L-DOPA undergoes further oxidation being a sacrificial antioxidant molecule. [1] Segura-Aguilar J., Paris I., Muñoz P., Ferrari E., Zecca L., Zucca F. A. (2014) J. Neurochem. 129, 898--915. [2] Shia Y. L., Benzieb I. F. F., Buswell J. A. (2002) Life Sci 71, 3047–3057. [3] Exner M., Hermann M., Hofbauer R., Kapiotis S, Gmeiner B. M. K. (2003) Free Radical Research 37 (11), 1147–1156. [4] Lorente C., Serrano M. P., Vignoni M., Dántola M. L., Thomas A. H. (2021) J. Photochem. Photobiol. 7, 100045. **Keywords:** L-DOPA, Biomolecules, Parkinson's

I.08 - Photophysical Properties of Photosensitizers derived from Protoporphyrin IX for Photodynamic Therapy

Maynne Duarte Suriani Franco [1], Mayara Caetano[1], Nayara Bessas[1], Ana Clara Martinho[1,1], Renata Galvão[1], Celso Rezende Júnior[1,1], Tayana Tsubone[1,1]
[1]Instituto de Química, Universidade Federal de Uberlândia (Minas Gerais, Brasil)

INTRODUCTION: Photosensitizer (PS) in the presence of visible light and oxygen, is the principle of Photodynamic Therapy (PDT), that causes photooxidation of biomolecules. PS are the essential part of PDT, in which most of them are currently composed of porphyrin. Due to their unique affinity for tumor tissues, porphyrins are excellent for delivering other active drugs to tumor tissues, promoting a synergistic anti-cancer effect of PDT and chemotherapy. Moieties such as piperazines and morpholine are added to PpIX structure due to antitumor activity. OBJECTIVES: Synthesize two new PS derived from protoporphyrin IX (PpIX), containing a methyl-piperazine or morpholine moieties. Evaluate their photophysical properties and their interaction with protein (HSA). MATERIALS AND METHODS: PSs (PpIX-methylpiperazine and PpIX-morpholine) were prepared from the amidation reaction between Pp IX and amines derived from piperazine and morpholine. Photophysical parameters such as molar absorptivity coefficient (ε), fluorescence quantum yield (φ_f) and singlet oxygen quantum yield of (φ_Δ) were determined. Aggregation tendency of PS in water/methanol mixtures monitored by UV visible spectra. Interaction between PS and human serum albumin (HSA) studied by fluorescence supression. DISCUSSION AND RESULTS: Fluorescence quantum yield (φ_f) values were similar between both PS (φ_f = 0,074 PpIX-methylpiperazine e 0,073 PpIX-morpholine. φ_Δ valuesobtained for PpIX-methylpiperazine (φ_Δ = 0.45 ± 0.05) was 2-fold greater than PpIX-morpholine (φ_Δ = 0.25 ± 0.01). This is probably due to more available amine electrons of morpholine to react with $_1O_2$ than methylpiperazine. Studies about the interaction of HSA with protoporphyrin IX derivatives revealed a higher interaction between HSA in the presence of PpIX-methylpiperazine ($K_a = 4.80x10^3$) than PpIX-morpholine ($K_a = 1.36x10^2$). CONCLUSION: The chemical structural modification of PpIX by the addition of morpholine or piperazine showed that morpholine moiety kept photophysical properties as absorptivity and φ_f, but decreased φ_Δ generation and HSA interaction.
Keywords: photoactivity, Photodynamic Therapy, Photosensitizers / **Supported by:** FAPEMIG APQ-007704-21, CAPES, CNPq, PPGQUI and UFU

I.09 - Photophysical properties and application in photodynamic therapy of pyranoflavylium cations

Lucas Mendel de Oliveira Silva Martins [1], Gustavo Thalmer M.Silva [1], Xuhui Wang [2], Cassio Pacheco Silva [1], Mauricio Da Silva Baptista[3], Frank Herbert Quina [1]
[1]Department of Fundamental Chemistry, University of São Paulo (São Paulo , Brazil), [2]School of Pharmaceutical Science and Technology, Tianjin University (, China), [3]Department of Biochemistry, University of São Paulo (São Paulo , Brazil)

INTRODUCTION: Anthocyanins are pigments found in flowers, fruits, leaves and are responsible for most of the purple, red and blue colors. The anthocyanins present in red wines slowly react with metabolites, giving rise to a class of structurally more complex pigments, known as pyranoanthocyanins. The chemical and photophysical properties of pyranoflavylium cations, synthetic analogs, and the capacity to sensitize formation of singlet oxygen in solution, suggested the potential for application in photodynamic therapy (PDT). OBJECTIVES: This work carried out an evaluation of the photophysycal proprieties of a synthetic pyranoflavylium analogue 8–Hydroxy–5-(4-methylphenyl)–2-phenylpyrano-[4,3,2-de]chromenium chloride (1), and study of its dark toxicity and phototoxicity against HeLa Cell line. MATERIALS AND METHODS: : Steady state fluorescence spectra were measured in a Varian Cary Eclipse. The fluorescence quantum yield (ΦF) was measured employing quinine sulfate (Φf=0.55) as standard . The singlet oxygen quantum yield was measured by direct detection of their phosphorescence decay using a TCMPC-1270-LED and 400 nm led source, and [Ru(bpy)3]Cl2 (Φ∆=0.57) as standard. For phototoxicity and dark toxicity, HeLa cells were seeded in two 96 well plates, incubated for 24 hours and incubated with 1 for one hour. The plate was irradiated at 450 nm using a Biolambda LED for 30 minutes. The dark control was kept in the dark for 30 minutes. Cellular viability was determined after 24 h using the MTT viability test. DISCUSSION AND RESULTS: For the pyranoflavylium 1, the quantum yields were Φ∆=0.16, and ΦF=0.13. The treatment of HeLa cells with 1 exhibited low dark toxicity but a strong phototoxicity effect, with a LD50 of 1.5 μM CONCLUSION: The pyranoflavylium 1 showed effectiveness and potential for application of this class of bioinspired chromophores in PDT

Keywords: Photodynamic therapy, Photophysics, Pyranoflavilyum cations
Supported by: CAPES

I.10 - Ruthenium Polypyridines as Photosensitizer for Photodynamic Therapy

Patrícia Alves de Matos [1], Marcos Eduardo G. do Carmo[1], Antonio O. de Toledo Patrocinio[1], Tiago Araújo Matias[1], Tayana Mazin Tsubone[1]
[1]Instituto de Química, Universidade Federal de Uberlândia (Minas Gerais, Brasil)

INTRODUCTION: Even though the “new” era of Photodynamic Therapy (PDT), following the work of Dougherty (1998), is already over 30 years, photosensitizer (PS) containing the same chemical structure characteristics (porphyrins, chlorines and phthalocyanines) are still the most used. In this context, Ru(II) polypyridine complexes have received attention due to their photophysical and biological properties, including high production of reactive oxygen species (ROS). TLD-1433 Ru(II) complex is the first inorganic complex to enter phase II clinical trials as FS for PDT against bladder cancer, which does not belong to the classes of porphyrins, chlorins and phthalocyanines. OBJECTIVES: Study two ruthenium polypyridine complexes, Rupby (cis-bis(4,4'-dimethyl-2,2'-bipyridine)-(4,4'-bipyridine)chlororuthenium(II), and Rubpe (cis -bis(4,4'-dimethyl-2,2'-bipyridine)(trans-1,2-(4-yridyl)ethylene)chlororuthenium(II) as PSs for application in PDT. There is a slight difference in ligand size arising from the insertion of an unsaturation in the 4,4'-bipyridine ligand between both PS. MATERIALS AND METHODS: UV-Vis absorption spectra and emission spectroscopy was used to evaluate the interaction of Rubpe and Rubpy with liposomes and Bovine Serum Albumin (BSA). Cell uptake and viability assays in tumor cells are ongoing studies. DISCUSSION AND RESULTS: The interaction between ruthenium (II) complexes and lipid bilayer showed that the addition of the ethylene group in the Rubpe ligand does not affect the interaction of compound. On the other hand, the binding of PSs with protein (BSA) showed that the interaction can be modulated by the presence of unsaturation between the pyridyl groups, so that Rubpe had a higher value of Kb in BSA compared to Rubpy at 35ºC. Photooxidation kinetics of BSA by Ru complex, indicated that the greater interaction of the complex increases the rate of oxidation. CONCLUSION: The addition of an ethylene group between the pyridyl groups favors the interaction of Rubpe with protein, however no difference in the binding of the two compounds was observed with the bilayer lipid.

Keywords: Photodynamic Therapy, Ruthenium complex, biomolecule interaction / **Supported by:** FAPEMIG, CAPES, CNPq, PPGQUI and UFU

I.11 - Versatile Fluorescent Nanotool Based on Quantum Dots and Enterolobium contortisiliquum Trypsin Inhibitor (EcTI)

Natália Melo[1], Weslley Felix[1], Mariana Cabrera[3], Clóvis Bezerra[4], Maria Luiza Oliva[4], Leydianne Patriota[1], Thiago Napoleão[1], Paulo Euzébio[2], Adriana Fontes[2], Maria Tereza Correia[1]
[1]Depto de Bioquímica, [2]Departamento de Biofísica e Radiobiologia, [3]Departamento de Química Fundamental, Universidade Federal de Pernambuco (PE, Brasil), [4]Departamento de Bioquímica, Universidade Federal de São Paulo (SP, Brasil)

INTRODUCTION: Serine proteases play crucial roles in life maintenance and have their activity controlled by their inhibitors. The dysregulation in activation/deactivation mechanism can trigger diseases. EcTI is a serine proteases inhibitor purified from *Enterolobium contortisiliquum* seeds that has, among others, anti-cancer activity. Therefore, the combination of EcTi with fluorescent nanoprobes can be a valuable tool for unraveling the interaction of this inhibitor with cancer cells. In this context, quantum dots (QDs) have been standing out as fluorescent nanoprobes due to their unique optical properties. OBJECTIVES: This study aimed to conjugate EcTI with QDs to obtain a highly fluorescent nanosystem and apply it to investigate the profile of serine proteases in cancer cell lines. MATERIALS AND METHODS: Carboxyl-coated QDs were conjugated to EcTI by a covalent procedure. QDs and conjugates were characterized by absorption and emission spectroscopies. The conjugation efficiency was evaluated through fluorescence correlation spectroscopy (FCS) and fluorescent microplate assay (FMA). The profile of specific serine proteases was analyzed in cervical adenocarcinoma (HeLa) and breast (MDA-MB-231) cancer cell lines through flow cytometry. DISCUSSION AND RESULTS: The QDs-EcTI conjugate presented high fluorescence, and its absorption spectra did not show noteworthy changes compared to bare QDs. From FCS analysis, the hydrodynamic sizes were estimated as 3.4 nm and 13.1 nm for bare QDs and QD-EcTI conjugate, respectively. Moreover, in FMA a relative fluorescence of ca. 3,815% was obtained compared to controls (only QDs or EcTI). Therefore, FCS and FMA confirmed that EcTi was conjugated effectively to QDs. The conjugate labeled about 62% of HeLa cells and 90% of MDA-MB-231, suggesting that the cells have different expression levels of proteases to which EcTI has affinity on their membrane. CONCLUSION: EcTI was effectively conjugated to QDs, making up a promising nanotool for studies on serine proteases in tumor cells through fluorescence-based techniques.

Keywords: Quantum dots, protease inhibitor, fluorescence / **Supported by:** CAPES, CNPq, FACEPE, INFABIC, and INCT-INFo

I.12 - Betulinic acid and photodynamic therapy: an innovative endeavor to improve tumor cell death

Maryana do Nascimento da Silva[1], Mauricio da Silva Baptista[2], **Waleska Kerllen Martins**[1]
[1]Biotecn. e Inovação em Saúde, Univ. Anhanguera de São Paulo (SP, Brasil), [2]Bioquímica, Univ. de São Paulo (SP, Brasil)

INTRODUCTION: Photodynamic Therapy (PDT) is a safe, minimally invasive, and emerging modality of cancer treatment in which tumor cells might activate the autophagy-lysosomal pathway to counteract the photoinduced damage. Recently, autophagy has emerged as a therapeutic target concerning its pro-survival or pro-death role. OBJECTIVES: Here, we proposed an original approach that switches the PDT-mediated cytoprotective autophagy to a pro-death mechanism in human carcinoma HeLa after combining PDT based on methylene blue (MB) with the pentacyclic triterpenoids - oleanolic acid (OA) and betulinic acid (BA). MATERIALS AND METHODS: We applied molecular approaches concerning autophagy (the autophagic-related transcription factor TFEB, and cathepsin B immunoassays, status of lysosomotropic vacuolization, and caspase-3 activity analysis), as well as evaluation of cell proliferation (MTT, CVS, and clonogenic assays). Following irradiation with MB (LED 660 nm, 20 J/cm2 - 380 W/m2), BA or OA was tested as an adjuvant agent. DISCUSSION AND RESULTS: Due to mitochondria damage, both triterpenoids should sensitize PDT-treated cells. However, only BA enhanced PDT efficacy. We observed lower gain (i.e., 21%) when 100 μM BA was combined with 2.0 μM MB-PDT, whilst when associated with 0.5 μM MB-PDT remarkably reduced the cell survival by 48%, a rate very similar observed with 2.0 μM MB-PDT alone. Spite 0.5 μM MB photoinduced mild lysosomal damage, HeLa remained viable due to the cytoprotective autophagy, which was related to TFEB nuclear translocation and cathepsin B recruitment. In the combined regimen, by damaging mitochondria and lysosomes BA jeopardized the cytoprotective photoinduced autophagy, leading to increased vacuolization and granularity, loss of lysosomes integrity, and ultimately the accumulation of autophagic vacuoles. Altogether these effects ended up in increased cell death by about 80%. CONCLUSION: We showed for the first time that BA combined with MB-PDT significantly alleviates tumor resistance and leads to increased cell growth inhibition and death. Our findings demonstrate that this protocol may tackle the autophagy-mediated resistance to PDT in malignant cells.

Keywords: damage in mitochondria and lysosomes, methylene blue, autophagy-associated cell death / **Supported by:** FAPESP; CAPES/PROSUP.

J - Glycobiology

J.01 - Magneto-Optical Multimodal Nanoprobe Based on Mannose-Binding Lectin

Paulo Euzébio C. Filho [1], Pedro Paulino[1], João Lima[1], Weslley Oliveira[1], Carinna Lima[1], Mariana Cabrera[2], Adriana Fontes[1]
[1]Departamento de Biofísica e Radiobiologia, [2]Departamento de Química Fundamental, Universidade Federal de Pernambuco (Pernambuco, Brazil)

INTRODUCTION: Quantum dots (QDs) are semiconductor nanocrystals with unique properties, such as highly photostable fluorescence and active surface for conjugation with biomolecules. In a complementary way, magnetic nanoparticles (MNPs) can be applied for the separation/detection of biological systems, as contrast agents for magnetic resonance imaging, and in hyperthermia. Thus, the union of QDs and MNPs may allow new advances in the biomedical field, especially if combined with biomolecules. Mannose-binding lectin (MBL) is a protein that plays an important role in human host defense. MBL can bind to carbohydrate residues, such as D-mannose, *N*-acetyl-D-glucosamine, and L-fucose present on cell surfaces, by its carbohydrate recognition domain (CRD), more effectively in the presence of Ca^{2+}. OBJECTIVES: This study aimed to prepare a multimodal nanoprobe from CdTe QDs, iron oxide MNPs, and MBL. MATERIALS AND METHODS: Bimodal nanoparticles (BNPs) were prepared by conjugation between carboxyl-coated QDs and amine-functionalized MNPs. Then, BNPs were conjugated to a recombinant MBL (rhMBL) at different concentrations, forming the BNPs-rhMBL-50 and BNPs-rhMBL-100 nanoprobes. All systems were characterized by absorption and emission spectroscopies and zeta potential analyses. The potential for biological application of BNPs-rhMBL was evaluated by flow cytometry using *Candida albicans* yeasts as the biological model. DISCUSSION AND RESULTS: Zeta potential analyses indicated that QDs were associated with MNPs, as well as the rhMBL to BNPs. The optical evaluation also confirmed the QD conjugation with MNPs. Regarding the cell labeling of *C. albicans* in Ca^{2+} enriched saline solution, BNPs-rhMBL-100 showed better performance (*ca.* labeling of 86% with a median of fluorescence of 14,382). Furthermore, BNPs-rhMBL-100 labeled *ca.* 26% of the yeasts in saline without Ca^{2+} and only *ca.* 0.3% of cells in saline with Ca^{2+} and EDTA, indicating that the BNPs-rhMBL-100 nanoprobe showed specificity, interacting with yeasts through the CRD. CONCLUSION: An effective and specific multifunctional nanoprobe (BNPs-rhMBL) was prepared, which holds great potential for glycobiological studies. **Keywords:** carbohydrates, iron oxide nanoparticle, quantum dots / **Supported by:** FACEPE, CNPq, CAPES, INCT-INFo.

J.02 - Interaction of SARS-CoV-2 Surface Protein (Spike Protein) with Heparin: Therapeutic Potential

Ana Luiza Silva [1], Robertha Mariana Rodrigues Lemes[1], Maria Cecilia Zorel Meneghetti[1], Marcelo Andrade Lima[1,2], Helena Bonciani Nader[1]
[1]INFAR, Universidade Federal de São Paulo (São Paulo, Brasil), [2]Molecular and Structural Biosciences, Keele University (Staffordshire, United Kingdom)

INTRODUCTION: Repurposing existing drugs that can prevent and treat novel coronavirus infections is a timely and attractive alternative. Heparin, a well-tolerated anticoagulant pharmaceutical, has been used safely in medicine for >80 years. Preliminary data show that the Surface Glycoprotein of SARS-CoV-2 binds to heparin. OBJECTIVES: To study and explore the properties of heparin as proof of concept for heparin-based antiviral drugs. MATERIALS AND METHODS: Cell lines (VeroE6, CLPS, ECV) were pretreated with heparin and infected with SARS-CoV-2. The groups corresponding 2, 24 and 48 hours of incubation. At the end of each incubation, supernatants were recovered and stored in lysis buffer and cells were lysed. The RNA was extracted and directed to the analysis of the expression. DISCUSSION AND RESULTS: In CLPS cells, the virus in the supernatant was higher than in the cell lysate, even though there is already time for cell multiplication and the possibility of new infections. A possible explanation is that the viruses in these cells were not successful. This hypothesis is supported by the drop in cell lysate over time suggesting that internalization was not successful. In ECV cells, which are human cells, we could see in 24h of infection, when cell multiplication has already occurred and, therefore, the opportunity to infect new cells, in the presence of heparin the virus is higher in the supernatant, suggesting that the pre-treatment prevents this new infection. 24h after infection, in the absence of pretreatment, we have no significant difference suggesting that the supernatant viruses are infecting new cells. This can be explained by the fact that when we offer heparin, there is competition with the viral protein S to the Heparan Sulfate chain of Heparan Sulfate Proteoglycans on the cell surface, thus inhibiting entry. CONCLUSION: Our data suggest heparin can hinder and even prevent the entry of the new coronavirus into human cells. **Keywords:** Heparin, Spike Protein, Therapeutic Potential / **Supported by:** FAPESP

J.03 - O-GlcNAc characterization during Tribolium castaneum Development

Bruno da Costa Rodrigues [1], Lupis Ribeiro[1], Adriane Todeschini[2], Wagner Dias[1], Rodrigo Fonseca[1]
[1]Instituto NUPEM, [2]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brazil)

INTRODUCTION: O-GlcNAc (O-linked β-N-acetylglucosamine) is a dynamic intracellular post-translational modification (PTM) that regulates several cellular processes. This PTM is catalyzed by the covalent attachment of GlcNAc on residues of serine/threonine in proteins by the enzyme O-GlcNAc transferase (OGT), while its removal is performed by O-GlcNAcase (OGA). This PTM is limited by the concentration of UDP-GlcNAc, the end-product from the hexosamine biosynthetic pathway (HBP) that, together with OGT and OGA levels and activity, will regulate protein O-GlcNAcylation. Although O-GlcNAc has been reported in all studied metazoans, functional analysis during development in Hexapoda is restricted to the model organism Drosophila melanogaster. OBJECTIVES: In this study, the role of O-GlcNAc during development was investigated in an emerging model system, the beetle Tribolium castaneum. MATERIALS AND METHODS: In situ hybridization and RT-qPCR were performed for mRNA expression analysis during Tribolium castaneum development. Parental RNAi was performed for knock down experiment analysis. DISCUSSION AND RESULTS: Comparison of the transcriptional profile of the HBP rate-limiting step enzyme glutamine-6-phosphate amidotransferase (GFAT) during developmental stages showed higher levels during larval stages. Interestingly, OGT is modulated during embryonic development with an increase of mRNA levels during gastrulation while OGA remained unaltered in all stages. In situ hybridization analysis revealed an embryonic limited ubiquitous pattern of OGA during egg development, while GFAT is expressed in both embryonic and extra-embryonic cells. Finally, parental knock down of OGT by RNA interference (RNAi) shows physiological impairment of oocyte maturation and a decrease in egg laying and embryonic survival, while OGA knockdown did not lead to physiologically and phenotypic significant changes. CONCLUSION: Altogehter, these results provide an important characterization of the O-GlcNAc machinery in the most diverse order of insects, and suggest a critical role of O-GlcNAcylation in a stage-specific manner during Tribolium castaneum development.
Keywords: Development, O-GlcNAc, Trbibolium castaneum

J.04 - CRL: a novel lectin isolated from Centrolobium robustum seed shows synergistic effect with tetracycline against Staphylococus aureus strains

Willian Fernandes dos Reis [1], Rômulo Farias Carneiro [2], Helton Colares da Silva [3], Alexandre Lopes Andrade [4], Edson Holanda Teixeira [4], **Mayron Alves de Vasconcelos** [1]
[1]Unidade Divinópolis , Universidade do Estado de Minas Gerais (MG, Divinópolis), [2]Departamento de Engenharia de Pesca, Universidade Federal do Ceará (CE, Fortaleza), [3]Faculdade de Educação, Ciências e Letras de Iguatu, Universidade Estadual do Ceará (CE, Iguatu), [4]Departamento de Patologia e Medicina Legal, Universidade Federal do Ceará (CE, Fortaleza)

INTRODUCTION: Plant lectins are proteins or glycoproteins of non-immune origin capable of binding reversibly and specifically to carbohydrates and glycoproteins, without changing their structure. Some studies showed lectins with antimicrobial properties and synergistic effect in combination with antibiotics. OBJECTIVES: The aimed was evaluated the purification and characterization of a novel lectin present in seeds of Centrolobium robustum (Vell.) Mart. ex Benth., and verify its synergistic effect against Staphylococcus aureus strains. MATERIALS AND METHODS: C. robustum seeds were collected in Belo Horizonte - MG. The lectin was purified by affinity chromatography in Sepharose-Mannose column. The hemagglutinating activity and inhibition was performed using 2% type O human erythrocytes. The molecular mass was evaluated by SDS-PAGE and gel filtration chromatography. Moreover, the Circular Dichroism (CD) spectroscopy was used to determine the secondary structure. The antimicrobial activity was evaluated by microdilution, and checkboard assay used to determine the synergistic effect with tetracycline. DISCUSSION AND RESULTS: The lectin named C. robustum lectin (CRL) was purified and inhibited by D-mannose, D-glucose, N-acetyl-D-glucosamine and tetracycline. CRL showed a molecular mass of 25 kDa, verified by SDS-PAGE, and approximately 48 kDa determined by gel filtration chromatography, its native form is probably a homodimer. The lectin proved to be thermostable, maintaining its hemagglutinating activity up to 60 ºC, and it was significantly reduced from 70 ºC. The lectin presented optimal pH values between 6.0 and 8.0. The secondary structure of CRL consisted of 6% α-helix, 57% β-sheet and 37% coil. Regarding antimicrobial activity, CRL showed no antimicrobial activity against S. aureus ATCC 25923 and S. aureus ATCC 700698. However, when CRL was used in combination with tetracycline, the lectin showed significantly reduction in the minimum inhibitory concentrations (MIC) values of the tetracycline for both S. aureus strains. CONCLUSION: The results obtained indicate that CRL improves tetracycline performance and may be promising in the development of new antimicrobial agents. **Keywords:** Plant lectin, Centrolobium robustum, antimicrobial / **Supported by:** Fundação de Amparo à Pesquisa do Estado de Minas Gerais and Conselho Nacional de Desenvolvimento Científico e Tecnológico

J.05 - GM2/GM3 Controls the Organizational Status of CD82/Met Microdomains: Further Studies in GM2/GM3 Complexation

Mariana Amorim Thaumaturgo da Silva [1], Ronan Christian Machado dos Santos Análise[1], Daniela Maria dos Santos Lucena[1], Hector Franco Barbosa Rhault Loponte[1], Frederico Alisson da Silva[2], Wagner Barbosa Dias[1], Roberto Dias Lins Neto[3], Adriane Regina Todeschini[1]
[1]Instituto de Biofísica Carlos Chagas Filho, Univ. Federal do Rio de Janeiro (RJ, Brasil), [2]Instituto de Microbiologia Paulo de Goes, Universidade Federal do Rio de Janeiro (RJ, Brasil), [3]Instituto Aggeu Magalhães, Fundação Oswaldo Cruz (PE, Brazil)

INTRODUCTION: Gangliosides are amphiphilic glycophingolipids found in cell membranes, composed of oligosaccharide units, exposed to the extracellular medium, associated with a hydrophobic ceramide, present in the plasma membrane. Grouped gangliosides can modulate cell adhesion inducing activation, motility and cell growth and are called “glycosynapse”. These are often associated with signal transducer proteins, growth receptors, integrins and tetraspanins, forming cell surface domains that contribute to the cell phenotype. We previously described the formation of a GM2 /GM3 heterodimer, which interacts with CD82 tetraspanin, controlling epithelial cell, motility by inhibiting integrin-hepatocyte growth factor-induced (cMet) tyrosine kinase signaling. OBJECTIVES: Comprehend how gangliosides controls the organization of CD82 and cMet in the cell membrane. MATERIALS AND METHODS: Tritium labeling was performed to verify the ability of GM2 and GM3 to form complexes in aqueous solution. To study the molecular basis of the GM2 and GM3 interaction, molecular modeling and simulations of the gangliosides in methanol and water were performed. Bladder cancer cell line (YTS1) and a normal bladder cell line (HCV29) were utilized. Cell line YTS1/CD82+ was obtained by stably transfecting the human CD82 gene in a pcDNA3 vector into YTS1 cells. To study the impact of ganglioside expression on the organization of the main components of the glycosynaptic domain, cells were pre-treated with D-threo-1-phenyl-2-palmitoylamino-3-pyrrolidino-1-propanol, an inhibitor of glucosylceramide synthase. Cells were than harvested, pelleted, resuspended in Brij 98 lysis buffer, and the lysate was centrifuged. The supernatant was subjected to sucrose density gradient ultracentrifugation to separate low density membrane fractions. Fractions were analyzed using western blot. DISCUSSION AND RESULTS: We observed that the GM3/GM2 complex alters the location of CD82 tetraspanin on the cell surface, thus increasing the phosphorylation of Src2 kinase. CONCLUSION: These results raise the possibility that microdomain organization dictates cell phenotype, suggesting that malignancy may result from microdomain data disorganization.
Keywords: glycosphingolipids, cancer, microdomains

J.06 - The Effect of Metabolic Incorporation of Exogenous Sugar Neu5Gc on EGFR Signaling Pathway in Colon Carcinogenesis

Amanda Carlos Paulino [1], Ana Luiza dos Santos Lopes[1], Adriane Regina Todeschini[1], Frederico Alisson Silva[1]
[1]Instituto de Biofisica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: Sialic acids (Sia) are a family of nine carbon-monosaccharides, present in the terminal portions of glycoconjugates, such as glycoproteins and glycolipids. They have an important role in extracellular signaling, mainly due to their electronegative nature, position and abundance on cell surface. N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc) are the main forms of sialic acids in mammals. Humans are deficient in Neu5Gc, since the enzyme CMAH, responsible for the hydroxylation of Neu5Ac into Neu5Gc, is inactive in humans. However, Neu5Gc can be detected in human tissues, due to its metabolic incorporation from foods that are rich in this Sia, such as red meat. In Brazil, colorectal cancer (CRC) is the third most prevalent cancer and red meat consumption was previously associated with the progression of CRC. Furthermore, nearly 50% sporadic CRC cases show changes in EGFR signaling pathway. OBJECTIVES: To investigate whether Neu5Gc incorporation could alter EGFR signaling pathway in CRC cells. MATERIALS AND METHODS: HCT-116 and SW480 human CRC cell lines were induced to incorporate Neu5Gc and stimulated with different concentrations of rEGF. We then performed an MTT assay to verify cell viability and immunoblotting analysis of proteins involved in EGFR signaling pathway. We also performed an immunoprecipitation assay of the EGFR to check for Neu5Gc incorporation at the receptor. DISCUSSION AND RESULTS: CRC cells exposure to exogenous Neu5Gc led to its incorporation in the EGFR. Western blot analysis revealed an increase in p-AKT (activated AKT) in cells incubated with Neu5Gc, suggesting an increased cellular response to rEGF. However, no changes in cell viability were observed between incubation Neu5Gc or Neu5Ac. CONCLUSION: Our initial results suggest that Neu5Gc incorporation can modulate the EGFR signaling pathway through increased activation in AKT and its target, supporting the need of further studies regarding the intake of Neu5Gc involved in CRC progression.
Keywords: Colorectal Cancer, Sialic acids, EGFR

J.07 - Heparin Action on Antiangiogenic Activity *In vitro* and *In vivo* : Potentiation of Anti-VEGF Effect by Modified Heparin

Bruna Zancanelli Fetter [1], Bianca Oliveira Augusto[1], Alex Treiger Grupenmacher[2], Diego Lisboa Araújo[2], Lucas Zago Ribeiro[2], Caio Vinicius Saito Regatieri[2], Juliana Luporini Dreyfuss[1]
[1]Bioquímica, Universidade Federal de São Paulo (São Paulo, Brasil), [2]Oftalmologia e Ciências Visuais, Universidade Federal de São Paulo (São Paulo, Brasil)

INTRODUCTION: Angiogenesis is the formation of new vessels from preexisting vasculature. The most studied pro-angiogenic regulator molecule is vascular endothelial growth factor (VEGF). Bevacizumab, a humanized anti-VEGF monoclonal antibody, was developed to bind to VEGF proving to be effective in reducing angiogenesis. Heparin, a sulfated glycosaminoglycan, also can modulate the activity of VEGF, having demonstrated through its chemically modified forms an antiangiogenic factor behavior. OBJECTIVES: In this context, the aim of the study is to evaluate the antiangiogenic potential of modified heparin (HepM) and the possible potentiation of the antiangiogenic action of the HepM-Bevacizumab complex for the development of new antiangiogenic therapies. MATERIALS AND METHODS: In *In vitro* assays were used Rabbit Aortic Endothelial Cells (RAEC) to investigate cell viability, proliferation, migration and tube formation assay. *In vivo* assays were performed using laser-induced choroidal neovascularization (CNV) model in lean Zucker rats (CEUA 9900220818) and analyzed in an immunofluorescence microscope. Was also performed the Surface Plasmonic Resonance (SPR) to evaluate the kinetics interaction of heparin, HepM and Bevacizumab with VEGF. DISCUSSION AND RESULTS: *In vitro* studies showed that the treatment of RAECs with HepM-Bevacizumab didn't show cytotoxicity at any concentration tested, on the other hand, this treatment promoted a significant reduction in the proliferation of these cells. It was also observed a significant reduction in the formation of capillary-like structures as well as in the migration of RAEC treated with HepM-Bevacizumab. *In vivo* studies showed a significant reduction in the area of CNV after intravitreal injection of HepM-Bevacizumab at all doses tested. The SPR results showed that heparin bind to VEGF (K_D: 36.4 μM), whereas HepM didn't bind to VEGF. Bevacizumab bind with high affinity to VEGF (K_D: 0.065μM) as expected. CONCLUSION: The HepM-Bevacizumab complex showed potentiate the antiangiogenic activity, being a drug candidate for new antiangiogenic therapies.
Keywords: Angiogenesis, anti-VEGF, Modified heparina / **Supported by:** FAPESP and CNPq

J.08 - Extraction, isolation, characterization and biological activity of sulfated polysaccharides present in ascidian viscera Microcosmus exasperatus

Ananda Bento [1], Simone Cardoso[2], Mauro Pavão[3], Mariana Stelling[1]
[1]Biotecnologia , Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro (RJ, Brasil), [2]Instituto de Física, Univ. Federal do Rio de Janeiro (Rio de Janeiro, Brasil), [3]Instituto de Bioquímica Médica, Univ. Federal do Rio de Janeiro (RJ, Brasil)

INTRODUCTION: Ascidians are marine invertebrate tunicates that synthesize sulfated glycosaminoglycans (GAGs) within their viscera. Ascidian GAGs are considered analogues of mammalian GAGs and possess great potential as bioactive compounds, presenting antitumor and anticoagulant activity. Due to their clinical potential, it is important to understand how ascidian GAGs are produced, their function within the ascidian organism and how they keep their bioactivity after extraction and purification processes. Mammalian heparin is one of the main compounds used to treat thrombosis and related diseases due to their anticoagulant activity. However, heparin presents adverse effects, in this context, ascidian GAGs are proposed as good alternatives for mammalian heparin and, therefore, should be carefully studied. OBJECTIVES: Our main objectives are to study the ascidian *Microcosmus exasperatus* regarding GAGs composition, structure, distribution within the viscera and also its biological activity. MATERIALS AND METHODS: Ascidians were collected by free diving. GAGs were extracted by proteolytic digestion and purified by ion-exchange liquid chromatography and characterized by agarose gel electrophoresis and enzymatic treatments. Anticoagulant activity was evaluated by aPTT assays. Cell cytotoxicity was evaluated by MTT assays using two tumor cell lines (LLC and MC-38). Tumor cell migration activity was evaluated *In vitro* by wound healing assays. DISCUSSION AND RESULTS: Our results show that *M. exasperatus* presents three distinct polysaccharides. These polysaccharides were fractionated into two fractions named PS 1 and PS 2. *M. exasperatus* produces a low anticoagulant dermatan sulfate (PS 2) and a heparin-like (PS 1) compound, which, interestingly, is not susceptible to heparinases treatment and does not present significant anticoagulant activity. PS 2 shows mild cytotoxicity in LLC tumor cells when in combination with manganese, however it has no effect on the invasive potential of LLC and MC-38 tumor cells. CONCLUSION: As a conclusion, we hope to establish *M. exasperatus* GAGs as suitable compounds for future preclinical studies in cancer and vascular disease areas. **Keywords:** Microcosmus exasperatus, sulfated glycosaminoglycans, ascidians / **Supported by:** CNPq, FAPERJ, CAPES, Fundação do Câncer, UFRJ and IFRJ

K - Molecular Mechanisms of Diseases

K.01 - NO Modulation In Cardiomyocytes After Conditioned Medium Produced By Renal Cells Under Hypoxia Conditions: Cardiorenal Syndrome 3 *In vitro* Model

Giovana Marchini Armentano [1], Joana Claudio Pieretti[1], Amedea B. Seabra[1], Marcela Sorelli Carneiro Ramos[1]
[1]Centro de Ciências Naturais e Humanas, Universidade Federal do ABC (São Paulo, Brasil)

INTRODUCTION: The cardiorenal syndrome type 3 (CRS3) is characterized by an acute renal injury that leads to cardiac damage. Experimental hypoxia, as a model of kidney injury, can damage the heart through the expression of genes related to the inflammatory process, in addition to regulating the expression of enzymes such as nitric oxide synthases and nitric oxide (NO) levels. OBJECTIVES: The aim of this study was to mimic CRS3 *In vitro* and verify the influence of inflammatory mediators and the role of NO, produced by renal cells preconditioned to hypoxia on cardiac cells. MATERIALS AND METHODS: HEK293 (renal cell lineage) was cultivated and submitted to hypoxia for 16 hours and 3 hours of reoxygenation using the GasPak EZ System (BD). The conditioned medium from HEK293 was transferred to H9c2 cells (cardiac cell lineage) for 24 hours, treated or not with 1 mM of L-NAME. RT-qPCR was performed to evaluate gene expression. NO levels were analyzed by amperometry technique. Data were expressed as mean ± standard deviation values of $p < 0.05$ were considered significant. DISCUSSION AND RESULTS: The H9c2 cells that received conditioned medium from HEK293, after hypoxia, had an increase of 87.27% in ANF expression, indicating a possible hypertrophy. Hypoxia increases NO levels in renal cells as well as in H9c2 after conditioned medium subjected to hypoxia. Treatment with L-NAME was able to decrease NO levels in HEK293 and H9c2 cells. Regarding the inflammatory profile, H9c2 showed no changes in the gene expression of IL-6 and IL-1β, which may indicate the participation of other inflammatory molecules that will be investigated later. CONCLUSION: Renal cell hypoxia is able to modulate cardiac trophism. Furthermore, the NO pathway plays an important role in modulating cardiac trophism in the experimental model.

Keywords: cardiorenal syndrome, hypoxia, inflammation / **Supported by:** CAPES

K.02 - ANALYSIS OF THE MECHANISM OF INTERACTION BETWEEN HUMAN RESPIRATORY SYNCYTIAL VIRUS MATRIX PROTEIN (M) AND TRANS-RESVERATROL BY NMR AND FLUORESCENCE SPECTROSCOPY.

Thaina Dos Santos Rodrigues [1], Isabella Otenio De Lourenço[1], Ícaro Putinhon Caruso[1], Fátima Pereira De Souza[1], Marcelo Andres Fossey[1]
[1]física, Universidade Estadual Paulista - Instituto De Biociências, Letras E Ciências Exatas - Câmpus De São José Do Rio Preto - Ibilce/Unesp (São Paulo, Brasil)

INTRODUCTION: Human Respiratory Syncytial Virus is the main cause of hospitalization for respiratory diseases among children under 5 years of age, being responsible for pneumonia or bronchiolitis. Currently, there are no effective vaccines or treatments against the disease. Among the proteins encoded by this virus, the Matrix protein is part of the internal structure of the vírion and is composed of 256 amino acid residues and involves the entire ribonucleoprotein complex, located between this complex and the viral lipid bilayer with primary function in the sprouting of the viral particle and spread of the virus. Resveratrol (trans-3,40,5-trihydroxystylbene) is a phytoalexin found naturally in many common food sources, blackberries and peanuts and has numerous benefits and effects on human health, including antiviral property. OBJECTIVES: The objective of this work was to characterize the interaction of matrix protein (M) and Resveratrol. MATERIALS AND METHODS: Then, the protein was obtained recombinantly, purified through affinity chromatography and molecular exclusion, verified by SDS-PAGE electrophoresis and the analysis of the secondary structure and interaction of the protein in the ligand were performed respectively by circular dichroism and fluorescence spectroscopy. DISCUSSION AND RESULTS: The purified protein with molecular weight (30 kDa). The circular dichroism (CD) spectra showed that the M protein is mainly composed of β-leaf structures, with 44% beta structures, 23% alpha helix structures, and 33% randoim coil structures. The results of protein/ligand interaction showed a hydrophobic type interaction where the Kb of the complex increases proportionally with an increase in temperature of 5,2x10-3 M-1, showing a medium/high interaction being n = 1, that is, 1 ligand for 1 protein. CONCLUSION: The work suggests the interaction of Resveratrol with the M protein, indicating that this antioxidant interferes with viral assembly and budding. Due to the scarcity of new drugs with antiviral action, Resveratrol may be an alternative for the treatment of HRSV infection.

Keywords: HUMAN RESPIRATORY SYNCYTIAL VIRUS , MATRIX PROTEIN (M), RESVERATROL-TRANS /
Supported by: CAPES

K.03 - Are we still the same as our parents? Potential generational involvement of H3K4me3 and H3K36me3 in susceptibility to the Depression or Resilience phenotype

Lucas dos Santos da Silva [1], Luiza Behrens[1], Camila Barbosa[1], Nicole Frohlich[1], Luana Possa[1], Roberto Almeida[2], José Cláudio Moreira[1]
[1]Departamento de Bioquímica, Universidade Federal do Rio Grande do Sul (Rio Grande do Sul, Brazil),
[2]Departamento de Ciências Biológicas, Universidade Federal de Ouro Preto (Minas Gerais, Brazil)

INTRODUCTION: Major Depressive Disorder (MDD) is a severe and multifactorial psychiatric disorder. However, some individuals do not develop MDD, characterizing resilience. Epigenetic mechanisms underlie susceptibility to MDD, and histone methylation may be involved. However, there are still no studies relating the lysine residues (K) 4 and 36 of histone 3 (H3) trimethylation with the inheritance of MDD or resilience. OBJECTIVES: Thus, our objectives were: 1) to evaluate the phenotype of 40 Wistar rats (20 males and 20 females) submitted to the CUMS model (F0 animals) and their offspring (F1 animals); 2) verify the levels of H3K4me3 and H3K36me3 in F0 and F1 animals; 3) relate residues H3K4me3 and H3K36me3 with inheritance for MDD or resilience MATERIALS AND METHODS: The phenotype of the animals was determined utilizing behavioral tests. We used the western blot technique to analyze the pattern of trimethylation in the hippocampus. Two-way ANOVA followed by Sidak analyzed behavioral tests. One-way ANOVA followed by Tukey analyzed the methylation pattern. DISCUSSION AND RESULTS: We found that part of the animals submitted to CUMS developed the depressive phenotype ($p < 0.05$), while another portion developed resilience ($p < 0.05$). When analyzing the trimethylation pattern in F0 animals, we observed that H3K4me3 and H3K36me3 were hypermethylated in male and female resilient animals ($p < 0.05$) in relation to depressive and controls. When we evaluated the offspring's behavior, we observed that the offspring of depressive parents inherited the depressive phenotype ($p < 0.05$). Meanwhile, offspring of resilient parents showed resilient behavior ($p < 0.05$). According to their parents, we found that the resilient male offspring showed hypermethylation of H3K4me3 and H3K36me3 ($p < 0.05$). CONCLUSION: Our results demonstrate that H3K4me3 and H3K36me3 are involved in the resilience phenotype. Furthermore, we observed that the offspring inherited their parents' behavior and resilient male animals inherited the trimethylation pattern of H3K4me3 and H3K36me3. Therefore, H3K4me3 and H3K36me3 can help in the resilience phenotype in a generational way.

Keywords: Major Depressive Disorder, Resilience, Heredity / **Supported by:** CNPq, CAPES and Propesq-UFRGS

K.04 - cis-[Ru(phen)2(3,4Apy)2]2+ as luminescent probe in the cross-aggregation and citotoxicity of amyloid-beta and human insulin.

Marco Antonio Tiburcio [1], Maria Laura da Cruz Garcia[1], Rose Maria Carlos[1]
[1]Química, Universidade Federal de São Carlos (São Paulo, Brasil)

INTRODUCTION: Alzheimer's Disease (AD) and Type 2 Diabetes Mellitus are the most common chronic disorders associated with the formation of amyloid aggregates, affecting thousands of people. This scenario is exacerbated by epidemiological, clinical, and animal analyzes that indicate that one disease constitutes a risk factor for the other. One of the imbalances reported in people with AD is the low consumption of glucose in the brain, causing AD to be known as Type III Diabetes. New generation of antidiabetics shows efficacy in animal models of AD encouraging research into proteins associated with these diseases considering that insulin as well as Aβ peptide (associated with AD) are proteins that form amyloid fibrils. OBJECTIVES: To evaluate the cytotoxicity of the various species generated during Aβ aggregation in SH-SY5Y neuroblastoma cells in the absence and presence of human insulin in monomeric and fibrillar form, using luminescent Ru(II) complexes to map the structures formed. MATERIALS AND METHODS: The cis-[Ru(phen)2(3,4Apy)2]2+ (RuApy) complex was synthesized by combining cis-[Ru(phen)2(Cl)2] with 3,4-diaminopyridine, the Aβ peptide and human insulin were used in monomeric and fibril form. The luminescence experiments were performed using the intrinsic emission of the proteins (λEX: 280nm), RuApy (λEX: 480nm) and ThT (λEX: 440nm) and the cytotoxicity experiments were revealed by the MTT technique. DISCUSSION AND RESULTS: After 48 hours the emission of the Aβ peptide is totally suppressed, with the formation of new bands at 330nm and 450nm. When incubated in SH-SY5Y cells, the monomeric form shows greater toxicity compared to fibrils, as it passes through all stages of aggregation, including toxic oligomers, with a less pronounced effect in the presence of insulin and the RuApy complex. CONCLUSION: The present study demonstrates the protective character of insulin in relation to the Aβ peptide in SH-SY5Y cells, as well as the RuApy complex, which increases cell viability when compared to Aβ.

Keywords: Ru(II) complexes, Amyloid-beta, human insulin

K.05 - Evaluation Of Cell Viability And Proliferation On Coculture Of Human Hepatic Cells Exposed To PM2.5

Jéssica Marques Obelar Ramos [1], Arieli Cruz de Souza[1], Hemelin Resende Farias[1], Lílian Corrêa Costa-Beber[1], Leo Anderson Meira Martins[1], Izabel Cristina Custódio de Souza[2], Fátima Theresinha Costa Rodrigues Guma[1]
[1]Departamento de Bioquímica, Universidade Federal do Rio Grande do Sul (RS, Brasil), [2]Departamento de Morfologia, Universidade Federal de Pelotas (RS, Brasil)

INTRODUCTION: Particulate Matter with a diameter ≤ 2,.5 μm (PM2.5) is an atmospheric air pollution fraction which already been associated with hepatocellular carcinoma (HCC). The main PM2.5 effect in liver occurs in hepatocytes, which represent 80 % of the entire organ. Another cell type involved in the tumor microenvironment of HCC is hepatic stellate cells (HSCs). Hepatic injuries can promote tumor HSCs activation to myofibroblast from a quiescent state. Cell communication between hepatocytes and HSCs can be accessed through mixed cell cultures, and this could elucidate the effect of PM2.5 on the tumor microenvironment. OBJECTIVES: Evaluate the effect of PM2.5 on cell viability and proliferation in monoculture or coculture of hepatocytes and HSCs. MATERIALS AND METHODS: Human cell lines LX2 (HSCs), and HepG2 (hepatocyte derived from HCC) were cultivated isolated or in coculture. On the day after, the cultures were exposed to PM2.5 (blank, 20 and 50 μg/mL) for 24 hours. The cytotoxicity was evaluated by LDH leakage. Cell proliferation was determined by counting the nuclei dyed with DAPI. Cell death was evaluated using an annexin-V/PI detection kit. Differences between groups were calculated using one-way analysis of variance (ANOVA) followed by Dunnett post hoc test, with statistical significance considered for p≤0,05. DISCUSSION AND RESULTS: Our results show that exposure to PM2.5, at least at the time and doses used in this study, did not cause significant cytotoxic effects, as measured by LDH leakage, in both cells, in monocultures or in cocultures. However, we observed a significant increase in cell proliferation in cocultures exposed to 20 μg/mL of PM2.5 ($p = 0.0391$, versus blank), and reduced rates of early apoptosis on hepatocytes cocultured with HSCs in 20 and 50 μg/mL ($p = 0.0012$ and $p = 0.0010$, versus blank respectivly). CONCLUSION: Our results suggest that PM2.5 can induce characteristics of tumor cells, like high proliferative capacity and apoptosis reduced rates.
Keywords: Cancer , Hepatocyte, Pollutants / **Supported by:** CAPES, CNPq

K.06 - Understanding the Role of the Deubiquitinase USP2b as a Cancer Promoter

Soraia Ferreira [1], Silvia Daniéle Rodrigues[2], Silvio Marques Zanata[1]
[1]Departamento de Biologia Celular, Univ. Federal do Paraná (Paraná, Brasil), [2]Department of Medicine, Weill Cornell Medicine (New York, USA)

INTRODUCTION: Deubiquitinases (DUB) are proteases that regulate the functions of the target protein by removing its ubiquitin chain. The ubiquitin specific peptidase 2 (USP2) is a DUB that has three described isoforms (a, b and c). Among USP2 isoforms, the USP2a has been associated with poor prognosis in tumor pathogenesis, due to their interference with cell cycle proteins, oncoproteins, and on tyrosine kinase receptor degradation pathway, like EGFR (epidermal growth factor receptor). However, the role of USP2b isoform is still unknown. OBJECTIVES: The aim of this study was investigate if USP2b could have the same behavior in promotion of cancer. Then, the USP2b overexpression was used to evaluate the degradation of the EGFR, the promotion of proliferation and the cell survival under the treatment of cyclophosphamide. MATERIALS AND METHODS: For all analysis, HeLa cells were transiently transfected with USP2a, USP2b and empty vector in serum-free medium and after 16h the supernatant was replaced. For the proliferation analysis, the cells were cultivated for 48h and the rate of proliferation was measured by crystal violet assay. For the EGFR degradation analysis, after transfection and starvation time, the cells were stimulated with EGF for different times and the stabilization of receptor was evaluated by western blot. For survival analysis, the cells were treated with 400 uM cyclophosphamide for 24h and the MTT assay was used to measure cellular metabolic activity to indicate the cell viability. DISCUSSION AND RESULTS: Our data showed that USP2b overexpression improves the proliferation rate of HeLa and increases EGFR stabilization, furthermore this isoform prevents the cell death when these cells were challenged with cyclophosphamide, a drug employed as chemotherapy. CONCLUSION: Our findings suggest that USP2b could be an important pharmacological target for cancer treatment due to the importance of EGFR signaling pathway to promote the growth and spread of cancer cells.
Keywords: Deubiquitinase, Cancer, USP2b
Supported by: CAPES

K.07 - Assessment of the IgG Antibody Profile of Individuals Infected with SARS-CoV-2 and Determination of Serological Prognostic Markers for Disease Severity

Tayná Evily de Lima [1], Matheus Vitor Ferreira Ferraz[2], Carlos Alexandre Antunes de Brito[3], Pamella de Brito Ximenes[4], Carolline de Araújo Mariz[1], Lícya Samara Xavier[1], Roberto Dias Lins Neto[1], Isabelle Freire Tabosa Viana[1]
[1]Department of Virology and Experimental Therapy , Aggeu Magalhães Institute, Oswaldo Cruz Foundation (PE, Brazil), [2]Department of Fundamental Chemistry, [3]Hospital das Clínicas, Federal University of Pernambuco (pe, Brazil), [4]Hospital dos Servidores do Estado de Pernambuco, Hospital dos Servidores do Estado de Pernambuco (pe, Brazil)

INTRODUCTION: Several risk factors are known to be implicated with disease severity during SARS-CoV-2 infections. However, prognostic markers to appropriately indicate individuals with higher probability to develop more severe symptoms, and therefore, those who would potentially require more aggressive medical interventions, are still not completely understood. OBJECTIVES: To define the SARS-CoV-2-specific IgG antibody profiles of subjects infected with the B lineage (Hu-1) and to identify serological prognostic markers for disease severity as the infection progresses. MATERIALS AND METHODS: Serum samples were collected from 100 individuals admitted to a reference hospital in Recife with SARS-CoV-2 infection confirmed through qRT-PCR. Samples were collected daily from admission to discharge or death. SARS-CoV-2 receptor-binding domain (RBD) protein was produced *In vitro* through transfection of HEK293T cells and purified through chromatography. An indirect enzyme-linked immunosorbent assay (ELISA) was set up and used to determine the IgG antibody profile of the enrolled subjects. Routine laboratorial measurement data was used to determine risk factors associated with disease severity, and to develop a classification prognosis based on machine learning algorithms. DISCUSSION AND RESULTS: Our RBD-based ELISA exhibited sensitivity of 92.45% and specificity of 97%. Among the IgG positive individuals, 55% progressed to the mild form of disease, while 45% progressed to severe phenotypes. Severe disease was associated with pre-existing conditions, such as hypertension (69%) and asthma (21%). Individuals who presented anemia (81%), lymphocytopenia (28%), leukocytosis (17%) and increased levels of CK (14%), AST (69%) and blood glucose (86%) during hospitalization presented a higher rate of disease severity. Using a combination of linear discriminant analysis and decision tree algorithms, we were able to classify disease severity, from laboratory data only, with 100% accuracy. CONCLUSION: Antibody profile and laboratory data of COVID-19 patients were used to characterize markers associated with disease severity, while machine learning algorithms to classify disease prognosis.
Keywords: SARS-CoV-2, RBD-based ELISA, Disease severity / **Supported by:** FACEPE, CNPq, Fiocruz, INOVA-Fiocruz, Rede Genômica-Fiocruz.

K.08 - Increased NADase Activity and Nicotinamide Excretion may Account for Decreased NAD+ Levels in the Brain During Zika Virus Infection in Neonate Mice

Julianna Dias Zeidler [1], Emanuelle Vasconcellos de Lima[2], Tamires Rocha da Silva[2], Geórgia do Nascimento Saraiva[3], Matheus Atella de Oliveira[1], Stefany Alexandre Bezerra[2], Julia Clarke[4], Giselle Fazzioni Passos[2], Juliana Camacho Pereira[1], Andrea Thompson Da Poian[1]
[1]Instituto de Bioquímica Médica Leopoldo de Meis, IBqM, [2]Faculdade de Farmácia, [3]Instituto de Microbiologia Paulo de Goés, [4]Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: As a result of Zika Virus (ZIKV) epidemics, thousands of children today suffer from congenital ZIKV syndrome, and many more are at risk. Therefore, it is essential to understand what aspects of ZIKV infection contribute to neurological damage to uncover therapeutical targets. A recent study has identified NAD+ metabolism disruption during Zika virus (ZIKV) infection in the fetal brain. However, the molecular mechanisms by which it takes place remain elusive. OBJECTIVES: To investigate that, we used a mouse model of ZIKV infection in neonates, which corresponds to an infection in the third trimester of pregnancy in humans. MATERIALS AND METHODS: On the third day after birth, Swiss mice were subcutaneously infected with 106 PFU ZIKV or mock and euthanized 13 days post-infection. Tissues were processed for NAD+ levels measurement (by enzymatic coupled cycling assay), NADase activity (etheno-NAD+ assay), and changes in NAD+ transcriptome (qPCR). DISCUSSION AND RESULTS: We found that NAD+ levels decreased by about 20% in the brain but not in the lung and spleen of ZIKV-infected mice compared to mock-infected mice. Accordingly, total NAD+ hydrolase activity increased by 40% in the brain of ZIKV-infected mice, but not in the spleen and lung. We also found a significant increase in mRNA expression of NAD+ hydrolases, which generate nicotinamide as a byproduct. Accordingly, we found a 3-5-fold increase in gene expression related to the nicotinamide excretion pathway. Also, the expression of key genes of the NAD+ de novo synthesis pathway increased by 2-4-fold in ZIKV-infected brains. However, there was no significant change in the expression of nicotinamide salvage pathway genes. CONCLUSION: Our data suggest that NAD+ levels decrease in the brains of ZIKV-infected mice, possibly due to increased activity of NAD+-degrading enzymes and increased nicotinamide metabolization through the excretion pathway without sufficient compensation of NAD+ synthesis pathways. We are currently working on further experiments to confirm this hypothesis.
Keywords: Zika virus infection, NAD+ metabolism, neuroinfection / **Supported by:** FAPERJ, CAPES, CNPq

K.09 - INTERLEUKIN-10 GENE PROMOTER REGION POLYMORPHISM IS NOT ASSOCIATED WITH THE FREQUENCY OF VENTRICULAR EXTRASYSTOLES IN CHRONIC CHAGAS DISEASE

Joao Paulo da Silva Liberalino[1,2], Dayane Carla Costa Paiva Dantas[1], Valeria Duarte de Almeida[1], Fernando Liberalino Fernandes[1,2], Gilson Aquino Cavalcante[1,2], Amanda Estevam Carvalho[1,2], Micássio Fernandes de Andrade[2], Cleber Mesquita de Andrade[1], Ellany Gurgel C. do Nascimento[1], Christiane Medeiros Bezerra[3], **Thales Allyrio A. de M. Fernandes [1,2]**

[1]Ciências Biomédicas, Universidade do Estado do Rio Grande do Norte (RGN, Brasil), [2]SBBq, Programa Multicêntrico de Pós-Graduação em Bioquímica e Biologia Molecular (RN, Brasil), [3]Departamento de Microbiologia e Parasitologia, Universidade Federal do Rio Grande do Norte (RN, Brasil)

INTRODUCTION: Functional genetic polymorphisms involved in the immune response may modulate the clinical variability in Chagas disease (CD). Several studies have proposed a protective role for IL-10 in CD pathophysiology. Two previous publications found an association between the cardiac form of CD and the A allele in rs1800896 single nucleotide polymorphism (SNP), which is correlated with lower production of IL-10 by immune system cells. However, possible associations between this SNP and electrocardiographic findings have not yet been assessed. OBJECTIVES: This study aimed to evaluate the association between rs1800896 and the frequency of ventricular (VE) and supraventricular extrasystoles (SVE) and the occurrence of non-sustained ventricular tachycardia (NSVT) in a 24-hour Holter monitoring of CD patients. MATERIALS AND METHODS: It was conducted a cross-sectional study with 103 chronic CD patients, composed of 55 males and 48 females, with a mean age of 47,51 ± 12,34 years. They were genotyped by the polymerase chain reaction-restriction fragment length polymorphism method. P-value < 0.05 was considered significant. DISCUSSION AND RESULTS: It was found 41 patients presenting the AA genotype, 52 AG and 10 GG. The expected and observed genotype frequencies obeyed the Hardy-Weinberg equilibrium. The statistical analyses conducted showed an association between the AA genotype and VE frequencies higher than one episode per hour (p = 0,011). However, the same did not apply to VE frequencies higher than 10 episodes per hour, to which is attributed higher clinical importance. Individuals with the AA genotype had higher VE frequencies as a group, but that finding did not have statistical significance (p = 0,101). Also, no relationship was established between rs1800896 and SVE frequencies or the occurrence of NSVT. CONCLUSION: This study did not found a significant association between the AA genotype of rs1800896 with higher VE frequencies. However, other studies are necessary to support these findings.

Keywords: Chagas Disease, Interleukin-10, Single Nucleotide Polymorphism / **Supported by:** CAPES; CNPq

K.10 - Protective Role of IL-4 Receptor in Adriamycin-Induced Nephropathy

Giulianne Bastos Serpa [1], Caio Vettorazzi Barboza Menário[1], Rodrigo Pacheco da Silva de Aguiar[1], Diogo de Barros Peruchetti[1], Douglas Esteves Teixeira[1], Rodrigo Alves da Silva Peres[1], Christina Maeda Takiya[1], Ana Acacia de Sá Pinheiro[1,2], Celso Caruso Neves[1,2,3]

[1]Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro (RJ, Brazil), [2]Rio de Janeiro Innovation Network in Nanosystems for Health, Rio de Janeiro Innovation Network in Nanosystems for Health (RJ, Brazil), [3]National Institute of Science and Technology for Regenerative Medicine, National Institute of Science and Technology for Regenerative Medicine (RJ, Brazil)

INTRODUCTION: Chronic kidney disease (CKD) is a chronic-degenerative disease associated to high-rate mortality and morbidity. Proteinuria is a well-known marker of CKD progression. Some authors have highlighted the role of protein endocytic machinery in the proximal tubule on the development of tubule-interstitial injury (TII) and, consequently, on the progression of CKD. Previous evidence demonstrated the protective role of interleukin-4 receptor (IL-4R) in the development of TII induced by albumin overload. OBJECTIVES: We aimed to study the role of IL-4R in the TII development in the early step of CKD. MATERIALS AND METHODS: CKD induced in mice by an intravenous single-dose of adriamycin (ADR, 10 mg/kg) was used (CEUA: IBCCF098-A13/20-045-17). IL-4Rα chain knockout mice were used (IL-4R-/-) having wild type (WT) BALB/c mice as a control. Four groups were generated: WT (n=3), WT/ADR (n=5), IL-4R-/- (n=3) and IL-4R-/-/ADR (n=3). Two weeks after ADR injection the animals were kept in a metabolic cage for urine and plasma samples collection. After that, mice were euthanized, and kidneys were collected for histological approach. All data are presented as mean ± standard deviation. DISCUSSION AND RESULTS: IL-4R-/-/ADR developed more severe proteinuria than WT/ADR (WT:0,73±0,78 mg/24h; IL-4R-/-:0,12±0,13 mg/24h; WT/ADR:25,23±15,66 mg/24h; IL-4R-/-/ADR:62,52±17,92 mg/24h). Interestingly, this proteinuria was associated with increased activity in the marker of TII, urinary γ-glutamyltransferase (WT:90,23±22,73 U/g creatinine; IL-4R-/-:31,87±32,99 U/g creatinine; WT/ADR:140,80±57,09 U/g creatinine; IL-4R-/-/ADR:273,70±61,00 U/g creatinine) without changes in blood urea nitrogen (WT:90,52±6,16 mg/dL; IL-4R-/-:54,53±16,39 mg/dL; WT/ADR:110,30±67,62 mg/dL; IL-4R-/-/ADR:97,22±3,73 mg/dL) or plasma creatinine (WT:0,90±0,18 U/g creatinine; IL-4R-/-:0,33±0,57 U/g creatinine; WT/ADR:0,87±0,22 U/g creatinine; IL-4R-/-/ADR:0,51±0,41 U/g creatinine), markers of glomerular function. Moreover, IL-4R-/-/ADR showed glomerular tuft area expansion in comparison with WT/ADR. There was no difference between WT and IL-4R-/-. CONCLUSION: These findings indicate that absence of IL-4R aggravates the TII and highlight the role of IL-4 as a new therapeutic target for CKD treatment.

Keywords: Adriamycin, Chronic kidney disease, IL-4 / **Supported by:** CAPES, CNPq e FAPERJ

K.11 - Role of Tetrahydrobiopterin Metabolism in Abdominal Pain in Inflammatory Bowel Diseases

Jennyffer Souza[1], **Hugo Tolentino[1]**, Bruna Lenfers Turnes[1], Ananda Christina Staats Pires[1], Sara Rizzatti Marques[1], Laís Niero [1], Débora da Luz Scheffer[1], Andrés Trostchansky[2], Vivian Menegassi[1], Alexandra Latini[1]
[1]Department of Biochemistry , Federal University of Santa Catarina (Santa Catarina, Brazil), [2]Facultad de Medicina, Universidad de la Republica (Montevidéu, Uruguay)

INTRODUCTION: Inflammatory bowel diseases (IBD) cause a weighty socioeconomic burden and severely compromise quality of life. IBD's physiopathology is not fully elucidated, contributing to the scarcity of effective treatments. We have shown for the first time that the levels of tetrahydrobiopterin (BH4), an obligatory cofactor for the synthesis of biogenic amines and nitric oxide are pathologically increased in chronic pain. Exacerbated BH4 levels were found in tissues and fluids of animals submitted to multiple neuropathic pain models, and correlated with nociceptive hypersensitivity. The use of QM385, a specific inhibitor of BH4 synthesis developed by our group, normalized BH4 levels and induced analgesia. OBJECTIVES: To investigate the involvement of BH4 metabolism in the physiopathology of abdominal pain in IBDs. MATERIALS AND METHODS: Male C57BL/J6 mice received 2% sodium dextran sulfate (DSS) for six days, inducing IBD. The clinical disease activity, histopathological scores, abdominal mechanical hypersensitivity, biochemical markers of inflammation, and the BH4 metabolism were assessed. Controls received filtered water. Some DSS mice also received QM385 (3 mg/kg) for three days. Clinical scores, urine and plasma samples obtained from IBD-affected individuals were used to validate the animal data. DISCUSSION AND RESULTS: DSS-treated mice showed clinical disease activity and histopathological scores compatible with experimental IBD. Inflammatory markers and BH4 metabolism were increased in the blood and also in colonic samples from DSS mice. The use of QM385 restored BH4 to normal levels and attenuated the clinical and biochemical presentation of DSS-treat mice. CONCLUSION: The data strongly suggest the exacerbation of the BH4 metabolism is also involved in IBD-associated abdominal pain. The use of inhibitors of BH4 synthesis seems a promising avenue to treat IBDs more effectively. The levels of BH4 in fluids may represent a non-invasive tool for evaluating the degree of IBD inflammation and the effectiveness of pharmacological treatments in clinical practice.
Keywords: Biomarkers, Inflammatory Pain, Ulcerative Colitis / **Supported by:** FAPESC /Programa de Pesquisa para o SUS); Conselho Nacional de Desenvolvimento Científico e Tecnológico

K.12 - Immune Checkpoint Protein PD-L1 expression in Non-Small Cell Lung Cancer with NRF2 Activating Mutations

Karoline Almeida Lima [1], Marcelo Luiz Brunatto Falchetti[1], Helena Souza Zancanaro[1], Daniella Serafin Couto Vieira[2], Lisleia Golfetto[2], Danusa Leidens[2], Nauana Somensi[3], Jonathan Paulo Agnes[1], Raquel Nascimento das Neves [1], Alfeu Zanotto-Filho[1]
[1]Departamento de Farmacologia, Laboratório de Farmacologia e Bioquímica do Câncer - Universidade Federal de Santa Catarina (Santa Catarina, Brasil), [2]Departaento de Patologia, Hospital Universitário Polydoro Ernani de São Thiago - Universidade Federal de Santa Catarina (Santa Catarina, Brasil), [3]Departamento de Bioquímica, Centro de Estudos em Estresse Oxidativo - Universidade Federal do Rio Grande do Sul (Rio Grande do Sul, Brasil)

INTRODUCTION: Non-small cell lung cancer (NSCLC) is characterized by mutually exclusive mutations in several signaling pathways. Mutations in the KEAP1/NRF2 pathway occur in 20-30% of NSCLC, and have been associated with upregulation of redox-related proteins - such as thioredoxin-reductase 1 (TXNRD1) - and poor prognosis in NSCLC patients. Patients with high expression of PD-L1 have been characterized as a subgroup that responds positively to immune checkpoint inhibitors (ICI). In this context, recent literature shows that mutations in the KEAP1/NRF2 pathway confer better response to ICI therapy. OBJECTIVES: Therefore, this work aims to evaluate expression of PD-L1 in tumors with hyperactive KEAP1/NRF2 pathway, and whether there is a NRF2-dependent control of PD-L1 expression in NSCLC. MATERIALS AND METHODS: Firstly, we used bioinformatics to evaluate PD-L1 expression and KEAP1/NRF2 mutation gene signature in 1,145 NSCLC patients from The Cancer Genome Atlas (TCGA). We also evaluated PD-L1, NRF2 and TXNRD1 by immunohistochemistry in a cohort of 95 patients diagnosed with NSCLC (Ethics approval CAAE: 83271317.1.1001.5347 and 47699320.0.0000.0121). In addition, we determined PD-L1 mRNA expression by RT-qPCR in KEAP1-mutant A549 lineage treated with pharmacological inhibitor of NRF2 (ML-385) or NRF2 knockdown (NFE2L2 siRNA). DISCUSSION AND RESULTS: The results showed none significant correlation between PD-L1 expression, KEAP1/NRF2 mutation, and NRF2 gene signature expression in TCGA cohort. Immunohistochemical analysis corroborated this data, with no association between TXNRD1/NRF2 and PD-L1 protein scores. Finally, NRF2 inhibition and NFE2L2 knockdown did not alter PD-L1 expression in A549 cells when compared to control cells, suggesting that mutations in the KEAP1/NRF2 pathway do not dictate PD-L1 expression in lung cancer. CONCLUSION: Our data thus far indicate that hyperactive NRF2 pathway is not determinant of PD-L1 expression in NSCLC, indicating that response to ICI therapy in NSCLC patients is not directly related to NRF2-dependent PD-L1 expression.
Keywords: lung cancer, PD-L1, NRF2 / **Supported by:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES.

K.13 - LPS Increases Protein Content of De Novo Lipogenesis Enzymes in Hepatocytes by activating Rictor/mTORC2

Bianca Franco Leonardi [1], Albert Souza Peixoto[1], Tiago E. Oliveira[1], Erique Castro[1], William Tadeu Festuccia[1]
[1]Departamento de Fisiologia e Biofísica, Instituto de Ciências Biomédicas I - Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: Non-alcoholic fatty liver disease (NAFLD) is a group of highly prevalent liver diseases that comprises from a simple steatosis to more severe conditions such as steatohepatitis, cirrhosis and hepatocellular carcinoma. Despite the recent advances in NAFLD physiopathology, the molecular mechanisms and signaling pathways that contribute to NAFLD onset and progression are not completely understood. Several pieces of evidence indicate that intestinal dysbiosis and lipopolysaccharides (LPS) play a major role in NAFLD development exacerbating lipid deposition, inflammation, oxidative stress, mitochondrial dysfunction, among other phenotypes commonly found upon NAFLD. It has been suggested that mechanistic target of rapamycin complex 2 (mTORC2) signaling and de novo lipogenesis are increased in NAFLD, but the involvement of LPS with the activation of these pathways is not known. OBJECTIVES: Herein we investigated the involvement of Rictor/mTORC2 as a mediator of the hepatic actions of LPS. MATERIALS AND METHODS: C57BL6/J mice bearing or not Rictor/mTORC2 deficiency in hepatocytes (albumin-Cre) were fed with a control diet and acutely (30 and 120 min) or chronically (7 days) treated with LPS in different doses (0.1, 1 and 5 mg/kg, ip). After this period, mice were evaluated for body and tissue weight, serum parameters and hepatic intracellular signaling. DISCUSSION AND RESULTS: Acute LPS (0.1mg/kg) administration increase phospho-Akt (Ser473) and SCD1 content without affecting enzymes of de novo lipogenesis FAS and ACC. In contrast, when LPS was administered chronically, in addition to the increase in the content of mTORC2 product phospho-Akt (Ser473), it also increased the content of SCD1, FAS and ACC, liver mass, serum triglycerides and hepatic IL-1β levels in wild type, but not in mice bearing hepatocyte deficiency of Rictor/mTORC2. CONCLUSION: Rictor/mTORC2 is an important mediator of LPS actions upon liver content of lipogenic enzymes.
Keywords: NAFLD, mTORC2, inflammation / **Supported by:** FAPESP

K.14 - Rho pathway: a fragile point in the GBM resistance to radiotherapy

Yuli Thamires Magalhães[1], **Fábio Luís Forti** [1]
[1]Departamento de Bioquímica, Instituto de Química-Universidade de São Paulo (SP, Brazil)

INTRODUCTION: Glioblastoma (GBM) is the most aggressive brain tumor characterized by rapid cellular infiltration of brain tissue and is routinely treated with ionizing radiation (IR), but therapy resistance is inevitably recurring. The actin cytoskeleton of GBM cooperates with its high invasive capacity but remains unclear whether Rho GTPases modulate DNA damage repair and sensitivity to therapies OBJECTIVES: To show that Rho GTPase pathway plays a role in the IR-resistance of GBM through modulation of DNA repair mechanisms in a p53-dependent manner MATERIALS AND METHODS: GBM cells with p53 wild-type (WT) or p53 mutated (MUT) status were subjected to Rho inhibition by C3 toxin or inhibition actin polymerization by Cytochalasin D (CytoD), followed by irradiation. DNA repair was evaluated by comet assays, γH2AX foci formation and functional NHEJ repair assays. p53 modulation was carried out by knockdown in WT cells and pharmacological reactivation by PRIMA-1 in MUT cells. WT cells were then subjected to rigorous cycles of low doses of IR, to obtain resistant sublines DISCUSSION AND RESULTS: Rho inhibition increases the sensitivity of gliomas to IR by increasing DNA double-strand breaks (DSB) and delaying DNA repair by NHEJ in p53 WT cells. The p53 knockdown reverses this phenotype by reduction of p21 expression and Rho activity, whereas the p53 reactivation in MUT cells with PRIMA-1 reverses these effects. The p53 and Rho interdependence resides on the nuclear p53 translocation facilitated by G-actin and enhanced by IR. IR-resistant p53 WT cells show altered morphology and stress fiber formation: the inhibition of Rho or actin polymerization decreases cell viability in a p53-dependent manner reversing the resistance phenotype. The p53 silencing reverses the sensitization of IR-resistant cells caused by Rho inhibition CONCLUSION: Targeting Rho GTPase pathway components significantly diminishes GMB resistance to IR, in a p53-dependent manner, suggesting the potential value of these proteins as therapeutic targets
Keywords: Rho GTPases, Glioblastomas, Radiotherapy

K.15 - Investigation of the Oxylipin Profile in Amyotrophic Lateral Sclerosis in the SOD1-G93A Rat Model

Hector Oreliana Fernandes Faria [1], Adriano Chaves-Filho[1], Rosangela Silva Santos[1], Rodrigo Santiago Lima[1], Lucas Souza Dantas[1], Isabella Pinto[1], Larissa Diniz[1], Rodrigo Lucas de Faria[1], Gabriela Ernandes Humizawa Poiato[1], Alex Inague[1], Karen Campos Fabiano[1], Marisa Helena Gennari de Medeiros[1], Isaias Glezer[2], Marcos Yukio Yoshinaga[1], Sayuri Miyamoto[1]

[1]Bioquímica, Instituto de Química Universidade de São Paulo (São Paulo, Brasil), [2]Bioquímica, Escola Paulista de Medicina, Universidade Federal de São Paulo (SP, Brasil)

INTRODUCTION: Amyotrophic lateral sclerosis (ALS) is a devastating disease leading to progressive degeneration of motor neurons, resulting in muscle paralysis and patient death. Most ALS cases are sporadic and about 5-10% of the cases are attributed to genetic factors, however, the disease mechanism is largely unknown. Oxidized fatty acid derivatives (oxylipins) are potent bioactive molecules acting in the regulation of metabolism and inflammation OBJECTIVES: Our study aims to map the profile of oxylipins in the plasma of SOD1-G93A rats to understand the presence of these molecules in the pathophysiology of ALS. MATERIALS AND METHODS: We used Sprague Dawley wild type (WT) and SOD1-G93A rats with 70 days (asymptomatic) and 120 days (symptomatic) of life. The plasma samples were mixed with antioxidant, butylhydroxytoluene and desferal, and internal standards. Oxylipin were enriched by SPE and the samples were analyzed by ESI-TOF-MS interfaced with HPLC. The statistical analyzes were performed with Metaboanalyst. DISCUSSION AND RESULTS: Our analysis identified 56 oxidized fatty acids, among which 17 oxylipins were significantly altered. Interestingly, 11 oxylipins derived from arachidonic acid formed by inflammatory and free radical pathways were elevated, while the lipokynes, 12(13)-DiHOME and 9(10)-DiHOME derived from linoleic acid, were decreased in the symptomatic ALS animals compared to WT and asymptomatic animals. CONCLUSION: Our data reveals oxylipin alterations related to systemic inflammation, oxidative stress, as well as alterations in oxylipins that could be potentially linked to the hypermetabolic phenotype of ALS patients. Further studies are being conducted to investigate lipokine alterations and their functional role in ALS disease development.

Keywords: oxylipin, amyotrophic lateral sclerosis, mass spectrometry / **Supported by:** FAPESP - CEPID Redoxoma CNPq, CAPES, NDR Inc.

K.16 - Effects of Metformin Administration On Behavioral And Metabolic Parameters In A Familial Hypercholesterolemia Mouse Model

Natália Baltazar do Nascimento [1], Hémelin Resende Farias[1], Tainá Schons[1], Matheus Scarpatto Rodrigues[1], Jade de Oliveira[1]

[1]Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Univ. Federal do Rio Grande de Sul (RS, Brasil)

INTRODUCTION: Cholesterol metabolism alterations, i.e., hypercholesterolemia, well known cardiovascular risk factors, have also been related to cognitive and behavioral impairments characteristic of neurodegenerative diseases. Familial hypercholesterolemia (FH) is a prevalent disease caused by the dysfunction of cholesterol metabolism. Recent studies have associated mTOR inhibition with conservation of blood-brain barrier integrity, cognitive damage attenuation, and systemic and cerebral inflammation reduction in dementia and FH animal models. In this regard, Metformin has presented a critical therapeutic potential because of its ability to inhibit mTOR through AMPK. OBJECTIVES: Evaluation of Metformin treatment effects on cognitive disturbances and metabolic parameters in C57BL/6 low-density lipoprotein (LDL) receptor-deficient (LDLr-/-) mice, a widely used animal model of FH. MATERIALS AND METHODS: LDLr-/- and wild-type (WT) male and female mice (3-month-old, 24-26 g) received a daily oral administration via gavage of Metformin (200 mg/kg) or Saline for 30 days. All animals were weighed weekly. Mice were submitted to the object reallocation task in the last week of treatment. Following the last day of treatment, animals fasted for 12 hours and were then euthanized by cardiac exsanguination. The plasma obtained from the collected blood was used to determine total cholesterol and triglycerides levels. DISCUSSION AND RESULTS: Total cholesterol levels of the LDLr-/- mice were significantly higher than the WT groups. We did not observe a significant difference between experimental groups regarding total body weight and triglycerides levels. LDLr -/- mice administered with Saline presented memory damage in the object reallocation task. On the other hand, metformin exposure ameliorated memory in LDLr -/- mice but could not reverse the memory prejudice. CONCLUSION: Metformin treatment showed a promising effect on the memory impairment observed in hypercholesterolemic mice, which was not via a cholesterol-lowering outcome.

Keywords: Familial hypercholesterolemia, LDLr -/- mice, Metformin

K.17 - MOLECULAR DIAGNOSIS OF A BRAZILIAN NAIL-PATELLA SYNDROME PATIENT

Jéssica Hilário Bonomo [1], Marilia Thomé Nadalini[1], Amélia Arcângela T. Trindade[2], Anderson Ferreira da Cunha[1]
[1]Departamento de Genética e Evolução, Universidade Federal de São Carlos (SP, Brasil), [2]Departamento de Medicina, Universidade Federal de São Carlos (SP, Brasil)

INTRODUCTION: Nail-Patella syndrome is a rare genetic disorder caused by mutations affecting the *LMX1B* gene, which encodes for a LIM-Homeodomain transcription factor involved in the development of limbs, kidney, eyes and neurons in vertebrates. It is characterized by combination of most or all of the following: fingernail absence or dysplasia, patellar absence or hypoplasia, elbow dysplasia, and presence of iliac horns. Renal and ophthalmological findings are also commonly found in patients. OBJECTIVES: In this study, we aimed to establish the molecular diagnosis for a Nail-Patella Syndrome patient presenting with classical skeletal findings and proteinuria. MATERIALS AND METHODS: Amplifications and purification of the LMX1B exons were performed using PCR and gel electrophoresis, and these were sequenced via the Sanger method. Analysis of the sequence was performed using BLASTn (NCBI) and variants were compared to the dbSNP (NCBI) database. DISCUSSION AND RESULTS: After molecular analysis, we found a missense mutation in exon 5 (p.V265L), which had been previously described in another NPS patient who presented with proteinuria (rs1588307477). A correlation between renal findings in NPS patients and mutations located in the homeodomain sequence region is well established. Worldwide, before this study, this variant has been described only twice. In Brazil, there is a lack of studies about NPS patients, and this is the first evidence of this variant in this population. CONCLUSION: This result corroborates with previous literature regarding the correlation between mutations in the homeodomain region and renal involvement in the Nail-Patella Syndrome, and reaffirms the importance of genetic diagnosis early to ensure better prognosis of these patients and, consequently a better outcome.
Keywords: Nail-Patella Syndrome, LMX1B, human / **Supported by:** CAPES, FAPESP

K.18 - Host Immune Response Participates In Malaria Acute Kidney Injury: Role Of CD8+ T Cells

Douglas Esteves Teixeira[1], Alessandro de Sá Pinheiro[1], Leandro de Souza Silva[1], Sarah Aparecida dos Santos Alves[1], Rodrigo Pacheco da Silva-Aguiar[1], Diogo B Peruchetti[1], Tatiane Almeida Pádua[4], Mariana Conceição de Souza[4], Celso Caruso-Neves[1,2,3], Ana Acacia de Sá Pinheiro[1]
[1]Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro (RJ, Brasil), [2]FAPERJ, Rio de Janeiro Innovation Network in Nanosystems for Health-NanoSAÚDE/FAPERJ, Rio de Janeiro, Brazil (RJ, Brasil), [3]INCT, National Institute of Science and Technology for Regenerative Medicine, Rio de Janeiro, Brazil (RJ, Brasil), [4]Bio-Manguinhos, Fundação Oswaldo Cruz (RJ, Brasil)

INTRODUCTION: Malaria acute kidney injury (MAKI) is characterized by glomerular and tubular damage caused directly by the parasite and the activation of the host immune response. But the role of T cells in MAKI is unclear. OBJECTIVES: We aimed to evaluate the participation of T cells in the pathogenesis of MAKI. MATERIALS AND METHODS: We used C57BL6 mice, infected with *Plasmodium berghei* ANKA that received treatment with FTY720 (FTY), an immunosuppressor, or depletion of CD8+ T cells, with anti-CD8α antibody. We performed adoptive transfer (AdTr) of splenocyte-derived T cells from infected to healthy acceptor. DISCUSSION AND RESULTS: FTY reduced infection-induced proteinuria (3-fold) and UPCr (protein to creatinine ratio, 2.8-fold). We observed a reduction in the activity of urinary γ-GT, a marker of tubular injury, while it did not alter the markers of glomerular injury (creatinine clearance, plasma urea and plasma creatinine). These results indicate that T cells induce renal tubulointerstitial injury. Using AdTr experiments, we observed an increase in T cell homing to the kidneys, spleen and brain. In the kidneys, AdTr induced proteinuria (2-fold), increased UPCr (2.3-fold) and urinary γ-GT activity (1.6-fold). AdTr did not change glomerular function. AdTr induced an increase in pro-inflammatory cytokines, IL-17 (1.3-fold), IL-6 (1.4-fold), INF-γ; (1.3-fold). We observed an increase in renal CD8+ T cells (1.5-fold) together with an increase in the marker of cytotoxic T cell activation, perforin-1 (2.0-fold) in renal tissue. These results show that the presence of malaria-responsive T cells induces a tubular injury in the absence of infection. Interestingly, CD8+ T cell depletion reduced proteinuria (3.1-fold), UPCr (2.0-fold) and urinary γ-GT (1.6-fold), but did not change the markers of glomerular injury. These results indicate that malaria-responsive CD8+ T cells migrate to the kidney inducing renal tubular damage. CONCLUSION: This work adds new information about the exacerbated activation of T cells during malaria infection.
Keywords: malaria acute kidney injury, Renal tubular injury, Plasmodium sp
Supported by: Faperj, CNPq, Capes

K.19 - HUMAN HSP27 REDUCES TOXIC AGGREGATES IN HUMANIZED YEAST MODEL FOR HUNTINGTON'S DISEASE

Vittoria Camandona [1], Isabella Silva[1], Ribamar Ferreira-Junior[1]
[1]Laboratório de Envelhecimento e Biologia Molecular, Escola de Artes, Ciências e Humanidades da Universidade de São Paulo, São Paulo, Brazil (São Paulo, Brazil)

INTRODUCTION: Human Hsp27 is a small heat shock protein involved in redox homeostasis, protein degradation (ubiquitin-proteasome pathway) and shows anti-apoptotic activity. Among the cellular functions of Hsp27 and its ubiquitous expression (all tissues), some studies demonstrate effects on neurodegenerative diseases such as Huntington's Disease (HD). HD has no cure and is caused by a mutation in the HTT gene, leading to the mutant huntingtin protein (mHTT), containing 36 or more glutamine (Q) repeats. Studies have shown that HSP27 reduces the toxic oxidative effects of mHTT, suggesting Hsp27 acts on the degradation of the mHTT protein. OBJECTIVES: to analyze the effect of human HSP27 on humanized *Saccharomyces cerevisiae* model for Huntington's Disease. MATERIALS AND METHODS: strains of *S. cerevisiae* expressing human HSP27 in two distinct levels and polyQ proteins, that contain 17 amino acids on the N-terminal of exon 1 HTT gene followed by 25 (control 25Q) or 103 (mutant 103Q) glutamine repeats fused to GFP, were constructed. Quantitative protein expression analyses were performed by Western Blot experiments and protein aggregation of 25Q and 103Q was also quantified by fluorescence microscopy. The monitoring of cell survival was done by Chronological Longevity *in situ* assay. DISCUSSION AND RESULTS: Regardless of the level of expression of human HSP27, there was an increase in the chronological longevity of the cells. Aggregates of 103Q protein was reduced in 66.33% (p=0.005) according to WB assays and 32.17% (p=0.034) by fluorescence microscopy analysis. These findings were more prevalent in cells that overexpressed HSP27. CONCLUSION: HSP27 acts in the 103Q degradation pathway and moderately increased the longevity of HD model cells. These findings contributes to an important understanding of the functions of HSP27 and its role in HD. Furthermore, as neurodegenerative diseases share a common etiology (protein aggregates formation), it's possible that HSP27 acts in other contexts through a similar mechanism.
Keywords: HSP27, Huntington's disease, *Saccharomyces cerevisiae* / **Supported by:** CAPES and FAPESP

K.20 - Identification of long noncoding RNAs involved in the cancer stem phenotype in pancreatic cancer

Gabriel Lucas da Fonseca[1], **Emily bronze dos santos** [1], Thalita Bueno Corrêa [1], Daniela Sanchez Basseres[1]
[1]Departamento de Bioquímica, Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal disease characterized by aggressive metastatic dissemination. One of the most important hallmarks of metastatic capability is the acquisition and maintenance of a stem-like phenotype by cancer stem cells (CSCs), a process that is still poorly understood in PDAC. OBJECTIVES: Considering that acquisition of stem-like features is achieved primarily by epigenetic mechanims, our goal was to identify long noncoding RNAs (lncRNAs) involved in promoting the CSC phenotype in PDAC. MATERIALS AND METHODS: We analyzed the global transcriptional profile of lncRNAs in primary cultures derived from 5 PDAC patient-derived xenograft (PDX) tumors comparing tumorspheres (CSC-enriched) with adherent cells (non-CSC-enriched). We analyzed the RNAseq raw data using the RSEM and DeSeq2 pipelines to identify differentially expressed lncRNAs and validated their differential expression by qPCR in AsPC-1 tumorsphere cultures. In addition, we investigated their expression in single cell RNAseq data of 57530 cells obtained from 24 PDAC samples and 11 non-tumor pancreatic samples and used publicly available PDAC patient datasets (PAAD-US and PACA-AU) to investigate the association of differentially expressed lncRNAs with patient survival. DISCUSSION AND RESULTS: We identified 19 upregulated lncRNAs and 6 downregulated lncRNAs in PDAC CSCs. Of the upregulated lncRNAs, LINC01559 was the most promising. Its expression was increased in AsPC-1 tumorspheres and a high expression of LINC01559 was significantly associated with shorter PDAC patient survival. Finally, single cell RNAseq data showed a higher expression of LINC01559 in type 2 ductal cells, a cell population that is present only in tumor samples and that shows an overexpression of markers of worse prognosis in PDAC. CONCLUSION: We have identified lncRNAs differentially expressed in PDAC CSCs and our results indicate that LINC01559 may represent a promising PDAC biomarker or therapeutic target, as it is associated with PDAC aggressiveness and a worse clinical prognosis.
Keywords: LncRNAs, Cancer Stem Cells, Pancreatic Ductal Adenocarcinoma / **Supported by:** FAPESP, CNPq, CAPES

K.21 - Macrophages Mediate Endothelial Dysfunction When Exposed To Fine Particulate Matter *In vitro*

Rafael Kazmirczak de Moraes [1], Lílian Correa Costa-Beber[1], Hémelin Resende Farias[1], Jade de Oliveira[1], Fatima Theresinha Costa Rodrigues Guma[1]
[1]Departamento de Bioquimica, Universidade Federal do Rio Grande do Sul (Rio Grande do Sul, Brasil)

INTRODUCTION: The exposure to fine particulate matter (PM2.5), an atmospheric pollutant, is a risk factor for cardiovascular diseases. Macrophages are the first defense against inhaled particles and mediate endothelial inflammatory response. Once injured, there is an increase in the endothelial permeability and generation of oxygen reactive species (ROS) and a reduction in the nitric oxide bioavailability. The loss of nitric oxide favors endothelial dysfunction and the development of atherosclerosis. OBJECTIVES: In this study, we aimed to evaluate if macrophages could mediate the endothelial dysfunction induced by PM2.5. MATERIALS AND METHODS: PM2.5 retained in glass fiber filters was partially extracted with PBS and further centrifuged at 1000g for 15min. This solution (1g filter/125 mL PBS) was diluted in DMEM 10% FBS ten times. We exposed a murine macrophages cell line (RAW264.7) to PM2.5 for 48h and used PBS as a Control. Further, we exposed a hemangioendothelioma cell line (EOMA) to the conditioned medium (10% of the 48h-exposed RAW medium in fresh medium), using fresh medium as a Control, for 48h. Cell viability was verified through MTT assay and nitrite levels by Griess method, both spectrophotometrically. ROS production was verified by DCF fluorescence. DISCUSSION AND RESULTS: Exposure to PM2.5 increased ROS production and nitric oxide in macrophages, indicating a pro-oxidative medium. Then, to investigate whether such alterations in the macrophage medium could be mediating endothelial dysfunction, we exposed vascular endothelial cells to the conditioned medium and evaluated some parameters indicative of endothelium integrity. We found an increased production of ROS and nitric oxide, after exposure to the conditioned medium of macrophages exposed to the pollutant. We also assessed the cell viability, and it was decreased when compared to the fresh medium, indicating endothelial damage. CONCLUSION: Taken together, these results indicate that macrophages mediate endothelial dysfunction caused by the PM2.5 leading to cardiovascular damage.

Keywords: redox imbalance, cardiovascular, coditioned médium / **Supported by:** Capes, FAPERGS

K.22 - 1H NMR Metabolomics Reveals Dysregulation in Choline Metabolites In Severe COVID-19

Marcos Almeida[1], Livia Teixeira[2], Eugenio Holtz[2], Fernando Bozza[3], Patrícia Bozza[2], Ana Paula Valente[4], Gilson Costa dos Santos Jr[5], Tatiana El-Bacha Porto[1]
[1]Instituto de Nutrição Josué de Castro, Institute of Nutrition Josué de Castro, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil (RJ, Brasil), [2]Departamento de Fisiologia e Farmacodinâmica, Fundacao Oswaldo Cruz, Departamento de Fisiologia e Farmacodinâmica, Rio de Janeiro, Brazil (RJ, Brasil), [3]Instituto de Pesquisa Clínica Evandro Chagas, Fundacao Oswaldo Cruz, Instituto de Pesquisa Clínica Evandro Chagas – IPEC, Rio de Janeiro, Brazil (Rio de Janeiro, Brasil), [4]Instituto de Bioquímica Medica, Instituto de Bioquímica Medica, Federal University of Rio de Janeiro, Brazil (RJ, Brasil), [5]Department of Genetics, IBRAG, Department of Genetics, State University of Rio de Janeiro, Rio de Janeiro, Brazil; (Rio de Janeiro, Brasil)

INTRODUCTION: To understand the molecular mechanisms related to the SARS-CoV-2 infection calls for a biologic system approach. In this context, metabolomics provids the metabolic signature of different organs and fluids in different conditions and might works as a tool to elucidate the metabolic pathways associated with the infectious process. OBJECTIVES: To use untargeted 1H Nuclear Magnetic Resonance (NMR) metabolomics to evaluate the metabolic shift associated to COVID-19 severity, providing an important basis for better understanding COVID-19 metabolic alterations and disease progression. MATERIALS AND METHODS: Samples from adults subjects (≥18 years of age) with severe COVID-19 (n=36) and control (n=15) were used. Severe COVID-19 was confirmed by presenting chest infiltrates on computed tomography scan and requiring respiratory support with either noninvasive oxygen supplementation or mechanical ventilation. 1H NMR analysis of plasma samples was performed on a Bruker Advance III 500.13 MHZ at 300K, coupled with a cooled automatic sample case at 280K DISCUSSION AND RESULTS: Discriminant metabolite profile was confirmed with Principal Component Analysis scores plot and the most pronounced changes in plasma metabolites were related to an increase in VLDL, a decrease in choline-related metabolites (phosphocholine, phosphatidylcholine) and an increase in several amino acids, including branched-chain amino acids leucine and isoleucine in subjects with severe COVID-19. The decrease in choline-related metabolites suggests an impairment in 1 Carbon metabolism and the alterations in amino acids are possibly related to insulin resistance in severe disease. Additionally, disruptions in 1 Carbon metabolism might be related to the alterations in VLDL, as phosphatidylcholine is the major phospholipid in lipoproteins. CONCLUSION: Our study provides a possible explanation for the observed metabolic biases associated to COVID-19 severity, and that the disruptions in 1-Carbon metabolism and lipoprotein biosynthesis might be associated with the pathophysiology of this disease.

Keywords: COVID-19, Metabolomics, NMR / **Supported by:** Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro e Capes

K.23 - Triple-Negative Breast Cancer inflammatory secretome regulation by mutant p53 and ERK1/2 MAPK

Raquel Nascimento das Neves [1], Karoline Almeida Lima[1], Helena Souza Zancanaro[1], Alexander James Roy Bishop[2], Alfeu Zanotto Filho[1]
[1]Departamento de Bioquímica , Universidade Federal de Santa Catarina (Santa Catarina, Brasil), [2]Greehey Children's Cancer Research Institute, University of Texas Health at San Antonio (Texas, Estados Unidos)

INTRODUCTION: TP53 is the most frequently mutated gene in human cancer. Particularly, the triple-negative breast cancer (TNBC) subtype displays TP53 mutation in 80% of patients. Unlike other breast cancer subtypes, TNBC patients currently lack an approved effective targeted therapy. Notably, most TNBC acquires TP53 mutations, which, additionally to the loss of canonical p53 functions, result in gain-of-function (GOF), activating different cellular mechanisms involved in carcinogenesis. OBJECTIVES: Evaluate the mutp53GOF role and inflammatory response in TNBC cell lines. MATERIALS AND METHODS: To understand the role of mutp53GO we used siRNA to deplete the TP53 gene, and evaluated the cell malignance through MTT, scratching, and transwell assay. The cytokines levels were measured by ELISA, protein immunocontent by Western Blot, and transcription factors activity by gene reporter assay. DISCUSSION AND RESULTS: Loss of p53 did not significantly impact cell viability. However, mutp53GOF knockdown made cells susceptible to treatment with a genotoxic alkylating agent. The invasion assay demonstrated that mutp53GOF depletion decreased the invasiveness potential of TNBC cells which was substantiated by reduced cell migration. Mutp53GOF knockdown decreased the expression of several pro-inflammatory cytokines, such as IL8, IL6, and CXCL2, but not molecules known to be involved in breast cancer malignancy, such as PGE2 and MMP1, which are upregulated by the ERK1/2MAPK signaling pathway as determined in TNBC cells treated with the MEK1/2 inhibitor UO126/sorafenib, that showed a decreased of cytokines production. Furthermore, combined mutp53GOF knockdown with MEK/ERK1/2 pathway inhibition caused a more pronounced IL8/IL6 inhibition when compared to both treatments alone. Interestingly, neither mutp53GOF knockdown impacted MEK/ERK1/2 phosphorylation status nor did UO126/sorafenib alter p53 immunocontent. Reporter assays showed that mutp53GOF promotes NFkappaB reporter activation, and MEK/ERK1/2 controls both NFkappaB and AP-1 transcription factors, associated with the expression of the secretome components evaluated herein. CONCLUSION: These results indicate that mutp53GOF cooperates with the ERK1/2MAPK pathway to promote inflammatory secretome in TNBC cell models.
Keywords: Breast cancer, Inflammation, Gain-of-function / **Supported by:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES

K.24 - Melanoma-Derived Small Extracellular Vesicles Isolation Pipeline

Sophia Nora Baptista [1], Helon Guimarães Cordeiro[1], Carmen Veríssima Ferreira-Halder[1]
[1]Departamento de Bioquímica e Biologia Tecidual, Universidade Estadual de Campinas (São Paulo, Brasil)

INTRODUCTION: Melanomas are extremely aggressive tumors that originate from melanocytes. Currently, it is a well stablished fact that the development and progression of tumors is strongly influenced by the tumor microenvironment. Several studies have shown that melanoma-derived cells are associated with the regulation and reprogramming of diverse cell types through the participation of tumor microenvironment's constituents such as small extracellular vesicles (sEVs). These particles are smaller than 200nm and play an essential role in cell-cell communication, making them excellent targets for the study of oncogenic associated events. OBJECTIVES: To stablish a protocol for the isolation of melanoma-derived sEVs. MATERIALS AND METHODS: For the sEVs isolation, Skmel-28 cells were cultured in DMEM containing 10% fetal bovine serum for 24h, followed for more 24h in serum-free medium. After this period, the medium was collected, concentrated by ultrafiltration (MWCO 10 kDa) and sEVs were isolated by Total Exosome Isolation Reagent (Invitrogen). sEVs were characterized by nanoparticle tracking analysis (NTA). Cell viability and morphology were assessed, after 24 hours in serum-free medium, by MTT reduction and crystal violet staining, respectively. DISCUSSION AND RESULTS: Skmel-28 cells maintained in the absence of serum for 24h, did not display major changes in morphology and viability as checked by formazan production and crystal violet staining. NTA showed a high concentration (7,2x10^8 particles/mL) of sEVs released by Skmel-28 cells, which displayed a mean diameter of 164nm. CONCLUSION: The results indicate that cell viability was maintained after 24h of serum deprivation, with small changes in morphology and MTT reduction capacity. The size of the vesicles is in agreement with the expected for sEVs and the yield was significant. Importantly, our protocol appears to be scalable, which is crucial for the biofunctional analysis of sEVs.
Keywords: Melanoma, Isolation Protocol, Small Extracellular Vesicles
Supported by: FAPESP - Fundação de Amparo à Pesquisa do Estado de São Paulo

K.25 - Rictor/mTORC2 is a major mediator of hepatic steatosis, steatohepatitis and hepatocellular carcinoma progression induced by PTEN deletion in hepatocytes

Álbert Souza Peixoto [1], Mayara Franzoi Moreno[1], Gustavo Renan Gilio[1], Érique de Castro[1], Caroline Aparecida Tomazelli[1], Thayna dos Santos Vieira[1], Tiago Eugênio Oliveira[1], Milene Ortiz Silva[1], Adriano Chaves Filho[2], Beatriz Pereira da Silva[2], Marcos Yukio Yoshinaga[2], Sayuri Miyamoto[2], William Tadeu Lara Festuccia[1]
[1]Depto de Fisiologia e Biofísica, Instituto de Ciências Biomédicas – Univ. de São Paulo (SP, Brasil), [2]Depto de Bioquímica, Instituto de Química - Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: Nonalcoholic fatty liver disease (NAFLD) is a group of diseases that affects the liver that includes from a simple steatosis, up to non-alcoholic steatohepatitis (NASH), which can eventually progress to cirrhosis and hepatocellular carcinoma (HCC). Constitutive activation of the PI3K-mTORC2-Akt pathway induced by Pten deletion in hepatocytes promotes the development of severe hepatic steatosis that later progresses to NASH and HCC. OBJECTIVES: Investigate the involvement of mTOR complex 2 (mTORC2) in the progressive development of NAFLD, and HCC induced by Pten deletion in hepatocytes. MATERIALS AND METHODS: Mice with specific Pten deletion associated or not with Rictor deletion exclusively in hepatocytes at 8, 24 and 48 weeks of age were evaluated for body and tissue weight, glycemic homeostasis, liver morphology, intracellular signaling, mitochondrial activity and lipidoma. DISCUSSION AND RESULTS: Pten deletion in hepatocytes promoted significant hepatomegaly due to intense hepatic steatosis, associated with the activation of de novo fatty acid synthesis and triacylglycerol synthesis processes, as evidenced by the increased hepatic mRNA and protein content of enzymes involved in these processes. Pten deletion also increased mitochondrial function, promoted oxidative stress and fibrosis, as well as change in hepatic lipidoma characterized by accumulation of neutral lipids such as triacylglycerol, diacylglycerol and cholesterol, reduced phosphatidylethanolamine, cardiolipin, sphingomyelin, ceramides, and increased phosphatidylcholine. Rictor/mTORC2 deletion reduced hepatomegaly and hepatic steatosis induced by the Pten deletion in hepatocytes, such effects were associated with a reduction in mRNA and protein content of enzymes involved in de novo fatty acid synthesis and triacylglycerol. Rictor/mTORC2 deletion re-established glycemic homeostasis and protected mice from liver damage, oxidative stress, fibrosis, lipidome alterations and development of HCC induced by Pten deletion in hepatocytes. CONCLUSION: Rictor/mTORC2 is an important regulator of several metabolic processes in the liver, including glycolysis, gluconeogenesis, glycogenesis, de novo lipogenesis, and mitochondrial biogenesis, and thus plays a key role in NAFLD-HCC progression. - K**eywords:** NAFLD, PTEN, mTORC2 / **Supported by:** FAPESP

K.26 - Proteomic Effects of IGF1R-IRS1/2 Pathway Inhibition in Chronic Myeloid Leukemia

Virgínia Campos Silvestrini [1], Natasha Peixoto Fonseca[3], Alessandra Pinto Vargas[1], Bruna Alves Fenerich[3], Antônio Bruno Alves Silva[3], Izadora Archiolli[1], Ana Paula Masson[1], Fabiola Traina[3,2], Vitor Marcel Faça[1,2]
[1]Dept. Biochemistry and Immunology, Ribeirão Preto Medical School (SP, Brasil), [2]Blood center of Ribeirão Preto, Blood center of Ribeirão Preto (SP, Brasil), [3]Department of Medicine, Medical School of Ribeirão Preto, Division of Hematology (SP, Brasil)

INTRODUCTION: Chronic myeloid leukemia (CML) is characterized by the presence of the oncoprotein BCR-ABL1, which constitutively activates the tyrosine kinase activity triggering a neoplastic transformation of hematopoietic stem cells. Although some reports using tyrosine kinase inhibitors have represented an advance in the treatment of CML, up to 20% of patients are resistant to those inhibitors. In this sense, other studies aiming proteins that bind indirectly to the BCR-ALB1 have identified insulin receptor substrates (IRS) as potential targets, representing a new therapeutic strategy for CML. OBJECTIVES: To evaluate proteomic alterations during the cellular resistance process in CML. MATERIALS AND METHODS: Peripheral mononuclear blood cells from a T315I mutation patient were treated with 6.4 µM NT157 for 48 hours. Proteomic analysis of these samples was performed using a Q-Exactive HF high resolution mass spectrometer coupled to a chromatographic system. Data processing was carried out using Maxquant. Overall 3244 proteins were confidently identified with FDR < 1%. Label-free quantitative analysis highlighted a list of 116 upregulated and 85 down regulated proteins in cells treated with NT157. DISCUSSION AND RESULTS: NT157 treatment decreased cell viability and increased apoptosis (82%) in primary cells carrying T315I mutation. Also, the downregulated set was enriched for phosphoproteins and, among these, the BCR protein directly involved in CML pathogenesis. Using BAF3 BCR-ABL and BCR-ABL T315I cell lines, we also validated these findings showing a downregulation in the IRS1 pathway and c-ABL. Despite the large number of proteins identified, shotgun proteomics and phosphoproteomics of cell lines are ongoing to better understand the pathways involved in survival and resistance processes. CONCLUSION: Pharmacological inhibitor NT157 displayed remarkable antineoplastic effects in CML primary cells and cell lines with T315i mutations, including decreased cell viability and increased apoptosis. Our findings support that using NT157 monotherapy, emerges as a potential therapeutic approach for resistant CML. **Keywords:** proteomics, Chronic Myeloid Leukemia, NT157

K.27 - Cytotoxicity of Thioridazine is Mediated by G-Protein Coupled Dopamine Receptor in Leukemia Cells

Rayssa de Mello Lopes [1], Laura Francisca Leite do Prado de Souza[1], Fabio Dupart Nascimento[2], Ivarne Luís dos Santos Tersariol[2], Tiago Rodrigues[1]
[1]Centro de Ciências Naturais e Humanas, Universidade Federal do ABC (São Paulo, Brazil), [2]Departamento de Biologia Molecular, Universidade Federal de São Paulo (São Paulo, Brazil)

INTRODUCTION: Phenothiazines are drugs used in the treatment of schizophrenia and emesis due to the antagonism of dopamine receptors (DR) in the central nervous system. However, several off-target actions have been described for these drugs, including antitumor effects *In vitro* and *In vivo* . We previoulsly showed the involvement of mitochondrial permeabilization and calcium homeostasis disruption in phenothiazine-induced cell death. Also, our and other studies have shown the ability of these drugs to induce autophagy, but the molecular mechanisms of autophagy induction by phenothiazines are not completely understood. OBJECTIVES: To investigate the role of autophagy in phenotiazine-induced apoptosis in leukemia cells, i.e., whether phenothiazine-induced autophagy contributes for cell survival or death. Also, we investigated whether the antagonism of DR by phenothiazines play a role in this process. MATERIALS AND METHODS: Leukemia cells (1x10 ^{5} /mL) were cultivated in supplemented RPMI 1640 medium. Cell viability was evaluated by the MTT and trypan blue assays. Autophagy was estimated through the LC3 I / II conversion by Western blot and lysosomal permeabilization was evaluated by flow cytometry using acridine orange. Chemical agonist/antaftergonist of dopaminergic receptors, as well as, modulators of downstream pathways were also used. DISCUSSION AND RESULTS: After 24h incubation, phenothiazines exhibited a concentration-dependent cytotoxicity in leukemia cells and the most potent derivative was thioridazine (TR). TR induced autophagy-associated apoptosis in K562 cells and this effect was enhanced by DR antagonist haloperidol. Moreover, this effect was diminished by the DR agonist dopamine or by the adenilyl cyclase inhibitor SQ 22536. CONCLUSION: These findings indicate that the antitumor action of TR involves the antagonism of G-protein coupled DR, blocking its downstream signaling.
Keywords: autophagy, cancer, G-PCR / **Supported by:** FAPESP, CNPq

K.28 - ViVe: Combination of Violacein with Vemurafenib for Overcoming Melanoma Survival

Beatriz Aires Lopes [1], Giselle Zenker Justo[2], Nelson Durán[3], Carmen Veríssima Ferreira Halder[1]
[1]Biochemistry and Tissue Biology , University of Campinas (SP, Brazil), [2]Department of Pharmaceutical Sciences and Department of Biochemistry, Federal University of São Paulo (São Paulo, Brazil), [3]Department of Structural and Functional Biology, University of Campinas (SP, Brazil), [4]Nanomedicine Research Unit (Nanomed), Federal University of ABC (SP, Brazil)

INTRODUCTION: Chemotherapy resistance and metastasis are the main challenges in the treatment of melanoma, which are very complex and, until now, poorly understood processes. Our group has been investigating the antitumoral property of Violacein, a compound produced by *Chromobacterium violaceum*, found in the Amazon River. This compound displays a strong capacity to trigger apoptosis of a broad type of tumors at nM range (500-1000). Specifically in relation to melanoma, this pigment disrupts the autophagy, a process that provides advantages to this cancer. OBJECTIVES: To assess the ability of violacein to sensitize melanoma to Vemurafenib treatment (ViVe protocol). MATERIALS AND METHODS: 2D and 3D cultures of Skmel-28 cells (*BRAF* mutated) were maintained in DMEM medium containing 10% fetal bovine serum and antibiotics. After 48h of spheroids (3D) obtention using the Bio-Assembler™ n3D system, they were submitted to treatment for 48h. The viability of cells cultured in 2D was examined by MTT reduction and cytometry, and Live/Dead® kit in the case of 3D model. Spheroid area and density were analyzed by using the ImageJ software. DISCUSSION AND RESULTS: In 2D culture, ViVe protocol promoted a decrease in cell viability by 41% after 24h of treatment. Also, an increase of cellular population in early apoptosis (6%) was detected. In 3D culture, it was also observed the diminishing of cell viability and morphological changes in the spheroids, as indicated by an increase in optical density. CONCLUSION: ViVe protocol is effective in turning off signals that favor melanoma survival. Noteworthy the disruption of the spheroid organization, as an indication that the treatment has a potential to ensure high bioavailability, besides to destroy the center of the tumor mass.
Keywords: Melanoma, Violacein, Vemurafenib
Supported by: Fapesp - Fundação de Amparo à Pesquisa do Estado de São Paulo

K.29 - Extracellular Vesicles in Colorectal Cancer Cells: The Unconventional Release of LMWPTP and Impact on Human Fibroblast Biology

Stefano Piatto Clerici [1], Sílvio Roberto Consonni[1], Carmen Veríssima Ferreira-Halder[1]
[1]Bioquímica e Biologia Tecidual, Universidade Estadual de Campinas (SP, Brasil)

INTRODUCTION: Colorectal cancer (CRC) is in the top 10 cancers most prevalent worldwide. Low molecular weight protein tyrosine phosphatase (LMWPTP) participates in the metastatic process and resistance acquisition to chemotherapy in different types of tumors. In the context of CRC, LMWPTP is associated with malignant evolution and a less favorable prognosis. Research on tumor-derived extracellular vesicles (EVs) suggests its importance in mediating cell-to-cell communication and thus, potentially affecting cancer progression via multiple pathways. OBJECTIVES: In the present study, we hypothesized that EVs derived from different CRC cell lines (HCT116 and HT29) differ in their ability to reprogram normal human fibroblasts through a process called tumor education. MATERIALS AND METHODS: The EVs derived from CRC cell lines were isolated by a combination of ultrafiltration and polymeric precipitation, followed by characterization based on morphology (transmission electron microscopy), size and concentration (nanoparticle tracking analysis), and the presence or absence of EV and non-EV markers (immunoblotting's). The uptake of EVs (PKH26 labeled), migration, and molecular pathways were analyzed in human fibroblasts co-cultured with CRC-derived EVs. DISCUSSION AND RESULTS: HT29 cells displayed a higher concentration of small extracellular vesicles (sEVs CONCLUSION: The data suggested an unconventional way of LMWPTP secretion via HT29-derived sEVs in addition to that, the sEVs secreted by CRC cells can educate normal human fibroblasts to activate them in a state that confers migratory capacity.
Keywords: small extracellular vesicles, colorectal cancer, LMWPTP.**Supported by:** Sao Paulo Research Foundation (FAPESP) under grants 2018/03593-6 (SPC) and 2015/20412-7 (CVF-H).

K.30 - Matrix Metalloproteinases on Severe COVID-19 Lung Disease Pathogenesis

Pedro da Silva-Neto[1,2], Valéria do Valle[3], Carlos Fuzo[1], Talita Fernandes[4], Diana Toro[1,2], Jonatan de Carvalho[6,1], Vinícius Pimentel[1,5], Lucia Faccioli[1], Marcelo Dias-Baruffi[1], Ana Paula Fernandes[4], Raquel Gerlach[3], **Carlos A. Sorgi[6,2,5]**
[1]Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto (São Paulo, Brazil), [2]Instituto de Ciências Biológicas, Universidade Federal do Amazonas (Amazonas, Brazil), [3]Departamento de Biologia Básica e Oral, Faculdade de Odontologia de Ribeirão Preto (São Paulo, Brazil), [4]Departamento de Enfermagem Geral e Especializada, Escola de Enfermagem de Ribeirão Preto (São Paulo, Brazil), [5]Departamento de Bioquímica e Imunologia, Faculdade de Medicina de Ribeirão Preto (São Paulo, Brazil), [6]Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto (São Paulo, Brazil)

INTRODUCTION: Patients with COVID-19 predominantly have a respiratory tract infection and acute lung failure is the most severe complication. While the molecular basis of SARS-CoV-2 immunopathology is still unknown, it is well established that lung infection is associated with hyper-inflammation and tissue damage. Matrix metalloproteinases (MMPs) contribute to tissue destruction in many pathological situations, and the activity of MMPs in the lung leads to the release of bioactive mediators with inflammatory properties. OBJECTIVES: We sought to characterize a scenario in which MMPs could influence the lung pathogenesis of COVID-19. MATERIALS AND METHODS: This observational, analytical, and prospective study was conducted using stringent and reasonable inclusion and exclusion criteria. In total, non-COVID-19 subjects (n = 13), who were hospitalized and intubated due to different clinical primary conditions, along with patients in severe/critical illness (n = 39), intubated and hospitalized in intensive care unit (ICU) who tested positive for SARS-CoV-2 infection. DISCUSSION AND RESULTS: Although we observed high diversity of MMPs in lung tissue from COVID-19 patients by proteomics, we specified the expression and enzyme activity of MMP-2 in tracheal-aspirate fluid (TAF) samples from intubated COVID-19 and non-COVID-19 patients. Moreover, the expression of MMP-8 was positively correlated with MMP-2 levels and possible shedding of the immunosuppression mediator sHLA-G and sTREM-1. Together, overexpression of the MMP-2/MMP-8 axis, in addition to neutrophil infiltration and products, such as reactive oxygen species (ROS), increased lipid peroxidation that could promote intensive destruction of lung tissue in severe COVID-19. CONCLUSION: Uncontrolled protease activity and improper expression of several MMPs were correlated to lung disease in severe COVID-19. Although considered plasma prognostic biomarkers, the MMP-2 and MMP-8 pathways in the lung could become the target of specific therapies, including those proposed to diminish inflammation, oxidative stress and tissue damage during COVID-19.
Keywords: Metalloproteinases, COVID-19, Lung / **Supported by:** FAPESP, CAPES, CNPq

K.31 - Inflammasome activation in the pulmonary parenchyma defines two distinct profiles associated with cytokine storm and worsening of lung function in COVID-19 patients

Keyla Santos Guedes de Sá [1], Luana Alexandrina Amaral[1], Camila C.S. Caetano[1], Amanda de Matos Becerra[1], Sabrina Setembre Batah[2], Isadora Mafra de Oliveira[1], Macel Koenigkam-Santos[3], Ronaldo Martins[1], Eurico Arruda[1], Alexandre Todorovic Fabro[2], Dario Simões Zamboni[1]

[1]Depto de Biologia Celular e Molecular e Bioagentes Patogênicos, Faculdade de Medicina de Ribeirão Preto (São Paulo, Brasil), [2]Depto de Patologia e Medicina Legal, Faculdade de Medicina de Ribeirão Preto (São Paulo, Brasil), [3]Depto de Imagens Médicas, Hematologia e Oncologia, Faculdade de Medicina de Ribeirão Preto (São Paulo, Brasil)

INTRODUCTION: Inflammasome activation is associated with disease severity in patients infected with SARS-CoV-2 and influenza, but the specific cell types that drive inflammasome activation as well as the inflammatory profile associated with inflammation-mediated disease exacerbation is unknown. OBJECTIVES: The aim of this project is understand the molecular mechanisms underlying the pathological processes that lead to death of COVID-19 and Influenza patients. MATERIALS AND METHODS: Here, we assessed lung autopsy of 47 fatal COVID-19 patients and 12 fatal influenza patients and examined inflammatory profile, inflammasome activation and correlated with clinical and histopathological patient's conditions. DISCUSSION AND RESULTS: We demonstrated the presence of more robust inflammasome activation in lethal cases of SARS-CoV-2 compared to Influenza and found a different profile of inflammasome-activating cells during these diseases. In COVID-19 patients, inflammasome activation is mostly mediated by macrophages and endothelial cells whereas in Influenza, type I and type II pneumocytes contribute more significantly. Analysis of gene expression allows classification of COVID-19 patients in two different clusters, cluster 1 (n=16 patients) died with higher viral loads and reduced inflammatory profile than oppose to cluster 2 (n=31). Illness time, mechanical ventilation time, pulmonary fibrosis, respiratory functions, histopathological status, and inflammasome activation differed in the two clusters. CONCLUSION: Our data reveal two distinct profiles in lethal cases of COVID19, indicating that the balance of viral replication and inflammasome-mediated pulmonary inflammation may lead to opposed clinical conditions, yet both lead to patient death. Understanding this process is critical for decisions concerning the higher efficacy of immune-mediated or antiviral-mediated therapies for the treatment of critical cases of COVID-19.

Keywords: COVID-19, Inflammasome, cytokine storm / **Supported by:** FAPESP

K.32 - Role of angiotensin II-modulated proximal tubule albumin endocytosis in the genesis of albuminuria and tubular injury in in early stage of diabetic kidney disease

Liz Goes Afonso [1], Diogo Peruchetti[1], Rodrigo Silva-Aguiar[1], Douglas Teixeira[1], Alvin Schmaier[2], Ana Acacia Pinheiro[1,3], Celso Caruso-Neves[1,3,4]

[1]Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro (, Brazil), [2]School of Medicine, Case Western Reserve University (, United States of America), [3]Rio de Janeiro Innovation Network in Nanosystems for Health, Rio de Janeiro Innovation Network in Nanosystems for Health (, Brazil), [4]National Institute of Science and Technology of Regenerative Medicine, National Institute of Science and Technology of Regenerative Medicine (, Brazil)

INTRODUCTION: Early stage of diabetic kidney disease (DKD) is characterized by albuminuria, tubular injury and increase in cortical angiotensin II (Ang II) level without changes in glomerular function and structure. These observations highlight the role of tubular segments in the genesis of DKD. However, how these components are interconnected is poorly known. One important clue come from the observation that high glucose (HG) decreased albumin endocytosis in proximal tubule epithelial cells (PTECs). Interestingly, changes in PT albumin endocytosis have been associated to the development of tubular injury. OBJECTIVES: We investigated the involvement of Ang II on the inhibitory effect of HG on the albumin endocytosis in PTECs and its correlation with albuminuria and tubular injury observed in DKD. MATERIALS AND METHODS: LLC-PK1 cells, a model of PTECs, were treated with HG (25 mM) during 48h. A streptozotocin (STZ)-induced diabetes animal model was used (CEUA-045/17). Male Wistar rats (8-10 wks-old) received a single intravenous injection of STZ (65 mg/kg) and renal parameters were measured after 8 weeks. DISCUSSION AND RESULTS: The inhibitory effect of HG on the albumin uptake (60%) was partially reversed by 10^{-7} M losartan, a specific AT_1R antagonist (n=6). PD123319 (AT_2R antagonist) at 10^{-8} M or A779 (Mas receptor antagonist) at 10^{-7} M did not change it (n=6). HG increased the Ang II level (2-fold, n=7). This effect was linked to the increase in the expression of angiotensin-converting enzyme (ACE) and the decrease in prolylcarboxipeptidase (n=3). Using a STZ-induced diabetes animal model, we observed a drop in PT albumin endocytosis correlated to the increase in albuminuria, urinary protein:creatinine ratio and γ-glutamiltransferase activity, marker of PT injury (n=9). Glomerular function was not changed. CONCLUSION: Our findings suggest that HG activates Ang II/AT_1R pathway leading to the decrease in PT albumin endocytosis leading to albuminuria and tubular injury observed in early step of DKD.

Keywords: diabetic kidney disease, albumin endocytosis, renin-angiotensin system

K.33 - Impaired expression of serine/arginine protein kinase 2 (SRPK2) affects melanoma progression in mice

Mônica Caetano[1], Gabriela Moreira[3], Gabriela Guimarães[2], Leandro Santos[2], Flávia Mendes[1], **Samuel Paiva** [1], Juliana Fietto[1], Mariana Boroni[2], Angela Saito[4], Gustavo Bressan[1]
[1]Departamento de Bioquímica e Biologia Molecular, Universidade Federal de Viçosa (MG, Brasil), [2]Divisão de Pesquisa Experimental e Translacional , Instituto Nacional do Câncer (RJ, Brasil), [3]Departamento de Biologia Molecular e genética, Universidade Estadual de Campinas (SP, Brasil), [4]Laboratório Nacional de Biociências, Centro Nacional de Pesquisa em Energia e Materiais (SP, Brasil)

INTRODUCTION: Cancer metastasis involves the processes of tumor cells migration and invasion at the surrounding tissue to colonize distant organs, and it is the main reason that makes melanoma one of the most aggressive tumors. The serine/arginine protein kinases 1 and 2 (SRPK1 and SRPK2) are classically related to pre-mRNA splicing control through SR proteins phosphorylation and have been found overexpressed in many types of cancer, including melanoma. Previously, we have demonstrated that the pharmacological inhibition of SRPKs impairs pulmonary colonization of metastatic melanoma in mice. OBJECTIVES: As the used compounds could target both, SRPK1 and SRPK2, here we aim to better clarify the involvement of these paralogs in metastatic melanoma patients. MATERIALS AND METHODS: Single-cell RNA sequencing data of human melanoma were analyzed. B16F10 cells were genome targeted by using CRISPR-Cas9 approach. Phalloidin staining was used to monitor actin dynamics in immunofluorescence assays. Tumors were induced in mice by injecting genome modified B16F10 cells subcutaneously or in caudal vein. DISCUSSION AND RESULTS: The Single-cell RNA sequencing data revelated that higher amounts of malignant cells expressing high levels SRPK2 correlates with poor prognosis. Consistently, CRISPR-Cas9 genome targeting of SRPK2, but not SRPK1, impaired the actin polymerization dynamics as well as the proliferative and invasive capacity of B16F10 cells *in vitro*. In further *In vivo* experiments SRPK2 genetic targeting, but not SRPK1, reduced tumor progression in both subcutaneous and caudal vein melanoma induction models. CONCLUSION: Together, these findings suggest different functional roles for SRPK1/2 in metastatic melanoma and highlight the relevance of pursuing selective pharmacological inhibitors for SRPK2.
Keywords: Melanoma, SRPK2, Metastasis

K.34 - Effects of Curcumin on the Fission Yeast Endocytic-Vacuolar Pathway

Daphne Alves Dias [1], Larissa Gleyce Machado Rego[1], Antônio Jesus Dorighetto Cogo[1], Arnoldo Rocha Façanha[1], Anna Lvovna Okorokova-Façanha[1]
[1]Lab. de Fisiologia e Bioquímica de Microrganismos, Univ. Estadual do Norte Fluminense Darcy Ribeiro (Rio de Janeiro, Brasil)

INTRODUCTION: Yeasts are widely used as model organisms to gain better understanding of plant and animal/human physiological and pathophysiological processes. Curcumin is a plant bioactive compound endowed with anti-oxidative, antiproliferative and antitumor activities. Several anticancer drugs modulate endoplasmic reticulum (ER) stress, and ER perturbations are intrinsic for tumor cells. OBJECTIVES: The aim of this study was to investigate the effect of curcumin on fission yeast proliferation and endosomal/vacuolar dynamics. MATERIALS AND METHODS: Schizosaccharomyces pombe mutant cells lacking ER Cta4 P5-ATPase exhibiting chronic ER stress, and wild-type cells were grown in the presence of curcumin (0, 10, 50 and 100 μM) and analyzed by fluorescence microscopy using CMAC, MDY-64 and FM4-64 vacuolar probes, and mitochondrial marker Mitotracker Red FM. Gene expression of the homologous human P5-ATPase ATP13A1 was analyzed using the Gtex, GEPIA, TCGA, UALCAN and STRING/GeneMANIA databases. DISCUSSION AND RESULTS: Curcumin inhibited cell growth and proliferation in a dose-dependent manner, and interfered with yeast cell polarity, morphology and cytokinesis. The mutant exhibited higher sensitivity to curcumin comparing to wild-type cells. Curcumin exposure resulted in intense vacuolar fragmentation, specifically in wild-type cells (2.4- and 1.5-fold increase in vacuoles per cell in wild-type and mutant, respectively). The effect of curcumin was prevented with low doses of FeSO4. In addition, curcumin prevented the mitochondria labeling and this effect was not restored by iron in cta4 mutant cells. CONCLUSION: Curcumin disturbs microtubules and endosomal/vacuolar dynamics, delayed cellular endocytosis and interfered with mitochondrial membrane potential, impacting the communication between endocytic pathway, ER and mitochondria, as indicated by gene expression/interaction data mining, which suggested P5-ATPase and ion signaling remodeling as part of adaptation mechanisms to ER stress and tumor metabolism reprogramming.
Keywords: protein-protein interactions, drug targeting, curcuminoids / **Supported by:** FAPERJ, CNPq, CAPES

K.35 - Chronic Hyperglycemia Compromises Communication between the Bone-brain Axis by Epigenetic Repression of the Gpr158 Receptor

Ericka P.da Silva[1], Geórgia da Silva Feltran [3], Sergio A. A. Santos[8], Rodrigo Cardoso de Oliveira[5], Rahyza Inácio Freire de Assis [6], Luis Antonio Justulin Junior[8], Denise Carleto Andia[1], Willian F. Zambuzzi[3], Alexandra Latini[7], **Rodrigo Augusto da Silva [1,2]**
[1]CEEpiRG, Program in Environmental and Experimental Pathology, Paulista University (SP, Brasil), [2]Department of Dentistry, University of Taubaté – UNITAU, Taubaté, São Paulo, Brazil (SP, Brasil), [3]LaBIO, Department of Chemical and Biological Sciences, São Paulo State University (SP, Brasil), [4]School of Dentistry, Health Science Institute, Paulista University (SP Brazil), [5]Department of Biological Sciences, University of São Paulo (SP. Brazil), [6]Department of Clinical Dentistry, Federal University of Espírito Santo (Espirito Santo, Brazil), [7]LABOX, Department of Biochemistry, Federal University of Santa Catarina (Santa Catarina, Brazil), [8]Department of Structural and Functional Biology, São Paulo State University (SP, Brazil)

INTRODUCTION: Diabetes mellitus (DM) is a chronic metabolic disease, mainly characterized by increased blood glucose and insulin dysfunction. In response to the persistent systemic hyperglycemic state, numerous metabolic and physiological complications have already been well characterized. However, its relationship to bone fragility, cognitive deficits and increased risk of dementia still needs to be better understood. OBJECTIVES: Thus, the effect of chronic hyperglycemia on the epigenetic transcriptional control of osteokines and their receptors in the bone tissue and the Central Nervous System was analyzed MATERIALS AND METHODS: Using a model of chronic hyperglycemia induction in Wistar rats by administering a single i.p. of streptozotocin (STZ - 55 mg/kg), the impact of chronic hyperglycemia on the bone-brain endocrine coupling, histological, histomorphometric and ultrastructural analyzes were performed in the bone tissue. As well as real-time PCR (qPCR) to determine gene expression and methylation status of genes encoding Osteokines and their receptors in bone and brain tissues. DISCUSSION AND RESULTS: The results demonstrated that chronic hyperglycemia negatively impacts bone biology and compromises the balance of the bone-brain endocrine axis. In bone tissue, ultrastructural disorganization, and destruction of the supporting tissue of the mandibular incisor were accompanied by global DNA hypomethylation and changes in gene expression of DNA-modifying enzymes. Still changes in the methylation status of *Ocn* and *Lcn2* genes in the femur was observed. Additionally, in the brain the hyperglycemic state modulated the gene expression of *Fgf23*, *Ocn* and *Lcn2* in the different regions. However, transcriptional regulation mediated by DNA methylation was observed only for the osteokine receptors, *Fgfr1* in the striatum and *Gpr158* in the hippocampus. CONCLUSION: Scientifically, this study is a pioneer in demonstrating that the chronic hyperglycemic state compromises the crosstalk between bone tissue and the brain, mainly affecting the hippocampus, through transcriptional silencing of the *Ocn* receptor, *Gpr158*.
Keywords: Osteocalcin, Bone-brain axis, Cognitive deficit

K.36 - Low Molecular Weight Protein Tyrosine Phosphatase Inhibitor Disrupts Stomach Cancer Hallmarks

Letícia Maria de Oliveira Magalhães [1], Alessandra Valéria de Souza Faria[1], Carmen Veríssima Ferreira-Halder[1]
[1]Department of Biochemistry and Tissue Biology, University of Campinas (São Paulo, Brazil)

INTRODUCTION: Stomach cancer is the fifth in incidence and the third in mortality in the world. Patients are often diagnosed late, with fewer therapeutic options and poorer survival. Some protein tyrosine phosphatases (PTPs) have been related to gastric carcinogenesis, and appear as potential therapeutic targets. Low molecular weight protein tyrosine phosphatase (LMWPTP) has been associated with some cancer hallmarks, such as sustaining proliferative signaling, resisting cell death, deregulating cell energy, and promoting invasion and metastasis. Recently, our group reported that the LMWPTP is overexpressed in stomach cancer patient biopsies. OBJECTIVES: To evaluate the action of the novel LMWPTP inhibitor I [N,N-diethyl-4-[4-[[3-(1-piperidinyl)propyl]amino]-2-quinolinyl]-benzamide] in the survival and migration of stomach cancer cells (Kato III). MATERIALS AND METHODS: Kato III cells were routinely grown in RPMI1640 medium (Lonza) supplemented with 10% Fetal Bovine Serum (Sigma-Aldrich), and 1% 100U/mL penicillin, 100ug/mL streptomycin (Pen-Strep, Life Technologies) at 37°C in a 5% CO2 humidified atmosphere. Cell viability was assessed by colorimetric (MTT reduction) and microscopy (calcein and ethidium homodimer) analysis; cell migration efficiency was checked by Scratch assay. DISCUSSION AND RESULTS: LMWPTP inhibitor I (0.5-1 µM) was able to overcome cell survival as assessed by MTT assay in 2D cell culture, and calcein-homodimer dyes in 3D model (spheroid). Another interesting findings were a decrease of intracellular vacuoles and cell migration. To test whether morphological changes and cell death are causally related to the mitochondrial damage, the organelle were stained with MitoTrackerRed, used to assess the integrity of the mitochondrial membrane potential. Treatment with the LMWPTP inhibitor I caused a diminishion of fluorescence signal intensity, in other words, this inhibitor somehow disrupts mitochondria function. CONCLUSION: We show, for the first time, that the inhibition of the LMWPTP might be a strategy to shut down hallmarks of cancer.
Keywords: Cancer hallmarks, LMWPTP, Stomach câncer / **Supported by:** Fapesp (Proc. 2020/04409-), CNPq, FAEPEX-Unicamp

K.37 - Stable Silencing of β3 Integrin Subunit Interferes with the Motility and Flow Adaptation of MDA-MB-231 breast tumor cells

Ana Carolina Caetano Nunes [1], Bianca Cruz Pachane[1], Larissa Thabata Gozzer[1], Aline Maria Machado[1], Wanessa Fernanda Altei[2,3], Heloísa Sobreiro Selistre de Araújo[1]
[1]Department of Physiological Sciences, Federal University of São Carlos (SP, Brazil), [2]Department of Radiation Oncology, Barretos Cancer Hospital (São Paulo, Brazil), [3]Molecular Oncology Research Center, Barretos Cancer Hospital (SPo, Brasil)

INTRODUCTION: Integrins are cell adhesion receptors involved in a two-way communication between intercellular and extracellular environments, thus aiding cells in adapting to different settings. In breast cancer, integrin β3 subunit is often linked with poor prognosis and increased likelihood for metastasis, as it aids processes of cell invasion and motility when coupled with αv subunit. The study of integrin β3 inhibition as a target for metastatic breast cancer is a viable approach, especially with *In vitro* techniques that mimic a physiological setting, such as interconnected co-culture flow system. OBJECTIVES: In this work, we investigated the alterations in motility and adaptation for culture under flow of triple-negative breast cancer cells submitted to either β3 integrin stable silencing or inhibition by DisBa-01, a recombinant RGD disintegrin. MATERIALS AND METHODS: ITGB3 was silenced in MDA-MB-231 by a third-generation lentivirus shRNA system in 293FT cells. Silencing was confirmed by RT-qPCR, western blotting, flow cytometry and immunofluorescence. Integrin β3 inhibitor DisBa-01 was recombinantly expressed and purified. Migration was assessed by wound healing and Boyden's chamber assays. Culture under flow was performed using the Quasi-vivo system (1h and 4h) with cells previously adhered to Matrigel matrix. Cell morphology was analysed by immunofluorescence. DISCUSSION AND RESULTS: RT-qPCR identified 84% of ITGB3 silencing, which was confirmed by other methods. Collective cell migration was drastically inhibited by DisBa-01 but not by MDA-MB-231-β3KD; however, individual cell migration was affected by DisBa-01 (85%) and MDA-MB-231-β3KD (78%). Parental MDA-MB-231 cells are more elongated under flow rate of 200µl/min at all tested incubation times. F-actin arrangement in MDA-MB-231-β3KD is distinct and adapts to flow after 4h. DisBa-01-treated MDA-MB-231 morphology remains unaltered by flow. CONCLUSION: β3 integrin inhibition promotes distinct responses in motility and adaptation to flow, highlighting the role of this integrin in tumor progression and metastasis.

Keywords: Cancer, Cell motility, Integrin β3 / **Supported by:** FAPESP, CAPES, CNPq

K.38 - Differential Expression of V-ATPase in HPV-associated Head and Neck Cancers

Larissa Freitas A. Gonçalves [1], Juliana do Couto Vieira C. dos Santos[1], Anna Okorokova-Façanha[1], Arnoldo Rocha Façanha[1]
[1]Laboratório de Biologia Celular e Tecidual, Univ. Estadual do Norte Fluminense Darcy Ribeiro (RJ, Brasil), [2]Laboratório de Fisiologia e Bioquímica de Microrganismos, Univ. Estadual do Norte Fluminense Darcy Ribeiro (R.J, Brasil)

INTRODUCTION: V-H+-ATPase is essential for physiological processes and pathophysiology of diseases including cancer and viral infection. Twenty-five human genes encoding different isoforms of thirteen subunits allow combinatorial assemblies of V-ATPase holoenzyme. Changes in V-ATPase expression and localization in cancer cells have been associated with antiapoptotic, metastatic and chemoresistance mechanisms. Our group recently identified molecular signatures inherent to specific expression of V-ATPase isoforms in multiple cancers. V-ATPase is required for human papillomavirus (HPV) infection, and E5 viral oncoprotein can interact with V-ATPase impairing endosomal acidification. OBJECTIVES: The aim of this study was to identify V-ATPase related molecular signatures to HPV-associated oncogenesis. MATERIALS AND METHODS: TCGA was used for clinical and genomic analyses of HNSCC patients. V-ATPase subunits associated with HPV+ was clustered in high (z-score >1) and low (<-1) expression groups. One thousand transcripts were clustered according to expression groups and PANTHER was used for GO enrichment analysis of proteins encoded by mRNA clusters. TCGA/GTEx databases were used to analyze the expression levels of V-ATPase genes. DISCUSSION AND RESULTS: HPV-associated head-and-neck carcinomas exhibited lower expression of subunits *B1, E2, C2, D, d1* while *e2* was augmented. The molecular function analysis revealed enrichment in membrane channel and transporter activity related to *ATP6V0E2*, indicating reprogramming of ion homeostasis and signaling. Transcriptional processes were observed in *ATP6V1D, ATP6V1E2, ATP6V0D1* and *ATP6V1C2* clusters. DNA metabolism and cytoskeletal activity were associated with *ATP6V1C2* and proteostasis regulation with *ATP6V1E2*. *ATP6V1B1* was mainly correlated with the immune system processes. Among subunits, *C2* showed higher expression in normal tissues, and higher expression of *ATP6V1C2* was associated with improved overall and disease-free survival. CONCLUSION: Our data suggest a metabolic reprogramming associated with viral carcinogenesis correlated with functional coupling of V-ATPase through the changes of subunits interaction at the V1-V0 interface, which mediate V-ATPase reversible disassembly.

Keywords: Head and neck carcinoma, HPV, V-ATPase / **Supported by:** FAPERJ, CNPq

K.39 - Evaluation of Doxazosin as a Potential Antiproliferative Agent in a Hepatic Stellate Cell Lineage

Renata Krüger Martins [1], Arieli Cruz de Sousa[1], Jéssica Marques Obelar Ramos[1], Fátima Costa Rodrigues Guma[1], Leo Anderson Meira Martins[2], Jarbas Rodrigues de Oliveira [3]
[1]Bioquímica, Universidade Federal do Rio Grande do Sul (RS, Brasil), [2]Fisiologia, Universidade Federal do Rio Grande do Sul (RS, Brasil), [3]Escola de Ciências da Saúde e da Vida, Pontifícia Universidade Católica do Rio Grande do Sul (RS, Brasil)

INTRODUCTION: The hepatic fibrosis is characterized by the accumulation of extracellular matrix (ECM), secreted by the hepatic stellate cells (HSC) after chronic liver injury, usually developing into cirrhosis. The HSCs occur in two states, quiescent and activated. They transdifferentiate from the quiescent state into the activated state, becoming proliferative, contractile and fibrogenic. This phenotype is responsible for the secretion of ECM as type I collagen, tissue inhibitor of metalloproteinases (TIMPs), and inflammatory cytokines, as TGF-β. Therapeutic strategies may involve the return to the quiescent state. Doxazosin is an α-1-adrenergic receptor antagonist, antihypertensive, with antifibrotic and pro-apoptotic effects. OBJECTIVES: This study assesses the effects of doxazosin through GRX, a murine lineage of HSCs. MATERIALS AND METHODS: The effects of doxazosin on cells were observed on a concentration curve as a function of the time of drug exposure, and then their effects on viability and proliferation parameters were determined using the MTT and Sulforhodamine B (SRB) assays. The time of 48 hours and the concentrations of 13.5, 18, 27 and 36 μM were defined for the remainder of the study. The results from the MTT assay were used to define the IC50. Apoptosis and necrosis were evaluated by Annexin and Propidium Iodide (PI) assays. DISCUSSION AND RESULTS: The cells suffered a significant decrease in viability and confluence according to the SRB and MTT assays. No apoptosis and necrosis alterations were identified by the Annexin and PI assay in the dose of 13.5 μM. The nuclear morphometric analysis showed the enlargement of the nuclei after the doxazosin treatment, possibly indicating the induction of cell senescence, but it isn't statistically significant. CONCLUSION: Due to the nuclear morphometric analysis and the absence of apoptosis alterations we expect to evaluate if these outcomes are caused by cellular senescence by analysis of senescence-associated β-galactosidase activity.
Keywords: hepatic fibrosis, myofibroblasts, α-1 adrenergic receptor

K.40 - Pharmacological Inhibition of MEK/ERK Reduces Hepatocellular Carcinoma Tumorigenesis in Mice with Deletion of Raptor/mTORC1 and PTEN in Hepatocytes

Érique de Castro [1], Albert Souza Peixoto[1], Natália Monteiro Pessoa[1], Gustavo Renan Gilio[1], Érika Vicência Monteiro Pessoa[1], William Tadeu Lara Festuccia[1]
[1]Department of Physiology and Biophysics, University of Sao Paulo (Sao Paulo, Brazil)

INTRODUCTION: The PI3K-mTORC2-Akt-mTORC1 signaling is involved in the hepatocellular carcinoma (HCC) development. Inhibition of PTEN in hepatocytes promotes activation of PI3K-mTORC2-Akt-mTORC1 signaling and induces HCC. Both mechanistic target of rapamycin complex 1 (mTORC1) activation and inhibition in hepatocytes promotes HCC. OBJECTIVES: To investigate the participation of mTORC1 and ERK in the development of HCC induced by PTEN deletion in hepatocytes. MATERIALS AND METHODS: Mice with deletion of Pten (L-PtenKO) associated or not with Raptor deletion (L-dKO) in hepatocytes, a component of mTORC1, were evaluated for liver mass and hepatic triacylglycerol (TAG) content, tumor number and size, glucose tolerance test (GTT) and serum parameters. DISCUSSION AND RESULTS: We found that L-PtenKO have HCC at 48 weeks of age, while L-dKO accelerates HCC development, with well-defined tumors at only 24 weeks. The liver of L-dKO mice has increased p-ERK. We hypothesized that inhibition of the MEK/ERK inhibits tumorigenesis in L-dKO. To test this hypothesis, wild-type (L-PtenWT), L-PtenKO and L-dKO mice at 24 weeks were treated or not with PD0325901 (PD, 10mg/kg), a potent MEK/ERK inhibitor. The liver mass and hepatic TAG content of L-PtenKO and L-dKO are increased in relation to L-PtenWT, and PD treatment did not change these parameters. At 24 weeks of age, 22% of L-PtenKO and 100% of L-dKO have tumors. PD treatment reduced tumor incidence in L-PtenKO (0%) and L-dKO (70%), and the number and size of tumors in L-dKO. We found that L-PtenKO and L-dKO have reduced glycemia and area under the curve (AUC) in GTT compared to L-PtenWT. Furthermore, PD reduced the AUC and glycemia in L-PtenKO and L-dKO. PD did not change the serum concentrations of TAG and alanine transaminase, but reduced cholesterol in L-PtenKO and L-dKO, and only reduced iron in L-dKO. CONCLUSION: Raptor/mTORC1 accelerates HCC tumorigenesis by ERK-dependent mechanisms.
Keywords: ERK, hepatocellular carcinoma, mTORC1 / **Supported by:** FAPESP #2020/16656-6 and 2020/04159-8

K.41 - Evaluation of the phenotypic plasticity in proteases expression during heterotypic signaling in melanoma

Uilla Barcick de Souza [1], André Zelanis Palitot Pereira[1]
[1]laboratório De Proteômica Funcional, Universidade Federal De São Paulo (São Paulo, Brasil)

INTRODUCTION: The main objective of this study is to identify molecular patterns associated with the cross-talk of stromal and tumoral cells that potentially contribute to the expression of proteases within the tumoral microenvironment. In this context, endothelial cells and fibroblasts actively participate in the secretion of factors that contribute to tumor growth and metastasis. OBJECTIVES: The main objective of this study is to identify molecular patterns associated with the cross-talk of stromal and tumoral cells that potentially contribute to the expression of proteases within the tumoral microenvironment. MATERIALS AND METHODS: Using a model of co-culture comprised by both tumoral (melanoma cell lines) and stromal cells (endothelial cells or fibroblasts), we evaluated functional outcomes related to protease activity by zymography. DISCUSSION AND RESULTS: Gelatin zymography revealed that although protease expression/activity was observed in the secretome of all cell lines, co-culture conditions of melanoma cells with stromal cells (fibroblasts or endothelial cells) resulted in distinct proteolytic profiles upon gelatin. In addition, we observed slight differences in proteolytic profiles of murine fibroblasts after co-culture with murine melanocytes or murine melanoma cells. CONCLUSION: Ongoing assays will reveal functional clues on the implications of the effect of such heterotypic signaling in the modulation of protease expression/activity in our cellular models of melanoma.
Keywords: heterotypic signalling, melanoma, proteases
Supported by: FAPESP/CAPES

K.42 - Iron Status in Obese Patients with COVID-19 infection

Gabriela Cruz Pereira [1], Jéssica Monteiro[1], Vitória Silva[1], July Alves[1], Raquel Campos[1], Kil Lee[1], Caio Asai[2]
[1]Dep. of Biochemistry, Federal University of São Paulo (SP, Brazil), [2]Polytechnic School, University of São Paulo (SP, Brazil)

INTRODUCTION: Increased ferritin and obesity are among the most reported predictors of severity of COVID-19. However, how altered ferritin levels influence on iron status has not been fully investigated. OBJECTIVES: This study aims to investigate the relationship between COVID-19 and iron status in obese patients (BMI≥30kg/m^2). MATERIALS AND METHODS: Anonymized clinical data of patients, who went to health institutions of São Paulo, Sírio-Libanês, Beneficência Portuguesa hospitals and Fleury Group were extracted from FAPESP COVID-19 DataSharing/BR repository. Python programming was used for data extraction and management. Data that were included were COVID-19 test, BMI, serum ferritin, serum iron and transferrin saturation. The generalized linear model was carried out for the comparison between groups. Generalized estimating equation was used for paired analyses. Pearson correlation was used to correlation between iron parameters. For all analysis, $p < 0.05$ was considered significant. DISCUSSION AND RESULTS: When analyzing iron levels and transferrin saturation stratified by BMI, regardless of diagnosis of COVID-19 and gender, reduced iron levels and transferrin saturation were observed in the obese population compared to eutrophic, while ferritin levels were increased. These results corroborate data from the literature which show higher prevalence of iron deficiency in the obese population and reinforce that ferritin is not an appropriate iron marker for obese population. When stratified by gender and COVID-19, significant increase in ferritin levels was observed only in women. COVID-19 infection increased the serum ferritin levels in both eutrophic and obese group. But larger increase was observed in obese group (117±124.7 ng/mL vs 134±152.2 ng/mL, p=0.002). Serum ferritin is also an inflammatory marker and its levels are increased by infection. Our data indicate that COVID-19 and obesity can have synergic effect on ferritin levels. CONCLUSION: Obesity is associated with lower iron levels, but regardless the iron levels, ferritin levels are increased by both obesity and COVID-19 in women but not in men.
Keywords: COVID-19, Iron status, Obesity / **Supported by:** FAPESP/CAPES

K.43 - Characterization of bone marrow mesenchymal stromal cell-derived extracellular vesicles from polycythemia vera

Juçara Gastaldi Cominal [1], Maira da Costa Cacemiro[1,2], Giuliana Minani Bertolino[2], Luciana Ambrosio[1], Maria Vitória Lopes Badra Bentley [1], Simone Kashima[2], Dimas Tadeu Covas[2], Lorena Lobo de Figueiredo Pontes[3], Maria Carolina de Oliveira Rodrigues[2,3], Belinda Pinto Simões[3], Hans-Jochem Kolb[4,2], Kelen C.R. Malmegrim de Farias[1,2], Fabiola Attié de Castro[1,2]
[1]Department of Clinical Analysis, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo (São Paulo, Brazil), [2]Center for Cell-Based Therapy, Regional Blood Center of Ribeirão Preto, Ribeirão Preto Medical School, University of São Paulo (São Paulo, Brazil), [3]Hospital das Clínicas, Ribeirão Preto Medical School, University of São Paulo (São Paulo, Brazil), [4]n/a, Kolb consulting (München, Germany)

INTRODUCTION: Polycythemia vera (PV) is a myeloproliferative neoplasm (MPN) characterized by overproduction of erythroid precursors. PV pathogenesis is associated with JAK2V617F mutation and alterations in bone marrow hematopoietic niche (BHN). Mesenchymal stromal cells (MSC) contribute to BHN homeostasis and regulation. Homeostasis of BHN is dependent of crosstalk between resident cells and extracellular matrix. In this context, extracellular vesicles (EV) from MSC seems to mediate intercellular communication. The MSC-derived EV have not been investigated or characterized in MPN. OBJECTIVES: To Isolate and characterize the MSC-derived EVs from PV patients and healthy subjects (CT). MATERIALS AND METHODS: MSC was isolated from bone marrow aspirates of 5 PV patients (median age= 57 years [31-62]) and 5 CT (median age=29 years [24-39]). MSC-derived EV were isolated from the MSC culture supernatant and collected by differential ultracentrifugation. EVs size and concentration were determined by nanoparticle tracking analysis and dynamic light scattering technique. EVs-derived RNA was extracted by Trizol. Angiogenic and inflammatory related genes (IL6, IL8, ANGPTL3, TNF, VEGFA and HIF1A) and microRNA (miR-21, miR-16-2, miR-15a, miR126, miR-199b) were analyzed by qPCR. The genes and microRNAs were chosen according to previous reports in the context of MSC-derived EVs in hematological malignancies. DISCUSSION AND RESULTS: The EVs-like structures found in PV patients had sizes ranging from 115 to 245 nm while those in CT had sizes ranging from 105 to 195 nm. Size and concentration of nanoparticles are similar in PV (median= 136.7 nm; median= 6.63x1010 particles/ml) and CT (median= 141.1 nm; median= 7.59x1010 particles/ml). PV and CT MSC-derived EV are enriched by IL6, VEGFA, TNF and HIF1A mRNAs. No differences in miR-21, miR15a and miR-132 expression levels were observed between PV and CT. CONCLUSION: We standardized an effective methodology to isolate and characterize MSC-derived EV. Our findings indicate that the size and concentration of MSC-derived EVs are similar in PV and CT.
Keywords: Mesenchymal stromal cells, extracellular vesicles, polycythemia vera / **Supported by:** CNPq, CAPES e FAPESP

K.44 - Manganese is a central element in tumor progression

Mariana Paranhos Stelling [1], Mariana Alves Soares[2], Simone Coutinho Cardoso[3], Carlos Alberto Pérez[4], Mauro Sérgio Gonçalves Pavão[2]
[1]DEPI, Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro (RJ, Brazil), [2]IBqM, Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro (RJ, Brazil), [3]IF, Instituto de Física, Universidade Federal do Rio de Janeiro (RJ, Brazil), [4]LNLS, Brazilian Center for Research in Energy and Materials (SP, Brazil)

INTRODUCTION: Manganese is a key element in cell proliferation and migration, two relevant aspects of tumor progression. OBJECTIVES: Our work investigates the role of manganese in tumor progression in an animal model of tumor growth and in an *In vitro* cell model. MATERIALS AND METHODS: *In vivo* studies were performed in a model of ectopic tumor growth. Briefly, Lewis lung carcinoma (LLC) cells were subcutaneously injected in C57BL/6 mice and tumor growth was accompanied up to 5 weeks (CEUA protocol number: 015/18). Samples were collected and analyzed for elemental distribution and concentration by high resolution X-Ray fluorescence. *In vitro* studies were performed using LLC cell line for the evaluation of cell behavior. We performed tumor cell growth and viability assays, transmigration and migration (wound healing) assays, as well as analyses of β1-integrin and syndecan-1 expression by immunofluorescence assays. DISCUSSION AND RESULTS: Consequences of such phenomenon were investigated *in vitro*, and we verified that short-term changes in manganese alter cell surface molecules syndecan-1 and β1-integrin, enhance collective cell migration and invasive behavior. Long-term increased levels of manganese do not affect cell growth and viability but enhance cell migration. We also observed that manganese is secreted from tumor cells in extracellular vesicles, rather than in soluble form. Finally, we describe exogenous glycosaminoglycans that counteract manganese effects on tumor cell behavior. CONCLUSION: In conclusion, our analyses describe manganese as a central element in tumor progression by accumulating in Mn-rich niches *In vivo* , as well as *in vitro*, affecting migration and extracellular vesicle secretion *in vitro*. Manganese accumulation in specific regions of the organism may not be a common ground for all cancers, nevertheless, it represents a new aspect of tumor progression that deserves special attention. **Keywords:** manganese, cancer, cell migration / **Supported by:** FAPERJ, CNPq, CAPES, Fundação do Câncer, IFRJ and LNLS

K.45 - Ethyl 6-methyl-4-(3-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-carboxylate (LaSOM 65) Shows Potential Antitumor Effect In Caenorhabditis elegans

Aline Silva [1], Isabelle Carrera[1], Luciano Kagami[2], Vera Lima[2], Daiana Ávila[1]
[1]Pharmacological and toxicological biochemistry, Universidade Federal do Pampa (Rio Grande do Sul, Uruguaiana), [2]Organic Medicinal Synthesis, Universidade Federal do Rio Grande do Sul (Rio Grande do Sul, Porto Alegre)

INTRODUCTION: The class of dihydropyrimidinones (DHPMs) has previously demonstrated important antitumor activity due to the study of the biological activities of monastrol as an inhibitor of mitotic kinesin Eg5. This finding led to the synthesis of monastrol derivatives with enzymatic activity far superior to the prototype. However, there are still few studies on the effects of DHPMs in living organisms, making further safety assessments for these compounds necessary. A promising model for toxicological and pharmacological analyzes is *Caenorhabditis elegans*, that by having its genome completely sequenced, it makes it possible to perform genetic manipulations, allowing the evaluation of pathways and mechanisms associated with the development of diseases in humans. OBJECTIVES: The present work aimed to evaluate a compound ethyl 6-methyl-4-(3-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-carboxylate (LaSOM 65) in *C. elegans*, in order to verify its safety and potential antitumor effect. MATERIALS AND METHODS: The worms N2 (wild type) and MT4244 *[unc-24(e138) let-60(n1046)]* were submitted to the treatment at the first larval stage in an acute form at concentrations of 200, 400 and 600 mM. After 48 h, the survival rate of the worms was evaluated and on the third adult day, the area and the number of multivulva phenotypes of the nematodes were verified. DISCUSSION AND RESULTS: The results obtained showed a reduction in worms survival rate (N2) after exposure to 400 and 600 mM, but this mortality was not observed in the MT4244 strain. The number of multivulva phenotype was decreased at the highest concentration tested. However, the pseudovulva area was not altered after exposure to the compound. CONCLUSION: Our results suggest a possible antitumor effect of LaSOM 65 due to a decrease in the multivulta phenotype. Mutations in the Ras (LET-60) signaling pathway exemplified in *C. elegans* are already established to promote carcinogenesis in humans. However, to better understand the effects of LaSOM 65 against tumor strains, other parameters will be investigated.

Keywords: dihydropyrimidinones, multivulva, Ras / **Supported by:** CAPES; CNPq

K.46 - Potential prognostic markers derived from proteolytic processing events in plasma samples from patients with melanoma

Murilo de Souza Salardani [1], Leonardo Cardili[2], Miyuki Uno[2], Roger Chammas[3], André Zelanis[1]
[1]Functional Proteomics Laboratory, Universidade Federal de São Paulo (São Paulo, Brasil), [2]Instituto do Câncer do Estado de São Paulo, Centro de Investigação Translacional em Oncologia (São Paulo, Brasil), [3]Faculdade de Medicina da Universidade de São Paulo, Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: Melanoma is aggressive skin cancer and a lethal melanocytic neoplasm with an increasing annual number of cases corresponding to 30% of cancers diagnosed in Brazil. Cutaneous melanoma is the 19th most common cancer worldwide, with an estimated incidence rate of 2.8-3.1 per 100.000. Given the inherent genomic diversity in cutaneous melanoma, the search for patterns related to its prognosis is essential for future approaches in the treatment of patients OBJECTIVES: Proteolytic processing is a major protein post-translational modification and it has a potential in the prospection of biological markers related to tumorigenesis. As it involves fragments being released into the blood of patients which, in turn, can reverberate as potential prognostic markers. MATERIALS AND METHODS: In this work, we apply an N-terminomic approach for prospecting prognostic markers in the plasma of patients with melanoma. Plasma samples were categorized into two groups: Systemic and Early/localized melanoma. Gelatin-zymography indicated higher activity in the samples from systemic disease groups. After depleting albumin and IgG, plasma samples were submitted to Terminal Amine Isotopic Labeling of Substrates (TAILS) and the resulting peptide lists were evaluated using bioinformatic analyses.Unique peptide signatures were observed for each disease group (systemic or localized). DISCUSSION AND RESULTS: Interestingly, regardless of disease stage, almost all peptide signatures were flanked by serine protease primary specificity. Functional assay using a broad chromogenic substrate for serine proteases confirmed such activity in all plasma samples CONCLUSION: The peptide set representing the prognostic signatures of melanoma identified in this study is currently being synthesizing for targeted proteomics assay using Selected Reaction Monitoring SRM) attempting to validate our findings in larger cohort of plasma samples from patients with melanoma.

Keywords: Melanoma, Proteolytic Processing , TAILS / **Supported by:** CAPES, FAPESP

K.47 - PM2.5-Induced Cytotoxicity Exacerbates Foam Cells Formation By Macrophages

Lílian Corrêa Costa-Beber [1], Hémelin Resende Farias[1], Rafael Kazmirczak Moraes[1], Jade de Oliveira[1], Fátima C.R. Guma[1]

[1]Departamento de Bioquímica, Universidade Federal do Rio Grande do Sul (RS, Brasil)

INTRODUCTION: The exposure to fine particulate matter (PM0.1-2.5 µm), an air pollutant, enhances the susceptibility to atherosclerosis. The phagocytosis of oxidized LDL by macrophages is a fundamental trigger for foam cells formation. Besides, macrophages apoptosis aggravate the thrombus formation and inflammation. In this context, the 70 kDa-heat shock proteins (HSP70) are powerful anti-senescence chaperones, closely related to cell survival and anti-inflammatory signaling. OBJECTIVES: We investigated if the atherogenic effect of PM2.5 could be related to an impairment in HSP signaling and cytotoxicity in macrophages. MATERIALS AND METHODS: PM2.5 retained in glass fiber filters was partially extracted with PBS and further centrifuged at 1000g for 15 min. This solution (1 g filter/125 mL PBS) was diluted in DMEM 10% FBS ten times. We exposed RAW264.7 mouse macrophages cell line to PM2.5 for 48 h, and used PBS as a Control. Triglycerides intracellular accumulation was verified by AdipoRed staining; Cell death by Annexin and PI kit; cell proliferation by Ki-67 immune-content; and HSP70 levels by immunocytochemistry in flow cytometer. DISCUSSION AND RESULTS: First, we established an *In vitro* model of foam cells, by exposing macrophages to PM2.5 for 48 h, and adding non-oxidized human LDL (50 µg/mL) at the last 24 h. As expected, the LDL induced an intracellular accumulation of lipids, which was exacerbated by the pollutant. Thus, we investigated if it could be related to an impairment in the apoptosis and proliferation. In fact, PM2.5 promoted macrophages apoptosis, whilst reduced the percentage of cells in proliferative phase. We also wondered if cytotoxicity could be related to an impairment in the HSP signaling. Curiously, the pollutant enhanced HSP70 levels, as an adaptive countermeasure to cytotoxicity. CONCLUSION: PM2.5-induced cytotoxicity exacerbates foam cells formation in macrophages, regardless of HSP70 levels.

Keywords: apoptosis, fine particulate matter, heat shock proteins

Supported by: CAPES-PROEX

K.48 - Tumor Microenvironment Metabolites Induces the Modulation of Macrophage Activity and Influences Lipid Metabolism.

Pedro Nobre Azevedo[1,2], Edson Gabriel Junior[3], Rodolfo Sellani[1,2], Diana Mota Toro[3,4], Pedro Vieira Silva Neto[3,4], Adriana Ferreira Lopes Vilela[1], Carlos Arterio Sorgi[1,2,4]

[1]Depto de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Univ. de São Paulo (SP, Brasil), [2]Depto de Bioquímica e Imunologia, Faculdade de Medicina de Ribeirão Preto, Univ. de São Paulo (SP, Brasil), [3]Depto de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo (SP, Brasil), [4]Instituto de Ciências Biológicas, Instituto de Ciências Biológicas, Univ. Federal do Amazonas (Amazonas, Brasil)

INTRODUCTION: Lipid Droplets (LD) are multifunctional organelles with many functions, such as cell signaling, activation, synthesis, and secretion of lipid mediators. Tumor-Associated Macrophages (TAM) are related to tumor development, being the tumor able to induce TAM phenotype, activity, influence the inflammatory mediator production and cell metabolic reprogramming. Indeed, the tumor can also modulate the metabolism of lipid mediators. OBJECTIVES: Demonstrate the influence of the murine melanoma (B16F10) tumor microenvironment metabolites on the polarization and activation of TAMs, the lipid mediators production, and its relation to LD formation. MATERIALS AND METHODS: Isolated cells from mice's bone marrow (C57BL/6) were differentiated into macrophages (BMDM) in M-CSF conditioned culture medium. After that, the BMDM were cultured with melanoma culture supernatant fractions (B16F10). Cytokines were determined by the ELISA method, lipid mediators by HPLC-MS/MS, and LD were measured using osmium staining dye. DISCUSSION AND RESULTS: It was observed that, in BMDM culture with melanoma supernatant (B16F10), the TAM resulted in pro-tumor function and pro-inflammatory cytokines production, as well as a large amount of LD. Analyzing the tumor microenvironment metabolites, a soluble factor was produced by B16F10 to induce the formation of cytokines and NO, with molecular weight greater than 30 kDa and sensitive to heat. However, the factor responsible for modulating lipid metabolism and LD formation in BMDM was present in the molecular weight fraction less than 3 kDa. CONCLUSION: The tumor microenvironment has a huge and complex variety of molecules, and TAM modulation occurs through soluble multi-factors, involving TAM-specific biological functions and lipid metabolism, favoring the tumor development.

Keywords: Lipid Metabolism, Melanoma, Lipid Droplets, macrophages / **Supported by:** FAPESP, CNPq, CAPES

K.49 - Protective Action of Cl-HIN Against Toxic Alterations in ChE Activity and RS Levels in Rats Exposed to Malathion

Arthur Acosta Cavalheiro [1], Edina da Luz Abreu[1], Anne Pinto Savall[1], Simone Pinton[1], Evelyn Cesca Vieira[1], Carol Brandão Quines[1]
[1]De Bioquimica, Universidade Federal do Pampa (Rio Grande do Sul, Brasil)

INTRODUCTION: Exposure to pesticides is currently an important national public health problem. A representative of that class, is the malathion (MAL), an organophosphates pesticides (OP) are widely used in agriculture, that when metabolized, it becomes highly toxic to humans for affect the liver, kidney, small intestine, urinary tract, and lungs. Based on this findings, new drugs or alternative approaches have been considered for malathion poisoning therapy. The (3Z)-5-Chloro-3-(Hydroxyimino)indolin-2-one (Cl-HIN) is an oxime which demonstrated some pharmacological effects on glucose metabolism regulation and cholinesterase reactivation in acute exposition to MAL. OBJECTIVES: This study was to investigate the effectiveness of Cl-HIN treatment against alterations in the liver induced by malathion subchronic exposure in rats over twenty days. MATERIALS AND METHODS: Male wistar rats (60 days old) received the following treatments once per day for 20 consecutive days: control group (corn oil via oral and tap water by intraperitoneal route (i.p.)); Cl-HIN group (5 mg/kg by the oral route); MAL group (1mg/kg i.p.); Cl-HIN+MAL (Cl-HIN 5 mg/kg + MAL 1 mg/kg). Twelve hours after the last administration the drugs rats were killed and their livers were removed to evaluated the cholinesterase (ChE) activity and reactive species (RS) levels. DISCUSSION AND RESULTS: Our results show that subchronic exposure to MAL inhibit the hepatic ChE activity and increased the RS levels, however the Cl-HIN treatment restored the ChE activity and protected against this alteration in RS levels. The primary mechanism of MAL toxicity involves the inhibition ChE activity which could favor the development of oxidative stress. Indeed, the Cl-HIN treatment protected against MAL hepatic toxicity by restored cholinesterase activity and was able to decreased the alterations in RS levels, suggesting a potent antioxidant activity. CONCLUSION: We demonstrated the beneficial effects of Cl-HIN against hepatic alterations induced by sub-chronic exposure to an organophosphate in rats.

Keywords: Environmental contaminants, hepatic damage, oximes / **Supported by:** CNPq

K.50 - Effects of doxazosin on hepatic stellate cell activation

Arieli Cruz de Sousa [1], Jéssica Marques Obelar Ramos[1], Renata Kruger Martins[1], Daiani Machado de Vargas[1], Leo Anderson Meira Martins[2], Jarbas Rodrigues de Oliveira[3], Fátima Costa Rodrigues Guma[1]
[1]Bioquímica, Universidade Federal do Rio Grande do Sul (RS, Brasil), [2]Fisiologia, Universidade Federal do Rio Grande do Sul (RS, Brasil), [3]Escola de Ciências da Saúde e da Vida, Pontifícia Universidade Católica do Rio Grande do Sul (RS, Brasil)

INTRODUCTION: Liver fibrosis is characterized by the accumulation of extracellular matrix after chronic liver injury and can lead to cirrhosis. Hepatic stellate cells (HSCs) play a crucial role during fibrogenesis. They transdifferentiate, or ‘‘activate’’, into proliferative, fibrogenic myofibroblasts (MFBs) that produce type I collagen, secrete inflammatory cytokines, and express high levels of alpha-smooth muscle actin (α-SMA). New therapeutic strategies are necessary to contain the proliferation of activated HSCs, induce their death by apoptosis and/or return to the quiescent state. One useful strategy may be adopting agents that are approved for the treatment of other diseases. Doxazosin is an antihypertensive α-1 adrenoreceptor antagonist, which also has antifibrotic and pro-apoptotic effects. OBJECTIVES: Herein we analyze the effect of doxazosin in the human cell line of HSCs (LX-2). MATERIALS AND METHODS: First, we used Image Cytometry to analyze the effect of different concentrations and times of doxazosin treatment upon cell confluence. The cells were treated with concentrations of 0.9-54 μM for 24, 48, and 72 hours. We observed a concentration- and time-dependent effect of the treatment. We defined 48 hours as the treatment time, and the chosen concentrations were 18, 27, 36, and 45 μM. Cell viability was determined by MTT assay and biomass by Sulforhodamine B. The apoptosis and necrosis evaluation was performed by Annexin and Propidium Iodide and the relative expression of α-SMA mRNA by qRT-PCR. The experiments were repeated at least three times, all in triplicate. DISCUSSION AND RESULTS: We found that doxazosin reduced LX-2 viability and culture biomass in a dose-dependent manner. Doxazosin (27 μM) increased apoptosis and significantly decreased the relative expression of α-SMA (*ACTA2* gene), which is a marker of MFBs. CONCLUSION: Doxazosin alters activated phenotype, reducing the expression of fibrogenesis markers and inducing apoptosis in HSCs. Taken together, these results suggest that doxazosin has a potential therapeutic effect on liver fibrosis.

Keywords: Hepatic fibrosis, myofibroblasts, α-1 adrenergic receptor / **Supported by:** CAPES and CNPq

K.51 - LINC00941 is an Oncogenic Long Non-coding RNA Regulated by KRAS in Pancreatic Cancer

Thalita Bueno Corrêa[1], Vitor Heidrich[1], Sandro Mascena Gomes-Filho[1], Diogo Vieira da Silva Pellegrina[1], Lutero Augusto Hasenkamp[1], Ester Risério Matos Bertoldi[1], Yuli Thamires Magalhães[1], Lilian Cristina Russo[1], Nicolas Carlos Hoch[1], Fabio Luis Forti[1], Eduardo Moraes Reis[1], **Daniela S. Bassères [1]**
[1]Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: Oncogenic KRAS-driven pancreatic ductal adenocarcinoma (PDAC) is a frequent and very aggressive disease, for which there are currently no effective therapies. Therefore, identification of KRAS targets with therapeutic potential is warranted. Even though long non-coding RNAs (lncRNAs) are important cancer biomarkers and can functionally affect the oncogenic process, lncRNAs involved in oncogenic KRAS-induced PDAC remain unknown. OBJECTIVES: We aimed to identify lncRNAs that play an important role in KRAS-induced PDAC. MATERIALS AND METHODS: We used RNAseq, exome sequencing and clinical datasets from PDAC cases generated by The Cancer Genome Atlas (TCGA) and/or the International Cancer Genome Consortium (ICGC) to identify differentially expressed lncRNAs according to KRAS status (mutated or wild type) and to evaluate the association of their expression with prognostic value. We also analyzed their differential expression in RNA-Seq data from 14 paired samples of PDAC tumors versus adjacent normal tissue. LncRNA expression was also evaluated in different cell subpopulations using PDAC single-cell sequencing data. LncRNA regulation by KRAS was validated by KRAS silencing in PDAC cell lines and in isogenic KRAS-mutant and KRAS-wildtype pancreatic cells (HPDE/HPDE-KR). Finally, LncRNA silencing was used in cell-based assays to evaluate functional impact. DISCUSSION AND ESULTS: We identified in the TCGA dataset 10 KRAS-associated lincRNAs. We confirmed in PDAC cell lines that mutant KRAS positively regulates expression of 4 lincRNAs. Of these, we found that LINC00941 expression is associated with worse PDAC prognosis and is expressed preferentially in a pancreatic ductal subpopulation that is found exclusively in PDAC tumor samples. siRNA-mediated silencing of LINC00941 in pancreatic cell lines reduces cell migration and invasion, decreases DNA repair ability and sensitizes PDAC cells to gemcitabine treatment, modulating the expression of genes involved in the DNA repair and chemorresistance pathways. CONCLUSION: Oncogenic KRAS promotes LINC00941 expression in PDAC. LINC00941 has prognostic and functional value and may represent a new PDAC biomarker or therapeutic target.
Keywords: KRAS, PDAC, lncRNA / **Supported by:** FAPESP, CAPES, CNPq

K.52 - Tetrahydrobiopterin in autism spectrum disorder: an unexplored avenue

Clóvis Lopes Colpani Filho [1,2], Sthephanie Luiz Ramos[1,2], Lucas Melfior[2], Mateus Santos Oliveira[2], Lillian Freitas Taruhn[2], Luísa de Oliveira Schmitt[1,3], Margrit Elis Muller[2], Thiago Ku. Nunes[1], Joana M.N. Gaspar[1], Alexandra Latini[1]
[1]LABOX - Bioquímica, Universidade Federal de Santa Catarina (Santa Catarina, Brasil), [2]Curso de Graduação em Medicina, Universidade Federal de Santa Catarina (Santa Catarina, Brasil), [3]Curso de Graduação em Farmácia, Universidade Federal de Santa Catarina (Santa Catarina, Brasil)

INTRODUCTION: Autism spectrum disorder (ASD) is a condition that describes individuals who have persistent deficits in social communication and social interaction with restricted, repetitive patterns of behavior, interest or activities, classified in a large spectrum, accordingly to symptoms. Nonetheless, ASD's physiopathological mechanisms are unclear, offering few treatment options for ASD. Evidence from the literature suggests that the tetrahydrobiopterin (BH4) pathway might be impaired affecting the activity of monoaminergic neurons that could explain some of the symptoms in ASD. OBJECTIVES: Thus, we conducted a systematic review to analyze whether BH4 administration could represent a possible pharmacological treatment for ASD. MATERIALS AND METHODS: A systematic review of the literature was conducted to identify articles in English with the following keywords, differently matched: autism, ASD, autism spectrum disorder, BH4, tetrahydrobiopterin, neopterin, NO, nitric oxide. These data bases were used: Pubmed, Cochrane, SCielo platforms. The analysis was performed between December 2020 through December 2021. DISCUSSION AND RESULTS: BH4 levels were reported to be lower in biological samples obtained from individuals affected by ASD, when compared to sex and age-paired controls. Neopterin was found to be higher in plasma and urine, but lower in the CSF, and nitric oxide was reported to be higher in all studies. Treatment with BH4 appears to improve the ASD related symptoms. BH4 metabolism was shown to be negatively modulated in ASD. While neopterin was increased in biological fluids, denoting inflammation, BH4 was diminished, possibly suggesting that BH4 production is being redirected to the synthesis of nitric oxide to perpetuate inflammation instead of supporting the synthesis of neurotransmitters. This is supported by various reports showing increased levels of nitric oxide in the fluids of ASD affected individuals. CONCLUSION: Since BH4 is an essential cofactor for monoaminergic neurotransmission, this molecule becomes a promising therapeutic off-label candidate, to treat ASD symptomatology, increasing the welfare of affected patients.
Keywords: Autism Spectrum Disorder, BH4 pathway, Nitric Oxide / **Supported by:** CNPq, FAPESC, PIBIC/UFSC.

K.53 - Curcumin's Antiproliferative Action on Fungi and Animal cells and Mechanistic Interconnections

Maysa de Almeida Ribeiro [1], Arícia Leone E. M. Assis[1], Gabriel C. Machado[1], Gildeíde A. Costa[1], Antonio Jesus Dorighetto Cogo[1,2], Anna Lvovna Okorokova Façanha[2], Arnoldo Rocha Façanha[1]
[1]Lab. de Biologia Celular e Tecidual, Univ. Estadual do Norte Fluminense Darcy Ribeiro (Rio de Janeiro, Brasil), [2]Laboratório de Fisiologia e Bioquímica de Microrganismos, Univ. Estadual do Norte Fluminense Darcy Ribeiro (Rio de Janeiro, Brasil)

INTRODUCTION: Plant tumorigenesis, when compared with the vast literature addressing animal/human carcinogenesis, encompass quite elusive mechanisms. ⟨I⟩Ustilago maydis⟨/⟩, a phytopathogen responsible for tumors in maize, induces tumorous galls in actively growing host tissues. OBJECTIVES: The aim of the present study was to evaluate the effect of curcumin on the growth of the fungus that generates plant tumorigenesis in comparison with the inhibition exerted by the same agent on melanoma cell lines, through a mechanism of action that involves antiproliferative and pro-apoptotic processes. MATERIALS AND METHODS: Fungal cells were isolated and incubated in YPD liquid medium for 36h, in parallel, plates were prepared with solid YPD medium with the respective concentrations of curcumin: 0, 5, 10, 20 and 50 µM. Here, this tumorigenic phytopathogen has also been analyzed in comparison with murine melanoma cell lines (B16F10), as a model of animal tumor cells. Data obtained from Scanning Ion-selective Electrode Technique (SIET) was used to analyze the H+ fluxes as affected by the curcumin treatments DISCUSSION AND RESULTS: At a concentration of 50 µM, the cells of ⟨I⟩U. maydis⟨/⟩exhibited morphological changes and reduced growth, evidencing the antifungal potential of curcumin in the same concentration range in which we showed the highest cytotoxic effect on murine melanoma. In both models, phytopathogenic and carcinogenic, curcumin induced antiproliferative effects at the concentration of 50 µM. Curcumin (20-50 µM) caused also significant reductions in cells H+ effluxes, that could be correlated with inhibitions of the V-ATPase and extracellular acidification. CONCLUSION: Since acidic tumor environment favors tissue damage, activation of lytic extracellular enzymes, and the acquisition of more metastatic and drug-resistant phenotypes, the curcumin effects on tumor cells H+ fluxes may prevent tumor proliferation and chemoresistance phenomena in both models.
Keywords: Malignant cells;, Pathogenic fungus;, Natural products for tumor chemotherapy;

K.54 - EGF Receptor Cleavage in EMT Induced by EGF

Guilherme Sastre de Souza [1], Virgínia Campos Silvestrini[1], Alessandra Pinto vargas[1], Izadora Archiolli[1], Camila de Souza Palma[1], Ana Paula Masson[1], Vitor Marcel Faça[1]
[1]Departamento de Bioquímica e Imunologia , Faculdade de Medicina de Ribeirão Preto - USP (São Paulo , Brasil)

INTRODUCTION: Epithelial mesenchymal transition (EMT) is related to tumor cells evolution leading epithelial cells to develop characteristics of mesenchymal cells like motility and loss of cell-cell adhesion. EMT occurs through reorganization and expression of cytoskeletal proteins, production of enzymes to degrade extracellular matrix and acquisition of locomotor properties. Cells develop motor phenotypes favoring detachment from original tumor and migration to other tissues culminating in metastasis development. Epidermal Growth Factor (EGF) stimulates EMT by binding to its receptor (EGFR), triggering a cell growth cascade, which controls cell division and survival. When it is not regulated, it may evolve to cancer. OBJECTIVES: Compare the expression of proteins involved in EMT on MCF-7 cells overexpressing SNAIL and/or indulced by EGF. MATERIALS AND METHODS: Based on proteomic data generated by our group, using MCF-7 cell line induced to EMT by overexpression of SNAIL and/or induced by EGF, we evidenced some proteins with the characteristic of having undergone a shedding process. In order to validate the process, MCF7 cells were induced to EMT by treatment with 10ng/mL rhEGF and subjected to subcellular fractionation. Western blotting (WB) was used to confirm EMT and EGFR cleavage. DISCUSSION AND RESULTS: According to our data 13 proteins suffered a decrease from five to ten times when induced by overexpression of SNAIL and/or induced by EGF when compared to control. Emphasis was given to EGFR_HUMAN, used in our studies as an inducer of the EMT, this cleavage could be the guide for EMT course. An increase of SNAIL expression confirmed EMT. Subcellular fractionation followed by WB showed no EGFR signal in the membrane fraction treated with EGF when compared to the non-treated group. CONCLUSION: These preliminary results confirmed EGFR cleavage during EMT. More studies with other fractions and EGFR labeling assays will help understanding chemical and biological processes undergoing receptor cleavage.
Keywords: MCF-7 , cancer, proteomics / **Supported by:** CAPES, FAPESP, CNPQ e FAEPA

K.55 - Functional relationship between Serine Arginine Protein Kinase 2 (SRPK2) and Thymosin Beta 4 (Tβ4) in Metastatic Melanoma

Juliana Regina Ribeiro de Souza [1], Gabriela Moreira[2], Leandro Santos[3], Gabriela Guimarães[3], Juliana Fietto[1], Mariana Boroni[3], Angela Saito[4], Gustavo Bressan[1]

[1]Departamento de Bioquímica e Biologia Molecular, Universidade Federal de Viçosa (Minas Gerais, Brasil), [2]Departamento de Biologia Molecular e genética/Centro de química medicinal, UNICAMP (São Paulo, Brasil), [3]Divisão de Pesquisa Experimental e Translacional, Instituto Nacional do Câncer (Rio de Janeiro, Brasil), [4]Laboratório Nacional de Biociências, Centro Nacional de Pesquisa em Energia e Materiais (São Paulo, Brasil)

INTRODUCTION: The Serine Arginine Protein Kinases (SRPK) are related to pre mRNA splicing control and have been found overexpressed in different tumors, including melanoma. Previously, we have identified a possible functional link between SRPK2 and Tβ4, an endogenous 4,9-kDa actin-hijacker peptide. This peptide operates in cell motility, epithelial-mesenchymal transition (EMT), and other multiple biological activities, including tumor metastasis. OBJECTIVES: The goal of this study was to investigate the functional relationship between SRPK2 and Tβ4 in the context of metastatic melanoma. MATERIALS AND METHODS: RNA-Seqdata from human melanoma patients were obtained from "The Cancer Genome Atlas— TCGA" database. Those patients were divided into primary and metastatic, and classified into high and low expressions of TMSB4X. Then they were compared in relation to the impact of those expressions in survival, through Kaplan-Meier 5-year survival curves, using log-rank and Cox regression as statistical tests. *In vitro* phenotypic assays of cell proliferation, migration, and colony formation were performed in wild-type or CRISPR-Cas9 modified B16F10 cells. Actin dynamics were investigated in immunofluorescence assays. SR protein phosphorylation was investigated through Western blotting and immunofluorescence assays. Metastatic melanoma was induced in mice through B16F10 cells injection. DISCUSSION AND RESULTS: Tβ4 expression correlates to poor prognosis in metastatic melanoma patients. Through *In vitro* assays, we observed that its effect in promoting cell proliferation, migration, and colony formation was impaired by SRPK pharmacological inhibitor or SRPK2 genetic depletion. Accordingly, Tβ4 promoted SR protein phosphorylation and actin lamellipodia organization, which was also negatively affected by SRPK functional depletion. Furthermore, treatments with a pharmacological SRPK inhibitor (SRPIN340) prevented Tβ4-tumor induced growth *In vivo* . CONCLUSION: Together, these results suggest that SRPK2 transduces the Tβ4 signal in tumor cells to affect actin organization and possibly pre-mRNA splicing, which should be better investigated in the context of metastatic melanoma. **Keywords:** Melanoma, SRPK2, Tβ4

K.56 - Impairment of Akt-dependent Prolylcarboxypeptidase Stability in Proximal Tubules: Involvement on the Subclinical Acute Kidney Injury

Diogo Barros Peruchetti [1], Rodrigo Pacheco Silva-Aguiar[1], Douglas Esteves Teixeira[1], Ana Acacia Sá Pinheiro[1,2], Alvin Schmaier[3], Celso Caruso-Neves[1,2,4]

[1]Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro (, Brazil), [2]Rio de Janeiro Innovation Network in Nanosystems for Health, Rio de Janeiro Innovation Network in Nanosystems for Health (, Brazil), [3]School of Medicine, Case Western Reserve University (, United States of America), [4]National Institute of Science and Technology of Regenerative Medicine, National Institute of Science and Technology of Regenerative Medicine (, Brazil)

INTRODUCTION: Subclinical acute kidney injury (subAKI) is characterized by tubule-interstitial injury (TII) and albuminuria. These processes involve the modulation of the albumin reabsorption in proximal tubule epithelial cells (PTECs) by increasing in angiotensin II (Ang II) concentration. Interestingly, the role of renal prolylcarboxypeptidase (PRCP), which is responsible to degrade Ang II into Ang-(1-7), in the development of renal injury has been highlighted in the Goldblatt hypertension model. OBJECTIVES: To investigate the role of PRCP in the development of TII in subAKI. MATERIALS AND METHODS: Two models were used: 1) murine (C57BL/6 mice) subAKI, produced by intraperitoneal injections of 10 g/kg/day BSA for 10 consecutive days (CEUA-043/18); and 2) LLC-PK1 cells, a porcine PTECs line. DISCUSSION AND RESULTS: The subAKI group presented increased proteinuria and UPCr (urinary proteins:creatinine ratio) associated to the decrease in PRCP expression (n=4). An inverse correlation exists between PRCP expression and proteinuria (R^2 =0.6357, P=0.0317). Alternatively, in LLC-PK1 cells, physiological albumin concentrations (0.01-1.0 mg/mL) increased total and luminal PRCP expression (n=3) as well as its stability (n=3). These effects were abolished by 10^{-7} M MK-2206, an Akt inhibitor (n=3). PRCP and LAMP1+ colocalized in lysosomes (n=3). When pathophysiological albumin concentration (20 mg/mL) were administered, there was decreased PRCP expression and stability (n=3). Moreover, MK-2206 treatment alone mimicked the inhibitory effect of high albumin concentration on PRCP stability while 10% FBS, an Akt activator, reversed it (n=3). ZPP at 10^{-5} M, a PRCP inhibitor, increased Ang II (n=3) and decreased albumin endocytosis (n=4) in a similar way to observed in subAKI animal model. CONCLUSION: Our findings suggest that reduction of cortical PRCP leads to an increase in Ang II that contributes to the development of subAKI. This process is mediated in part by an albumin-modulated Akt pathway in PTECs. **Keywords:** albuminuria, subclinical acute kidney injury, prolylcarboxypeptidase

K.57 - New Analgesic Lead Developed Based On Analysis Of Mutations In Patients With Congenital Insensitivity To Pain

Beatriz Caroline de Moraes [1], Helder Veras Ribeiro-Filho[2], Allan Pradelli Roldão[1], Victoria Regina da Silva Oliveira[3], Elaine Flamia Toniolo[3], Carolina Purcell Goes[1], Camila Squarzoni Dale[3], Paulo Sérgio Lopes de Oliveira[2], Deborah Schechtman[1]

[1]Department of Biochemistry, University of Sao Paulo (SP, Brazil), [2]Brazilian Center for Research in Energy and Materials, Brazilian Biosciences National Laboratory (SP, Brazil), [3]Department of Anatomy, Laboratory of Neuromodulation of Experimental Pain (LaNed), University of Sao Paulo, SP, Brazil (SP, Brazil)

INTRODUCTION: Chronic pain has become an important health issue for the last few decades, affecting nearly 30% of the population worldwide. Currently, the indiscriminate use of opioids causes innumerable side effects in the population, underscoring the necessity of new analgesics. Congenital Insensitivity to Pain with Anhidrosis (CIPA), is a rare autosomal recessive disease, characterized by loss of algesia and inability to sweat. This syndrome is caused by mutations in the gene NTRK1, that codes for Tropomyosin receptor kinase A (TrkA), the high affinity receptor for nerve growth factor (NGF). NGF signaling is key for the development of pain and neurite differentiation of sensory and nociceptive neurons. OBJECTIVES: In the present study we intend to investigate how mutations in TrkA affect pain-specific NGF-mediated signaling pathways in patients with CIPA. MATERIALS AND METHODS: To explore new drug targets, we investigated mutations in the NTRK1 gene. A systematic analysis of the mutations described in the literature in NTRK1 gene, accompanied by molecular modeling, was performed to characterize the effect of these mutations in NGF signaling. DISCUSSION AND RESULTS: Mutations that potentially decreased the interaction between TrkA and Phospholipase C γ (PLCγ) were identified in some CIPA patients. Based on this finding, we designed a cell permeable phospho-peptide (TAT-pQYP) that inhibited the interaction between TrkA and PLCγ that was tested in cells and in animal models of inflammatory pain. In mice, TAT-pQYP decreased mechanical and thermal sensitivity. These results showed that PLCγ activation in response to NGF is a key component for the sensation of mechanical pain. CONCLUSION: We demonstrate a strategy to develop new analgesic drugs for pain treatment based on analysis of CIPA-perturbed signaling pathways and developed a peptide that would specifically interfere with pain signaling and could eliminate side effects caused by totally blocking NGF signaling.

Keywords: cell signaling, pain, TrkA / **Supported by:** Capes

K.58 - Effect of the serine protease inhibitor rBmTI-A on inflammatory response in human lung epithelial cells

Graziele Cristina Ferreira[1], Bruna Rocha Almeida[2], Gilmara Barros de Lima[1], Erika Suzuki[2], **Sergio Daishi Sasaki** [1]

[1]Centro de Ciências Naturais e Humanas, Universidade Federal do ABC (São Bernardo do Campo, São Paulo, Brazil), [2]Microbiologia, Imunologia e Parasitologia, Universidade Federal de São Paulo (São Paulo, Brazil)

INTRODUCTION: rBmTI-A is a recombinant double domain Kunitz-BPTI serine protease inhibitor. rBmTI-A presents activity toward human neutrophil elastase (HNE), bovine trypsin, plasmin and human plasma kallikrein. In previous works, It was demonstrated that rBmTI-A is able to prevent the pulmonary emphysema development in mice induced by porcine pancreatic elastase (PPE), in that disease model the rBmTI-A presented an anti inflammatory effect. However, the mechanisms involved with this effect were not completely unraveled. OBJECTIVES: In this work, we investigated the action of rBmTI-A in A549 human lung epithelial cells induced to inflammatory response by PPE; furthermore, we analyzed the involvement of protease-activated receptor (PAR) in cytokine increasing resulted from the action of PPE. MATERIALS AND METHODS: The cells were incubated with rBmTI-A in different concentrations for two hours followed by addition of PPE (0.0125U/mL), after 16 hours, inflammatory cytokines quantification was performed using the culture supernatants by ELISA while the cells were submitted to cell viability assay by MTT, or to RNA extraction followed by inflammatory cytokines quantification using real time PCR. Similar procedure was accomplished with antagonist peptides PAR-1 and PAR-2 incubated for 30 minutes followed by addition of PPE (0.0125U/mL). DISCUSSION AND RESULTS: Our results showed that rBmTI-A at nM range promote decrease of cytokines expression in comparison with positive control group, IL-8, IL-6, MCP-1 and TNF-α were reduced in 56%, 20%, 45% and 64%, respectively. Concerning cytokines secretion in supernatant, rBmTI-A at 8 nM promoted 50% and 75% reduction of IL-8 and MCP-1, respectively. The PAR-1 and PAR-2 antagonist at 50μM were enough to reduce the gene expression and secretion of IL-8 in PPE stimulated cells. CONCLUSION: We conclude that the treatment with rBmTI-A was able to decrease the inflammatory process induced by PPE in A549 cells, additionally, we suggest that these cytokines increasing is related to the action on PAR-1 and PAR-2 receptors.

Keywords: rBmTI-A, inflammation, A549 cells / **Supported by:** FAPESP 2018/11874-5, CAPES process number 001

K.59 - Production of Capturing Agentes to Study Ubiquitination During Epithelial to Mesenchymal Transition

Ana Paula Masson[1], **Izadora Archiolli** [1], Virgínia Campos Silvestrini[1], Guilherme Sastre de Souza[1] ,Alessandra Pinto Vargas[1], Vitor Marcel Faça[1]
[1]bioquímica E Imunologia, Universidade De São Paulo (São Paulo, Brasil)

INTRODUCTION: Cancer is a set of multifactorial diseases consisting of malignant tumors that can invade adjacent tissues. One of the molecular mechanisms that occurs during metastasis is epithelial-mesenchymal transition (EMT). This process is controlled by multiple factors that trigger cells' ability to detach from a primary tumor, fall into the circulation and create secondary disease sites. During this process, target proteins such as SNAIL, HDAC, and others are altered by several post translational modifications, including ubiquitination. OBJECTIVES: Establishment of a specific capture strategy for ubiquitinated proteins through the production of an antibody for immunoaffinity purification. MATERIALS AND METHODS: We synthesized the peptide ESTLHLVLRLRGG, which represents ubiquitin C-terminus with the aim to explore the *In vitro* cell model of EMT. MCF7 cell line was stimulated with EGF 10ng/mL, inducing the critical stage of metastasis. Proteins were then extracted and western blotting and wound healing assays were performed for EMT validation. Ubiquitinated targets from obtained proteins will be isolated by immunoaffinity. DISCUSSION AND RESULTS: Peptide was synthesized through solid phase strategy and purified by HPLC. Its corresponding antibody was obtained by Rheabiotech. Induced EMT in MCF-7 cells was confirmed by western blotting. EMT and cell adhesion related proteins were monitored such as SNAIL overexpression and E-cadherin signal decrease. Wound Healing Assay was performed to evaluate the migratory capacities of MCF-7 due to EMT induction with EGF (0, 5, 10, 15, 20ng/mL). Results indicate that cells stimulated with EGF 20ng/mL significantly exhibit increased migration compared with control and other concentrations. Finally, the immune complex was prepared and submitted to Global Proteomics analysis for identification of new targets in tumor progression CONCLUSION: This study may reveal new targets for detecting tumor progression and potentially new targets for pharmacological inhibition of EMT.
Keywords: CANCER, EMT, UBIQUITINATION / **Supported by:** FAPESP

K.60 - Project Evaluation of the Association Between Polymorphism in miR-126 and miR-423 and the Clinical Evolution of the Patients with Chagas Disease

Fernando Liberalino Fernandes[1,2], Gilson Aquino Cavalcante[1,2], Amanda Estevam Carvalho[1,2], Valéria Duarte de Almeida[2], João Paulo da Silva Liberalino[2], Micássio Fernandes de Andrade[2], Ellany Gurgel Cosme do Nascimento[2], Cléber de Mesquita Andrade[2], Thales Allyrio Araújo de Medeiros Fernandes[2]
[1]SBBq, Programa Multicêntrico de Pós-Graduação em Bioquímica e Biologia Molecular (, Brasil), [2]Faculdade de Ciências da Saúde, Universidade do Estado do Rio Grande do Norte (Rio Grande do Norte, Brasil)

INTRODUCTION: The Chagas disease (CD) is caused by the protozoan *Trypanosoma cruzi* and is characterized by two phases; acute and chronic. The chronic phase is that in which the digestive, cardiac or cardiodigestive forms are mostly manifested and genetic factors may be associated with its clinical evolution. Thus, genetic polymorphisms may be related to the progression of the CD clinical form. MicroRNAs regulates various stages of cardiac development, and studies have shown the relationship of miR-423 and miR-423 on cardiac function, however, there are no studies relating miR-126 and miR-423 polymorphisms to the cardiac form of CD. OBJECTIVES: Thus, this project aims to analyze the association between non-coding RNA polymorphisms (miR-126 and miR-423) to clinical evolution of patients with CD. MATERIALS AND METHODS: For this purpose, a cross-sectional study will be performed among patients with the pathology confirmed by serological tests attended at ADOC from FACS-UERN. Peripheral blood samples will be obtained from patients, then genotyping of miR-126 and miR-423 polymorphisms will be performed through PCR-RFLP technique. Then, through the exams available in the attendance services, the clinical forms of the patients will be determined. Data will be accounted for and statistically analyzed. DISCUSSION AND RESULTS: Certain allele frequencies of the miR-126 and miR-423 genes are expected to be associated with the cardiac form in patients with CD. CONCLUSION: The development of this project is aimed identifying possible biological markers, as well as providing a better understanding of how the genetic factor may be linked to the clinical evolution of CD.
Keywords: Chagas Disease, Genetic Polymorphisms, Micro-RNA
Supported by: CAPES

K.61 - Evaluation of the effect of superparamagnetic iron oxide nanoparticles functionalized with Wedelolactona in modulating the aggregation of alpha-synuclein protein and neurotoxicity of aggregates formed.

Gabriela Ferraz Ribeiro [1], Luiz A. S. Oliveira[1], Carolina Braga[1]
[1]Campus Duque de Caxias, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: Parkinson's disease is considered an amyloidosis. There is thus, a need to find compounds that act as modulators of amyloid aggregation of α-syn protein, inhibiting the formation of toxic species and thus preventing death and maintaining cell morphology and viability. Previous projects of our group identified two possible compounds with the ability to inhibit α-syn aggregation *In vitro* and to undo mature fibers, namely, Wedelolactone (WED) and superparamagnetic iron oxide nanoparticles (SPIONs), respectively. WED, studied during my master's degree, has shown to reduce in approximately 90% thioflavin-T-binding, which is a marker for amyloid formation. While SPION was able to "break" pre-formed aggregates through treatment with hyperthermia, in addition to modulate aggregation. OBJECTIVES: In this work, we aim to functionalize the SPIONs with Wedelolactone compound, forming SPIONs@wed and to evaluate its role in the formation of alpha-synuclein protein aggregates, and in the neurotoxicity of the species formed in their presence. MATERIALS AND METHODS: The green synthesis of SPIONs was carried out by the sol-gel method, using coconut water as a framework for the reaction. The functionalization was carried out by incubating both substances for 3h with constant agitation and its confirmation occurred through UV/visible spectrophotometry. To evaluate the modulation of α-syn aggregation, aggregation kinetics were performed with binding to Thioflavin-T, HPLC and scanning electron microscopy - transmission mode. DISCUSSION AND RESULTS: So far, it was possible to notice that SPION@Wed has a superior modulation capacity than SPION and WED alone. While SPION 100 µg/ml and WED 5.6 µM reduce binding to thioflavin-T after 40 hours of kinetics by 80% and 30%, respectively, SPION@WED reduces it by 98%. It was also noted that different concentrations of SPION@WED appear to modulate the initial lag phase of aggregation kinetics differently. CONCLUSION: SPION's@Wed has been shown to be an interesting compound for inhibiting a-syn aggregation *in vitro*. **Keywords:** amyloid, Parkinson's disease, alpha-synuclein / **Supported by:** FAPERJ

K.62 - Disturbances of T cell phenotypic profile are observed in relapsing and non-relapsing patients with visceral leishmaniasis

Gabriela Corrêa e Castro [1,2], Maria Luciana Silva de Freitas[2], Ludmila de Paula[3], Maria Rita Teixeira Dutra[3], Leonardo Soares Pereira[3], Glaucia Cota[4], Alda Da Cruz[2], Joanna Reis Santos de Oliveira[1,2]
[1]Núcleo de Ciências Biomédicas Aplicadas, Programa Multicêntrico de Pós-Graduação em Bioquímica e Biologia Molecular, Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro (RJ, Brazil), [2]Instituto Oswaldo Cruz, Laboratório Interdisciplinar de Pesquisas Médicas - FIOCRUZ (Rio de Janeiro, Brazil), [3]Fundação Hospitalar do Estado de Minas Gerais, Hospital Eduardo de Menezes (Minas Gerais, Brazil), [4]CPqRR, Centro de Pesquisas René Rachou – FIOCRUZ (MG, Brazil)

INTRODUCTION: In Brazil, visceral leishmaniasis (VL) is caused by *Leishmania infantum*. Clinically, VL can vary from asymptomatic to severe form. Depletion of $CD4^+$T lymphocytes, polyclonal activation, microbial translocation and cytokine storm are involved in the VL immunopathogenesis, which can impair the response to the parasite. The factors that influence the different clinical outcomes concerning of remission/relapses are unclear. OBJECTIVES: Our aim was to evaluate the influence of activation/senescence/differentiation status of T cells on clinical outcome of VL regarding clinical remission/relapses. MATERIALS AND METHODS: Fifteen VL patients recruited from Hospital Eduardo de Menezes (BH-MG) were evaluated since the active phase until 12 months post-treatment (mpt). They were grouped as Relapsing (R-VL,n=5) and Non-Relapsing (NR-VL,n=10). Seven healthy controls (HC) were included. The percentage of naive, activated, senescent, central (T_{CM}) and effector (T_{EM}) memory T cells were evaluated by flow cytometry. DISCUSSION AND RESULTS: High levels of activated T cells were observed during active phase (p<0.05 compared to HC), although there was no significant difference between the groups. Interestingly, NR-VL reduced these levels on $CD8^+$T at post-treatment, while R-VL showed a decrease only at 12mpt. As expected, higher levels of senescent cells were verified in VL patients compared to HC throughout the follow-up. Regarding naive $CD4^+$T cells, R-VL remained with low percentages throughout follow-up, which can indicate an impairment in thymic output. In NR-VL, these percentages decreased at post-treatment, probably due to immune response to the parasite. Concomitantly, $CD4^+T_{EM}$ increased in NR-VL at post-treatment. At 12mpt, this group presented an augment in naive $CD4^+$T cells, while $CD4^+T_{EM}$ reached values close to HC. A decrease in $CD8^+T_{EM}$ was observed in R-VL group from 6mpt. Regarding $CD4^+$/$CD8^+T_{CM}$, higher levels were observed in R-VL compared to NR-VL at 12mpt. CONCLUSION: Our results suggest immunological disturbances among VL groups, especially an impairment of naive and T_{EM} compartments among R-VL patients, which could be related to relapses.
Keywords: *visceral leishmaniasis, relapses, T lymphocytes /* **Supported by:** FAPERJ, CNPq, IOC/FIOCRUZ, IFRJ

K.63 - Effect of zinc ions on the proliferation and differentiation of keratinocytes *in vitro*

Willian Moreira Miguel [1]
[1]Dep. de Biotecnologia, Escola de Artes, Ciências e Humanidades da Universidade de São Paulo (São Paulo, Brazil)

INTRODUCTION: The human skin is a stratified epithelium that consists of three layers: the epidermis, the dermis and the subcutaneous tissue. Epidermal cells, called keratinocytes, are constantly renewed, but pathological conditions, such as psoriasis, can affect the proliferation and differentiation of these cells. Several proteins participate in the proliferation and differentiation process of keratinocytes such as the epidermal kallikreins 5, 6 and 7 (KLK 5, 6 and 7). KLK7, an enzyme strongly inhibited by zinc (Zn2+) ions, also participates in the activation of pro-interleukin 1-beta, a cytokine involved in the inflammatory process observed in psoriasis. OBJECTIVES: The objective of this work was to study the effect of different concentrations of zinc ions on HaCaT cell line proliferation and death, as well as in the activity of epidermal kallikreins. MATERIALS AND METHODS: Cell death was evaluated by flow cytometry using propidium iodide. DISCUSSION AND RESULTS: Cell incubation with Zn2+ ions for 48 h, at all tested concentrations, did not result in changes in cell viability, suggesting that this ion is not cytotoxic. Using MTT assay, it was observed that the cultures grown in the presence of Zn2+ ions (1, 5, 10 and 25 µM) showed a decrease in the number of viable cells in comparison to the control for the same period. In relation to the KLK activity, cells cultured, for 1 day, in the presence of 25 µM Zn2+ ions displayed a decrease in KLK7 activity. In the presence of Zn2+ ions in the culture for 3 days, in general, there was an increase in the expression of involucrin, filaggrin, CK5, CK10 and CK14 proteins, evaluated by qPCR. CONCLUSION: Thus, this work opens perspectives for a better understanding the molecular events involved in both healthy epidermal differentiation and in the development of psoriasis, contributing for future therapeutic trials related to this disease based on the modulation of KLK activity.

Keywords: Keratinocyte, Kallikreins, Zinc

K.64 - Effect of Uncaria tomentosa Aqueous Extract on the Response to Palmitate- induced Lipotoxicity in Cultured Skeletal Muscle Cells

Bruna Leticia de Freitas [1], Jeniffer Farias dos Santos[1], Carla Roberta de Oliveira Carvalho[2], Viviane Abreu Nunes[1]
[1]Department of Biotechnology, School of Arts, Sciences and Humanities, University of Sao Paulo (SP, Brazil),
[2]Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of Sao Paulo (SP, Brazil)

INTRODUCTION: Type 2 diabetes mellitus (T2DM) is characterized by relative hypoinsulinemia and, frequently, associated with dyslipidemia, which corresponds to the increase in the triglycerides and fatty acids concentrations in tissues such as skeletal muscle, and to oxidative stress. The use of herbal medicines as Uncaria tomentosa (Ut) has been proposed as an auxiliary treatment for patients with T2DM, considering its possibility of overlapping conventional therapy. OBJECTIVES: The aim of this work was to evaluate the Ut aqueous extract effect on redox imbalance, oxidative stress and cell death induced by the free fatty acid (FFA) palmitate (PA), in skeletal myoblasts of C2C12 lineage. MATERIALS AND METHODS: Cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS), at 37°C humidified atmosphere and 5% CO2. The cells were incubated with PA in different concentrations, from 10 to 1000 µM, in the presence or absence of 250 µg/ml Ut aqueous extract, for 2, 6 or 24 h. The data obtained in the different assays were expressed as the mean ± standard error (SE), and the differences were evaluated by two-way ANOVA analysis, followed by the Bonferroni post-test using the software GraphPad8. DISCUSSION AND RESULTS: The cells treatment with Ut resulted in an increase of, at least, 50% cell viability in relation to the control. After these periods, oxidative stress was evaluated by fluorescence spectroscopy. The treatment of cells with Ut aqueous extract, for 6 h, followed by exposure to 500 µM PA, resulted in 38% less ROS formation than those incubated with only palmitate. CONCLUSION: In summary, the Ut aqueous extract promoted a raise in cell viability, reduced cell death and attenuated ROS formation in cultures incubated with 500 µM palmitate, which is known to be cytotoxic for muscle cells.

Keywords: Uncaria tomentosa, Palmitate, Reactive oxygen species / **Supported by:** FAPESP

K.65 - Project: Study of the Interaction of Hepatitis C Virus NS5A Protein with the GRB2 Protein

Manuel Bezerra de Menêses Neto [1], Luisa Hoffmann[1], Juliene Antonio Ramos[1]
[1]Genética Molecular, Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro (RJ, Brasil)

INTRODUCTION: It is estimated that hepatitis C virus (HCV) infection affects 58 million people worldwide. About 80% of those infected progress to chronic infection. After chronification, it is observed a natural progression of the disease characterized by a persistent inflammation, which generates tissue fibrosis, which can progress to liver cirrhosis and even to hepatocellular carcinoma (HCC). The HCV genome encodes 3 structural and 7 non-structural proteins. The non-structural protein NS5A is a phosphoprotein that has great importance for the replication, propagation, and pathogenesis of HCV. Several interactions of NS5A protein with human proteins have been described. One of these human proteins is the GRB2, which is an essential signal transducer for several basic cellular functions that acts as a critical intermediary in various pathways of oncogenic signaling. OBJECTIVES: Evaluate the interaction of the HCV NS5A protein with the human GRB2 protein and investigate its role in the natural evolution of the HCV infection. MATERIALS AND METHODS: The NS5A and GRB2 regions will be amplified through PCR which will later be confirmed by sequencing. Subsequently, these regions will be cloned into specific vectors, and the plasmids containing the NS5A and GRB2 regions will be transfected in Huh7.5 cells. Co-immunoprecipitation and two-hybrid assay techniques will be performed to evaluate the interaction of NS5A and GRB2 proteins. The role of GRB2 in viral replication will be evaluated in cells silenced for this protein. In addition, we will also evaluate the influence of this interaction on the PI3k-AKT pathway. DISCUSSION AND RESULTS: After a bibliographic review we are making the constructions of the vectors to confirm the interaction between the NS5A and GRB2 proteins. CONCLUSION: The interaction of NS5A with GRB2 may be an important pathway by which the HCV contributes to the development of HCC that is not yet understood.

Keywords: HCV-NS5A, GRB2, Hepatocarcinoma / **Supported by:** FAPERJ, IFRJ, CNPq and CAPES

K.66 - PROJECT: Evaluation of antifungal drug tolerance in Cryptococcus neoformans and Cryptococcus gattii

Mariana de Almeida Rosa Rezende [1], Gabriella Freitas Ferreira[1,2]
[1]Ciências Básicas da Vida, Programa Multicêntrico em Bioquímica e Biologia Molecular Universidade Federal de Juiz de Fora, campus Governador Valadares (MG, Brazil), [2]Pharmacy Department, Universidade Federal de Juiz de Fora campus Governador Valadares (MG, Brazil)

INTRODUCTION: The genus *Cryptococcus* is described with approximately 70 species, of which *Cryptococcus neoformans* and *Cryptococcus gattii* stand out as the most frequent causes of human and animal *cryptococcosis*. Pharmacological agents are limited. Treatment of microorganism (MOs) infections faces difficulties related to their adaptive capacity, capable of promoting tolerance mechanisms. Tolerance can be defined as when minimum inhibitory concentration (MIC) is the same as that of MOs susceptible to treatment, but their minimum killing time (MDT) to kill 99% of the microbial population (MDT99) is longer than in susceptible populations. Considering that adaptive mechanisms for tolerant behavior induce a false sense of cure, it is important to evaluate the tolerance mechanisms developed by *C. neoformans* and *C. gattii* strains. OBJECTIVES: Evaluate the tolerance profile of *Cryptococcus neoformans* and *Cryptococcus gattii* against antifungal drugs. MATERIALS AND METHODS: After tolerant strains identification, their mechanisms will be evaluated *in vitro*, through morphometric analysis, post antifungal effect, ergosterol quantification, zeta potential, EROS, lacase, melanization induction, phagocytosis induction, and ERG11 quantification. *In vivo* survival curve and determination of fungal burden of organs affected by *C. neoformans* and *C. gattii* will be evaluated. DISCUSSION AND RESULTS: At the end of the research, it is expected to be able to identify the profile of alterations in strains of *C. neoformans* and *C. gattii* by means of the methodology used, allowing future evaluations of the presence of these characteristics in infecting strains whose treatment has not proved resolutive.

Keywords: Tolerance, Cryptococcus, antifungal agentes / S**upported by:** CAPES

K.67 - Evaluation of Mir-215-5p and Mir-26b-5p as Biomarkers for Patients Chronically Infected with HCV.

Julio Cesar de Jesus Barbosa [1], Bianca Catarina Azeredo Cabral[2], Cristiane Alves Villela Nogueira[3], Rosane Silva[2], Luisa Hoffmann[1], Juliene Antonio Ramos[1]
[1]Laboratório de Genética Molecular, Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro (Rio de Janeiro, Brasil), [2]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil), [3]Serviço de Hepatologia, Hospital Universitário Clementino Fraga Filho (Rio de Janeiro, Brasil)

INTRODUCTION: Introduction: Hepatitis C virus (HCV) infection is one of the main causes of severe liver disease and most of those infected can develop chronic liver disease. Some patients will develop more complicated conditions such as cirrhosis and hepatocellular carcinoma. According to the World Health Organization, 58 million people are infected with the HCV, and at least 400,000 die each year. Hepatitis C treatment is based on direct acting antivirals and they are considered a revolution in treatment as they have shown over 90% of sustained response, short duration of treatment and minimal side effects. Viral factors such as resistance-associated variants may influence on therapeutic efficacy. Some host factors, such as microRNAs, have demonstrated their significant participation in the HCV life cycle. MicroRNAs are small non-coding RNAs that can act in the regulation of gene expression through base pairing. The altered expression of microRNAs is correlated with different pathological conditions in the human organism, so they are studied as potential biomarkers. Mir-215-5p showed increased expression levels in cirrhotic patients compared to patients with mild/moderate fibrosis while mir-26b-5p showed decreased expression levels. OBJECTIVES: Objectives: The objectives are to evaluate the expression of these microRNAs in patients chronically infected with HCV and to try to define a prognostic biomarker. MATERIALS AND METHODS: Materials and Methods: MicroRNAs will be extracted from serum samples and expression analysis will be by qRT-PCR. DISCUSSION AND RESULTS: Discussion and Results: We selected 48 patients with chronic hepatitis C, genotypes 1 and 3, and divided: mild/moderate fibrosis group (79,17%) and cirrhotic (20,83%). We expect to find promising results to try to standardize the expression profile of mir-215-5p and mir-26b-5p in patients chronically infected with HCV. CONCLUSION: Conclusions: The statistical analysis of these miRNAs and their interaction pathways, allows us to know the expression profile in different patients to define the appropriate treatment and obtain a better prognosis.
Keywords: MicroRNAs, HCV Infection, Biomarkers / **Supported by:** FAPERJ

K.68 - Molecular Mechanisms of FAM3B-Induced Breast Tumor Progression

Izabela Daniel Sardinha Caldeira[1], Patrícia Pereira Adriani[2], Viviane Abreu Nunes Cerqueira Dantas[1], Flavia Ramos Siqueira[1], José Ernesto Belizário[1], Humberto Miguel Garay Malpartida[1]
[1]EACH, Escola de Artes, Ciências e Humanidades (SP, Brasil), [2]Farmacologia, Instituto de Ciências Biomédicas (SP, Brasil)

INTRODUCTION: FAM3B/PANDER (Pancreatic-derived factor) is a secreted protein that was profoundly studied in different pathological conditions such as diabetes type II, metabolic syndrome as well in cancer progression. In breast, prostate, and esophagus cancer, FAM3B inhibits induced cell death, increases migration rates *In vitro* and *In vivo* promotes tumor growth, and induces metastasis, through epithelial-mesenchymal transition (EMT). Our previous results showed that FAM3B inhibits cell death and promotes metastasis in breast cancer cells, however, the molecular signaling in EMT-mediated tumor progression remains unclear. OBJECTIVES: Evaluation of FAM3B role in EMT and breast tumor progression. MATERIALS AND METHODS: MDA-MB-231 and MCF-7 cells were transfected with FAM3B-overexpressing plasmidial vector. Anchorage-independent growth and scratch wound assays were used to evaluate *In vitro* tumorigenicity and cell migration, respectively. Expression of genes involved in migration and in tumor invasion was assessed by real-time PCR and western blot at mRNA e protein levels, respectively. DISCUSSION AND RESULTS: When compared to control, cells overexpressing FAM3B displayed an increased anchorage-independent growth and increased cell migration rates, suggesting a putative role in tumor invasion. These results were accompanied by FAM3B promoting enhanced expression of genes involved in EMT and metastasis, such as Slug, Snail, TGF-β1, TGF-β2, TIMP-2, TGFBR-2, MMP-2 and MMP-9 at mRNA and protein levels. On the other hand, were no observed changes in Zeb-1, Zeb-2, Vimentin, Twist-1, TGFBR-1, E-cadherin, N-cadherin, and MMP-14 gene expression. CONCLUSION: Our results provide new insights into the role of FAM3B in the tumor progression and suggest that FAM3B may be a promising molecular target and diagnostic marker for breast cancer.
Keywords: FAM3B, Metastasis, Breast Tumor

K.69 - Temporal Effect of Cafeteria Diet on Cardiac Autonomic Modulation in Wistar Rats

Letícia São Julião Silva [1,2], Thais Marques Silva[1,2], Gleuber Henrique Marques Oliveira[1,2], Daniel Penteado Martins Dias[3], Valéria Ernestânia Chaves[1,2]
[1]UFSJ, Multicentric Program of Biochemistry and Molecular Biology (Minas Gerais, Brasil), [2]Campus Dona Lindu, University of São João Del-Rei (Minas Gerais, Brasil), [3]Medicina, Barão de Mauá University (São Paulo, Brasil)

INTRODUCTION: Hypercaloric diets contribute to the development of cardiovascular diseases and metabolic syndrome. Evidence shows that autonomic imbalance plays an important role in the pathogenesis of several cardiovascular diseases, increasing the risk of cardiac death. OBJECTIVES: To investigate the temporal effect of cafeteria diet on heart rate variability (HRV) using linear and non-linear methods. MATERIALS AND METHODS: Cafeteria diet group was fed a hypercaloric diet and control group was fed a balanced diet. Electrocardiogram was recorded at a 6-day interval, for 24 days. Through the records it was possible to evaluate the time domain (SDNN and RMSSD), frequency domain [Heart rate, low frequency (LF) and high frequency (HF)], multiscale entropy and symbolic dynamics. Adipose tissues were collected on the 24th day of the experiment after euthanasia. The results were analyzed by the t-Student test (P< 0.05). DISCUSSION AND RESULTS: The 24-day cafeteria diet increased the weight of retroperitoneal and epididymal adipose tissue. Cafeteria diet feeding for 6 days did not induce changes in HRV, however from the 12th day onwards, the animals showed an increase in the LF band with no change in the HF and time domain. There was an increase in the 0V and 2UV families from the 12th day onwards, while the multiscale entropy only increased on the 12th day of recording. The increase in the LF band and 0V family is indicative of an increase in sympathetic activity, while the decrease in the 2UV family suggests a decrease in parasympathetic activity. The changes seen in multiscale entropy are consistent with atrial fibrillation. CONCLUSION: Based on the results, we suggest that the cafeteria diet rats present an increased risk for developing cardiovascular diseases.
Keywords: Cafeteria diet, Heart rate variability, Autonomic nervous system
Supported by: CNPq, UFSJ

K.70 - Ring-shaped Neutrophils associated with echocardiographic parameters in patients with Chaga's disease

Amanda Estevam Carvalho [1], Valéria Duarte de Almeida [1], Gilson Aquino Cavalcante[1], Fernando Liberalino Fernandes[1], Luann Gabriel Porpino Sales[1], Pedro Afonso Oliveira Dias Rêgo[1], Micássio Fernandes de Andrade[1]
[1]Faculdade de Ciências da Saúde, Universidade do Esteado do Rio Grande do Norte (Rio Grande do Norte, Mossoró)

INTRODUCTION: Trypanosomiasis it's a neglected disease that affects about 6 to 7 million people worldwide. The cardiac form is the main cause of death and serious symptomatology in those patients. Immune cells are involved in the clinical evolution and prognoses of de chronic stages. Neutrophils are, between other functions, responsible in mediating chronic inflammation. Those cells can present a distinct nuclear morphology, with a ring-shaped nucleus. Ring-shaped Neutrophil's (RSN) have already been associated with some chronic pathologies such as cancers and myelodysplastic syndromes. There are no studies associating those cells with Chagas disease. OBJECTIVES: Correlate the presence of RSN with echocardiographic data from patients with Chaga's disease MATERIALS AND METHODS: 11 patients with indeterminate clinic form and 21 with chronic cardiac form where included, totalizing 32 patients. Morphological evaluation and differential count were executed by optical microscopy in blood smears. For correlation results, Spearman and Pearson tests were made between the median of RSN and echocardiographic data, such as left ventricular systolic diameter (LVSD), left ventricular diastolic diameter (LVDD), left ventricular ejection fraction (LVEF), Left Atrial Diameter (LAD), Cardiothoracic Index (CI) and left ventricular mass index (LVMI). DISCUSSION AND RESULTS: Negative correlation were observed between RSN and LVEF (*p=0,0031 e r= -0,7087). Otherwise, positive correlation between RSN and LVSD (*p= 0,0009 e r= 0,8040), LVDD (*p= 0,0250 e r= 0,5942), and LVMI (*p= 0,0351 e r= 0,5655). No correlation was found with LAD (p= 0,3857 e r= 0,2515) nor CI (p= 0,5441 e r=0,1948). CONCLUSION: RSN behaved directly proportional to the dilation degree of the cardiac chambers, expressed by the increase in the LVDD, LVSD and LVMI, and the decrease in the LVEF, which translate the heart failure syndrome. This shows the potential of these cells as a possible biomarker of clinical evolution to Chaga's disease.
Keywords: Polymorphonuclear, Ring-shaped neutrophil, American trypanosomiasis / **Supported by:** Capes

K.71 - Comparison Between Structural Region of Hepatitis C Virus Genotypes 1 and 3 and Its Clinical Implications

Maurício Tavares de Melo[1], Juliene Antonio Ramos[1], Bianca Catarina Azeredo Cabral [2], Cristiane Alves Villela Nogueira[3], Rosane Silva[2], Luísa Hoffmann[1]

[1]Laboratório de Genética Molecular, Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro (Rio de Janeiro, Brasil), [2]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil), [3]Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: **Introduction:** About 58 million people are infected with the hepatitis C virus (HCV) worldwide, which can cause various complications such as cirrhosis and hepatocellular carcinoma. The HCV has a single-stranded RNA genome that can be translated into structural and non-structural proteins. Currently, there are around 90 subtypes of the virus and 8 genotypes. Due to its high genetic diversity, there is no vaccine for the disease. The structural region of the HCV has been studied as a potential target for the development of diagnostic kits and vaccines. OBJECTIVES: The objective is to comparatively analyze the genetic diversity of the structural region (core, E1 and E2) of HCV genotypes 1a, 1b and 3a in patients with chronic hepatitis C with different clinical and laboratory characteristics. MATERIALS AND METHODS: For the study of twenty patients with chronic hepatitis C, the HCV whole genome was amplified using a customized panel (ThermoFisher) and a massive parallel Ion Torrent sequencing was performed. The data was analyzed using the CLC Genomics Workbench v.22.0.1 software and the structural regions were compared to HCV reference sequences. The differences were then evaluated, and the low frequency variants (≥2%) were detected. DISCUSSION AND RESULTS: Initially, we analyzed six samples of genotype 3a for the presence of amino acid exchanges. The core region demonstrated better coverage considering all samples. Hence, to improve the coverage and reading depth from all analyzed regions, the samples were resequenced. Therefore, we observed a greater number of non-synonymous amino acid exchanges and comparative tables from patients considered responders and non-responders to antiviral treatment were constructed. Also, it was found that the E2 region had more amino acid exchanges than core and E1. CONCLUSION: Studying the structural region of HCV is essential to better comprehension of genotypes differences and to assist in the development of effective vaccines.

Keywords: bioinformatics, biotechnology, hepatitis C / **Supported by:** IFRJ, CNPq, FAPERJ, CAPES

K.72 - PROJECT: Effects of Lavender and Sweet Orange Essential Oils on a Model of Postpartum Depression in Wistar Rats

Sílvia Ramos Silva[1], Cristiane Queixa Tilelli[1]

[1]Laboratório de Fisiologia Animal, Universidade Federal de São João Del Rei - Campus Centro-Oeste (Minas Gerais, Brasil)

INTRODUCTION: Postpartum Depression (PPD) is a mental disorder associated with stress. In Brazil, 26.3% of women in the puerperal period are affected by PPD. Essential oil (EO) is any group of natural active ingredients produced by plants. There is evidence that Lavender (*Lavandula augustifolia*) EO and Sweet Orange (*Citrus sinensis*) EO have antidepressant and anxiolytic effects. The therapeutic use of EOs is known as “aromatherapy”, a low-cost, easy-to-apply alternative therapy. Thus, aromatherapy can be an adjunct therapy to drugs already used to control PPD. Synthetic essences (SE) are mixtures of petroleum derivatives and components of the original EOs, with different production processes and complexity. An animal model for PPD uses Maternal Separation (MS), in which dams are daily separated from pups for 3 hours. OBJECTIVES: To investigate the possible anxiolytic effects of Lavender and Sweet Orange EOs and their respective SE against MS in dams through behavioral and biochemical tests of stress, anxiety and depression, comparing the results between EO, SE and saline exposition during the separation period. MATERIALS AND METHODS: Puerperal rats will be daily separated from their offspring from DPN2 to DPN14 and exposed to EO or SE during the 3 hours of separation, while control rats will be exposed to saline. A non-separated, non-exposed (naïve) group will also be conducted. Mothers will undergo behavioral tests 1 week after weaning. Open Field Test and Elevated Plus Maze Test: assess anxiety; Forced Swimming Test: assesses depression. Then, the animal will be euthanized to remove the blood for evaluation of Corticosterone, a blood serum indicator of stress. DISCUSSION AND RESULTS: Reduction in the pattern of anxiety and depression of the rats treated with OE in relation to the control; difference between the effects of EOs and SEs on behavioral and biochemical parameters. CONCLUSION

Keywords: Postpartum Depression, Essential Oil, Anxiety

L - Mechanobiology

L.01 - Chikungunya Virus: A Neglected Threat Looked Through A Physical Perspective

Ruana Cardoso-Lima [1], Maria Luiza A. Dorneles[1], Ralph Santos-Oliveira[2,3], Luciana Magalhães Rebelo Alencar[1]

[1]Laboraratory of Biophysics and Nanosystems, Physics Department, Federal University of Maranhão (MA, Brasil), [2]Nuclear Engineering Institute, Brazilian Nuclear Energy Commission (RJ, Brasil), [3]Laboratory of Nanoradiopharmacy, Rio de Janeiro State University (RJ, Brasil)

INTRODUCTION: The Chikungunya Virus (CHIKV) is an alphavirus with an RNA genome transmitted mostly through the *Aedes aegypti* mosquito. CHIKV was first isolated in 1952 and since then has been responsible for several epidemic outbreaks, especially in tropical areas. Still, not much is known regarding its physical mechanisms. This work aims to investigate the gaps surrounding the CHIKV, having physical virology as the main path. OBJECTIVES: Nanomechanical assays have been used as instrument to underline insights into the ultrastructure of viruses, and aid the development of treatments and prevention based on structural and mechanical intricacies. The goal of this study is to apply a physical approach to increase the knowledge of CHIKV's ultrastructural morphology and mechanical properties having the AFM as the main tool. MATERIALS AND METHODS: Force−distance curves were collected using probes with 0.4 N⁄m spring constant in Peak Force Quantitative Nanomechanics (QNM) mode on a Multimode 8 (Bruker). Structural analysis was performed in air. For the indentation analysis, the measurements were done on QNM Ramp Mode, and force curves were obtained on the top-center of each virion analyzed, with a force setpoint of 1 nN and tip velocity of 100 nm⁄s. DISCUSSION AND RESULTS: This study investigates the plasticity, morphology, and mechanical properties of CHIKV providing new information on its ultrastructure at nanoscale, offering an understanding of the viruses' behavior upon mechanical disruptions. The high-resolution topographic maps revealed the structures of the viral surface and its protein distribution, with rupture events revealing the mechanical response of the virus against applied loads. CONCLUSION: Currently, there are no therapies or commercial vaccines available against CHIKV. The results presented here add new data in the characterization of the physical and structural properties of CHIKV, corroborating models shown in the literature and providing new insights that can be useful in designing strategies to fight this infectious agent.

Keywords: Chikungunya, Nanomechanical Properties, Physical Virology

M - Microorganisms and Pathogens

M.01 - Malaria-induced acute kidney injury in BALB/C mice is associated with progressive glomerular dysfunction

Pedro Carneiro Abrahão de Souza [1], André Luiz Ferreira de alencar[1], Douglas Esteves Teixeira[1], Sarah Aparecida dos Santos Alves[1], Ana Acacia de Sá Pinheiro[1], Celso Caruso Neves[1,2,3]
[1]Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro (RJ, Brasil), [2]FAPERJ, Rio de Janeiro Innovation Network in Nanosystems for Health (RJ, Brasil), [3]INCT, National Institute of Science and Technology of Regenerative Medicine (RJ, Brasil)

INTRODUCTION: Malaria is a disease caused by infection with *Plasmodium* spp. and represents an important global health problem. Malaria-induced acute kidney injury (MAKI) is one of the main consequences in malaria patients being associated to death. However, the mechanisms underlying kidney impairment caused by malaria are still unclear. OBJECTIVES: We aimed to investigate the mechanisms involved in the genesis of renal damage induced by malaria. MATERIALS AND METHODS: BALB/C mice ageing 6-7 weeks-old were infected with *Plasmodium berghei* ANKA (CEUA008/18). Mice were allocated in metabolic cages throughout the experiment to collect blood and urine for analysis of renal function and parasitemia. DISCUSSION AND RESULTS: Infected animals showed parasitemia of 9% at the 5th day post-infection (p.i.), which progressively increased to 50% at day 15 and 100 at day 20. On the day 15, we observed a 60% mortality rate which progressed to 100% by day 20 p.i. Urinary flow, proteinuria (mg/24h), urinary proteinuria:creatinine ratio (UPCr) and proximal tubule injury marker urinary γ-glutamil transferase activity (γ-GT) were not changed in infected mice along the course of the infection. However, on day 12 p.i., increased plasma creatinine (2.3-fold), blood urea nitrogen (2.9-fold) and decreased in creatinine clearance (2.7-fold) were observed. We observed a positive correlation between parasitemia and plasma creatinine ($\rho = 0.62$,$p = 0.0015$) or BUN ($p = 0.81$, $p < 0.0001$) and an inverse correlation between parasitemia and creatinine clearance ($\rho = 0.62$,$p = 0.0040$). However, parasitemia was not correlated with proteinuria ($\rho = 0.4365$), UPCr ($\rho = 0.6681$) and γ-GT activity ($\rho = 0.8289$). CONCLUSION: Our data indicate that kidney damage caused by malaria infection involves a selective glomerular injury. These findings open new perspectives for understanding the genesis of MAKI.
Keywords: glomerular injury , kidney injury, malária / **Supported by:** Faperj, CNPq, Capes

M.02 - POSTTRANSSCRIPTIONAL MODIFICATIONS IN tRNAs FROM Trypanosoma brucei

Nathalia Silva de Lima [1], Luis Felipe Silva de Lima[1], Carla Polycarpo[1]
[1]Instituto de Bioquímica Médica, Univ. Federal do Rio de Janeiro, Instituto de Bioquímica Médica (Rio de Janeiro, Brasil)

INTRODUCTION: In trypanosomatids there is little evidence of gene expression control through the regulation of mRNA transcription, which is mainly done by post-transcriptional control. In other organisms, mechanisms of regulation of gene expression where tRNAs act as effectors have been reported. OBJECTIVES: We intend to investigate whether post-transcriptional modifications in tRNAs from *Trypanosoma brucei* nucleotides may be related to this event. MATERIALS AND METHODS: We grew procyclic and blood *T. brucei* cells, extracted the total RNA, purified the tRNAs and digested them with benzonase, releasing the nucleotides that were then dephosphorylated. Nucleosides were analyzed by LC-MS. DISCUSSION AND RESULTS: Our preliminary results show that there are important variations in modified bases between procyclic and blood *T. brucei*, with the largest variations observed for acp3U 3-(3-amino-3-carboxypropyl)uridine) and Gm (2'-O-methylguanosine) which are found in higher concentration in blood *T. brucei*, and D (Dihydrouridine), Y (pseudouridine), m2,2G (N2,N2-dimethylguanosine) AND m6A (N6-methyladenosine), which are present in higher concentration in *T.brucei* procyclics. The modification most found in both forms was pseudouridine (an average of 25% in both). CONCLUSION: While modifications present in one form usually is present in the other, there are quantity variations and it will be worthy to investigate if they make a difference during the metacyclogenesis process.
Keywords: Trypanosoma, tRNA, base modification
Supported by: FAPERJ, CNPq

M.03 - Influence of Growth and Purification Parameters in the Proteomic Content of Propionibacterium freudenreichii–Derived Extracellular Vesicles

Vinícius de Rezende Rodovalho[1,2], Brenda Silva Rosa da Luz[1,2], Julien Jardin[2], Valérie Briard-Bion[2], Gwenaël Jan[2], Yves Le Loir[2], Vasco Ariston de Carvalho Azevedo[1], Eric Guedon[2]
[1]Laboratório de Genética Celular e Molecular, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (Minas Gerais, Brazil), [2]Science et Technologie du Lait et de l'Oeuf, Agrocampus Ouest, Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (Rennes, France)

INTRODUCTION: *Propionibacterium freudenreichii* is a Gram-positive, probiotic bacterium with technological importance in the production of cheese, vitamin B12 and organic acids. It also presents health-promoting properties, associated to secreted metabolites, proteins and extracellular vesicles (EVs). EVs are spherical nanostructures delimited by a lipid bilayer and implicated in the transport of molecules. We previously demonstrated that EVs purified from *P. freudenreichii* cultures in milk ultrafiltrate (UF) by size-exclusion chromatography (SEC) contained immunomodulatory proteins and exerted anti-inflammatory roles *in vitro*. OBJECTIVES: The objective of this study was to evaluate whether the properties and content of EVs would vary in response to different environmental parameters, including growth media (UF and yeast extract-lactate – YEL) and purification methods (SEC and density gradient ultracentrifugation – UC). MATERIALS AND METHODS: Bacterial cultures in UF and YEL were centrifuged (6000 g, 15 min), filtered (0.22 µm top filters, Thermo Scientific) and concentrated (Amicon ultrafiltration units, 100 KDa). EVs were then recovered by SEC (qEV original, 70 nm; iZON) or UC (8-68% sucrose gradient). EVs morphology was evaluated with transmission electron microscopy, whereas size distribution and concentration were characterized with nanoparticle tracking analysis. Shotgun proteomics was performed by NanoLC-ESI-MS/MS. DISCUSSION AND RESULTS: Microscopic and size characterization confirmed the purification of typical EVs in all tested conditions. Cell culture assays showed that EVs exerted a medium-dependent anti-inflammatory activity via the reduction of NF-κB activation and IL-8 release. Shotgun proteomics allowed the identification of a diverse, condition-dependent set of proteins. The core proteome comprising 308 proteins was functionally enriched in energy and carbon metabolism, ribosomal structure and biogenesis, quorum sensing, protein export and peptidoglycan biosynthesis. CONCLUSION: Overall, we showed that EVs properties depend on the culture medium and purification method. This reinforces the importance of parameters optimization in the modulation of EVs potential therapeutic properties for the development of molecular delivery systems.

Keywords: proteomics, bacteria, vesicles / **Supported by:** CAPES (BR), INRAE (FR), Agrocampus Ouest (FR).

M.04 - Production of Bacterial Strains for Structural Studies of the Type III Secretion System

Clara Presotto Tavares [1], Germán Gustavo Sgro [1]
[1]Departamento de Ciências Biomoleculares , Faculdade de Ciências Farmacêuticas de Ribeirão Preto - Universidade de São Paulo (São Paulo , Brasil)

INTRODUCTION: Citrus canker disease is a major phytosanitary issue in citrus production, caused by *Xanthomonas citri* (Xac). Current control strategies are used with relative effectiveness, such as copper salts and other chemicals with inhibitory action on bacterial growth. These approaches have a negative impact on land use, as they heavily contaminate cultivated soils. Therefore, there is a need to seek alternative antimicrobial strategies capable of selectively target the species of interest, thus reducing environmental impacts. The infection by Xac depends on the bacterial Type 3 secretion system (T3SS), a membrane-embedded protein complex formed by more than 20 different subunits, responsible for the translocation of a variety of effectors. To date, the structure and functioning mechanism of phytopathogenic T3SSs are poorly known and understood. OBJECTIVES: We aim the production of several Xac strains for the overexpression and subsequent purification of its T3SS, to study and better understand the structure and functioning. MATERIALS AND METHODS: Molecular biology techniques were used to create a series of pNPTS vectors for inserting a StrepTag at the N- or C-terminus of HrcC, HrcD, HrcJ and HrcQ, four structural components of the T3SS from Xac. The vectors were introduced into Xac cells in order to produce different strains, each expressing a modified version of these proteins fused to a StrepTag. Clones were confirmed by DNA sequencing. Western Blot assays were performed to evaluate the expression level of the proteins under different conditions. DISCUSSION AND RESULTS: The target proteins were chosen for their localization in the T3SS apparatus, and StrepTag to follow their expression and to subsequently purify them. Our results indicate that we have successfully obtained the modified strains. CONCLUSION: The modification of the selected genes was successfully achieved in Xac. We present a summary of the results obtained, which will improve our knowledge about this T3SS.

Keywords: Citrus canker , Type 3 secretion system, Xanthomonas citri

M.05 - Could inositol hexakisphosphate kinase (IP6K) be a potential target for drug development against Chagas disease?

Marcelo Santos da Silva [1,4], Bryan Etindi Abuchery[1], Arthur de Oliveira Passos[1], Maria Cristina Machado Motta[2], Simone Guedes Calderano[3]
[1]Ciências Químicas e Biológicas, Universidade Estadual Paulista "Júlio de Mesquita Filho" (São Paulo, Brasil), [2]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil), [3]Laboratório de Ciclo Celular, Instituto Butantan (São Paulo, Brasil), [4]Bioquímica, Universidade de São Paulo (São Paulo, Brazil)

INTRODUCTION: Inositol pyrophosphates (PP-IPs) – mainly IP7, and IP8 – are involved in a wide range of processes in eukaryotes. However, the mechanism of action of PP-IPs is not yet fully understood. IP7 and IP8 are synthesized by pathways involving the participation of IP6K and PP-IP5K kinases, respectively. Trypanosomatids (single-celled eukaryotes parasites) have an ortholog gene for IP6K, but our analyses suggest that these organisms lost orthologs for PP-IP5K in the evolutionary course. OBJECTIVES: Our goal is to investigate the role of IP6K in metabolism of *Trypanosoma cuzi* (the causative agent of Chagas Disease). MATERIALS AND METHODS: Using the CRISPR/Cas9 approach and two rounds of 'sgRNA' transfection, we disrupted the single and double alleles of IP6K in *T. cruzi*, generating IP6K-/+ and IP6K-/- lineages, respectively. DISCUSSION AND RESULTS: IP6K inactivation causes several morphological effects in both lineages, such as rounding and wrinkling of the cell body, increased number of glycosomes, and mitochondrial enlargement. Notably, IP6K-/- lineage was unable to proliferate, and most *T. cruzi* cells died a few days after transfection, suggesting IP6K is essential to this organism. Curiously, IP6K-/+ lineage showed a slight cell cycle arrest at G0/G1 phase. However, the arrested population showed no DNA damage. Then, after estimate the cell cycle phases length and the doubling-time, we developed a pioneering assay to measure quiescent cells based on negative EdU (5-Ethynyl-2'-deoxyuridine) labeling. The result of this assay suggests that the presence of IP6K is important to keep *T. cruzi* committed with the cell cycle. CONCLUSION: Together, our preliminary data suggest that the loss of IP6K has harmful consequences for *T. cruzi*, which points this kinase as a potential target for drug development, given that its identity relative to its human homolog is ~ 15%. Furthermore, these findings can contribute to a better understanding of the pyrophosphorylation performed by IP7, an apparent non-enzymatic post-translational modification still little studied.

Keywords: Chagas disease, Inositol pyrophosphates, Quiescence / **Supported by:** FAPESP

M.06 - Ultrastructural characterization of the cell wall of microorganisms subjected to the attack of a cell penetrating peptide

Louise Eloa Araujo Souza[1], Emerson Rodrigo Da Silva[1,1]
[1]BIOFÍSICA , Universidade Federal De São Paulo (SÃO PAULO, BRAZIL)

INTRODUCTION: The pentapeptide RLRWR belongs to the group of the shortest cell penetrating peptides (CPPs) reported in literature. It also holds a tripeptide recently identified to have antimicrobial capabilities.[1] Although the interaction of CPPs with mammalian cells has been intensively researched, much less effort has been dedicated to the effects of CPPs on microorganisms. Herein, we study the action of RLRWR against the cell wall of model microorganisms. We investigate the structure of this peptide under various physicochemical conditions and provide high-resolution data on their attack against the wall of prokaryotic cells Escherichia coli, Pseudomonas fluorescencis and Bacillus subtilis. As a model eukaryotic microbe, we use the yeast Saccharomyces cerevisae OBJECTIVES: To investigate the topography and the infrared signature of cell walls of microbes subjected to antimicrobial attack by RLRWR MATERIALS AND METHODS: Microbes were cultivated in nutrient medium using standard techniques. Minimal inhibitory concentrations (MIC) and growth kinetic rates were determined by measuring the optical density of colonies incubated with different peptide concentrations. Atomic force microscopy combined with infrared spectroscopy (AFM-IR) was used to investigate both the topography and the local spectral signature of colonies fixed on appropriate substrates. DISCUSSION AND RESULTS: MICs were found at the interval 0.1-1 mg/ml, with a clear selectivity of the peptide against the Gram-positive B. subtilis species. AFM-IR data indicated the formation of peptide clusters at the cell walls of bacteria. The formation of pores was observed across the cells, with a predominance of these features in the Gram-positive organism. The spectral signature suggests that the clusters hold β-sheet enriched domains, endowed with amyloidogenic characteristics. CONCLUSION: the peptide showed stronger activity against B. subtilis cells, correlating with the formation of β-sheet enriched clusters at the cell wall surface, and suggesting that the interaction between RLRWR and the peptidoglycan layer is relevant for lysis.

Keywords: antimicrobial peptides, cell imaging, microbiology

M.07 - Microbial diversity soil-associated in two Syrah-producing farms of the São Paulo State

Graziela Silva Rezende [1], Emeline Boni Campanini[1], Marcelo Mendes Brandão [2], Iran Malavazi [1], Anderson Ferreira da Cunha [1]

[1]Departamento de Genética e Evolução, Universidade Federal de São Carlos (SP, Brasil), [2]Centro de Biologia Molecular e Engenharia Genética, Universidade Estadual de Campinas (SP, Brasil)

INTRODUCTION: The grape is a fruit that has been used for centuries for different purposes, whether for fresh consumption or for the production of different products. The quality of the fruit is of paramount importance in this market and is directly related to the conditions of cultivation and development of the plant, and the association between the plant and microorganisms is an alternative to improve the quality of the fruit and which has been extensively studied with the advent of metagenomics technology. Despite this, the microbiota associated with vines in Brazil needs further studies. OBJECTIVES: To analyze and compare the microbial diversity in the soil of two farms producing the Syrah grape variety MATERIALS AND METHODS: Soil samples were collected in two different farms: (i) Fazenda Terrassos (FT) and (ii) Fazenda Portal da Luz (FPL), located in the cities of Amparo and São Bento do Sapucaí in the state of São Paulo. Bacterial microbiota analyzes were performed by sequencing the V3-V4 region of the 16S rRNA gene (metabarcoding) and compared to nutrient analyses. DISCUSSION AND RESULTS: Soil analysis showed that the pH at both farms is acidic, ranging between 5.5 (FT) and 6.3 (FPL) with the highest amount of nutrients in the FPL. Consistent with these results, we found the Phylum Proteobacteria and Acid Bacteria (FPL-31e 27% and FT 33 and 24%) as the most abundant, with emphasis on the genera of the phylum Acidbacteria Gp4 (FPL-3.4% and ST-1.8%), Gp6 (FPL-4.8% and ST-3.12%), Gp7 (FPL-0.8% and ST-0.2%), and Gp16 (FLP-0.8% and ST-0.46%) and Phylum Proteobacteria the genus Bradyrhizobium (FLP-0.8% and ST-1.4%), significantly different between farms and directly related to fertility and soil quality. CONCLUSION: The results showed an important distribution of bacteria related to fertility with the nutrients found, evidencing their application as soil quality markers.

Keywords: Soil, Bacteria , Nutrients / **Supported by:** CAPES

M.08 - Identification of Human Papillomavirus (HPV) Oncogenic Genotypes in women with High-grade Cervical Intraepithelial Neoplasia and Human Immunodeficiency Virus (HIV)

Alessandra Silva e Silva[1], Luise Ramos Nobre[2,1], Priscila Ferreira de Aquino[1]

[1]Pesquisa, Leônidas & Maria Deane Institute, Oswaldo Cruz Foundation (Amazonas, Brasil), [2]Ensino, University Center of the North (Amazonas, Brasil)

INTRODUCTION: Cervical cancer (CC) is the most common cancer in the women population of the Amazonas state, representing a serious public health problem. Usually, this disease is related to the progression of cervical intraepithelial neoplasia (CIN), graded from I to III, being the stages II and III the high-grade lesion. The human papillomavirus (HPV) is the main etiological agent of CIN and CC. Another risk factor is the co-infection with the human immunodeficiency virus (HIV), where HIV+ patients have higher chances of developing this neoplasm. Thus, diagnosis using molecular techniques may improve the understanding of these co-infections, including the possible association with CIN. OBJECTIVES: To evaluate the prevalence of oncogenic HPV genotypes in HIV+ women diagnosed with high-grade cervical intraepithelial neoplasia. MATERIALS AND METHODS: This work has ethical approval under the number CAAE n. 39556220.2.0000.0004. First, it was collected tissues and blood samples from seropositive patients diagnosed with high-grade CIN. In addition, the patients were interviewed for sociodemographic and clinical data evaluation. Later, it was performed a molecular analysis through polymerase chain reaction (PCR) to detect HPV and its oncogenic subtypes (16 and 18). DISCUSSION AND RESULTS: In our cohort, we analyzed 8 patients and 62.5% were positive for the HPV16 oncogenic genotype infection, exclusively in cervical tissue. The positive women for this genotype were older than 30 years, had started sex in adolescence, and all (100%) were using some contraceptive method. Thus, women in the study were more affected by HPV16 and tissue samples were better to detect the virus. CONCLUSION: Therefore, the obtained data may help the comprehension, at the molecular level, of the regional context associated with the profile of patients infected by these two pathogens (HIV and HPV), especially in women diagnosed with high-grade cervical lesions.

Keywords: Cervical Intraepithelial Neoplasia, HPV, HIV

M.09 - Bacterial Two-Hybrid Assay for the Determination of Protein-Protein Interactions during Bacteriophage Infection

Verena Ariak Vieira Seabra [1], Chuck Shaker Farah[2], Germán Gustavo Sgro[1]
[1]Departamento de Ciências BioMoleculares, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo (SP, Brazil), [2]Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo (SP, Brazil)

INTRODUCTION: The Type IV pilus (T4P) from *Xanthomonas citri* (Xac) is a thin, flexible and resistant surface filament that is involved in different bacterial behaviors, including twitching motility, adhesion and biofilm formation. Previous studies have shown that the infection of Xac by the bacteriophage ΦXacm4-11 (Podoviridae) is T4P-dependent. However, it is not yet known which proteins from ΦXacm4-11 interact with the T4P of Xac, and which is the mechanism of recognition and injection of the phage genome during the infection. OBJECTIVES: We aim to identify possible protein-protein interactions involved in the recognition process between ΦXacm4-11 and Xac, through the use of two-hybrid assay in bacteria (BACTH). MATERIALS AND METHODS: The genomic DNA of Xac and ΦXacm4-11 were isolated using standard DNA extraction protocols. PCR and cloning were performed in the corresponding BACTH system vectors using case-specific restriction sites. The selected BACTH system is based on the reconstitution of the signal transduction pathway of cAMP in *E. coli*, using the catalytic domains of the enzyme adenylate cyclase (AC), that can be easily monitored. DISCUSSION AND RESULTS: Based on the recently determined cryoEM structure and sequenced genome of ΦXacm4-11, we decided to test several proteins that constitute both its tail and capsid. On the side of Xac, we selected a list of different pilins that can form its T4P, including major and minor ones. So far, the results indicate that we successfully cloned the selected genes into the corresponding vectors, and now we are ready to start with the two-hybrid assays. One-on-one combinations will be tested with the intention of finding an interaction hit. CONCLUSION: The cloning of the selected genes was successfully achieved, as confirmed by sequencing. Here, we present a summary of the results obtained, which will improve our understanding of the phage-mediated infective process.

Keywords: Bacteriophage, BACTH, Xanthomonas citri / **Supported by:** FAPESP

M.10 - The RNA-Binding Protein RBP42 from *Trypanosoma cruzi* Aggregates into Cytoplasmic Foci in Response to Cellular Stress Induced by Gamma Radiation

Daniela de Laet Souza [1], Daniela Ferreira Chame[1], Helaine Graziele Santos Vieira[2], Erich Birelli Tahara[1], Andrea Mara Macedo[1], Carlos Renato Machado[1], Glória Regina Franco[1]
[1]Bioquímica e Imunologia , Universidade Federal de Minas Gerais (Minas Gerais, Brasil), [2]Garvan Institute of Medical Research, Garvan Institute of Medical Research (Sydney, Australia)

INTRODUCTION: The parasite *Trypanosoma cruzi* is highly resistant to gamma radiation stress. The mechanisms underlying this resistance are still unknown, but RNA binding proteins may play essential roles in stress response by aggregating into cytoplasmic granules. OBJECTIVES: In this work, we studied the protein RBP42 in the context of gamma radiation stress, which causes an increase in its transcript levels. MATERIALS AND METHODS: To study this protein, we generated epimastigotes expressing 6xHis-tagged versions of TcRBP42 and GFP (control) and treated them with 500 Gy of gamma radiation. DISCUSSION AND RESULTS: By evaluating parasite growth in both normal and treated conditions, it was shown that the expression of the tagged proteins did not modify epimastigote proliferation in comparison to wild-type parasites. In all epimastigote lines tested, the treatment caused a similarly prolonged growth arrest that lasted for at least 144 hours. By performing immunofluorescence assays, we determined the gamma radiation effect on TcRBP42 protein localization. In normal conditions, TcRBP42 was detected distributed throughout the whole parasite cytoplasm. In contrast, the exposure to gamma radiation caused the accumulation of TcRBP42 into cytoplasmic foci at both 24 and 48 hours post parasite irradiation. Concerning our GFP control, the protein remained distributed throughout the parasite cytoplasm in all tested conditions. We also investigated if RNAs synthesized after stress induction took part in the TcRBP42 foci by labeling newly-synthesized RNAs. Under normal conditions, the newly-synthesized RNAs are concentrated in the nucleus and kinetoplast. In contrast, 24 hours after parasite irradiation, the newly-synthesized RNAs were distributed diffusely in the cytoplasm, not being detected 48 hours after stress induction. Additionally, the newly-synthesized RNAs and TcRBP42 foci did not colocalize. CONCLUSION: In conclusion, the protein TcRBP42 presented a differential distribution according to cellular conditions, accumulating into cytoplasmic foci in response to gamma radiation stress, suggesting that TcRBP42 may play some role in the ionizing radiation stress response.

Keywords: RNA binding protein, *Trypanosoma cruzi*, Gamma radiation stress / **Supported by:** CAPES, CNPq e FAPEMIG

M.11 - Characterization of new Picornaviruses and the antiviral defense in the Chagas disease vector *Rhodnius prolixus*

ingrid Alexandre de Abreu Brito [1], maira arruda cardoso[1], Tracísio Fontenele de Brito[1], Attilio Pane[1]
[1]Instituto de ciências biomédicas, Universidade Federal do Rio de Janeiro (Rio de janeiro, Brasil)

INTRODUCTION: *Rhodnius prolixus* is a triatomine insect and one of the main vectors of the protozoan *Trypanosoma cruzi*, the etiologic agent of Chagas disease. Despite the great medical relevance, little is understood about the *Rhodnius* virome. Until 2021, only the Triatoma virus had been identified and characterized in triatomines. Recently, we've identified 8 new viruses in ovarian tissues of *R. prolixus* (RpV1-8), through metatranscriptomic approaches. Our published data demonstrate that RpVs are vertically transmitted in *Rhodnius*, which contributes, at least in part, to the maintenance of persistent infections in the insect population. OBJECTIVES: The present work aims to investigate the triatomine viruses and antiviral defense mechanisms in *Rhodnius prolixus.* MATERIALS AND METHODS: Initially, we tested whether ovarian extracts enriched in RpVs could infect *T. cruzi*, considering that the parasite interacts with the microbiota of the *Rhodnius*. Subsequently, we analyzed by fluorescent in situ hybridization and qRT-PCR whether cell types from other insects such as *Aedes aegypti* and *Aedes albopictus* would support the infection by RpVs. To investigate the antiviral mechanisms in *Rhodnius*, we performed parental RNAi against *dicer 2* or *argonaute 2* in females of *R. prolixus.* DISCUSSION AND RESULTS: We verified that both *T. cruzi* and different mosquito cell lines are capable of withstanding RpVs infections. The dsRNAs are able to reduce the expression of the target genes, generating a knockdown, through parental RNA interference. We were able to verify, through qRT-PCR, that females injected with dsRNAs for *dcr-2* or *ago-2* had their survival significantly reduced. CONCLUSION: We conclude that, also in *Rhodnius* as in other insects, the RNAi pathway might be important to maintain the number of viral copies compatible with the survival of *Rhodnius*. Additionally, we found that the RpVs can infect *T. cruzi* and mosquito cells, suggesting that RpVs survive and can replicate in different hosts.

Keywords: Host restriction, *Rhodnius prolixus*;, siRNA / **Supported by:** CNPq, Faperj and Wellcome trust

M.12 - Surface Carbohydrate Assessment of Aeromonas spp. Using Cramoll Lectin Conjugated to Quantum Dots

Rafael Bastos Gonçalves Pessoa [1], Weslley Felix de Oliveira[1], Maria Tereza dos Santos Correia[1], Thiago Henrique Napoleão[1], Patrícia Maria Guedes Paiva[1], Paulo Euzébio Cabral Filho[2], Adriana Fontes[2], Luana Cassandra Breitenbach Barroso Coelho[1]
[1]Depto de Bioquímica, Univ. Federal de Pernambuco (PE, Brazil), [2]Depto de Biofísica e Radiobiologia, Univ.Federal de Pernambuco (, Brazil)

INTRODUCTION: The association between carbohydrate-binding proteins, lectins, and semiconductor nanocrystals, quantum dots (QDs), and their use as versatile fluorescent nanoprobes have been expanded the biotechnological strategies for comprehension of biological processes and diagnosis. The Aeromonas genus is responsible for several infectious outbreaks in aquaculture and also innumerable human health disorders worldwide. Due to taxonomical complexity, particular characteristics among the strains have been usually detected by molecular methods. Therefore, assessing their carbohydrate profile with Lectin-QDs conjugates can be a valuable tool for bacterial characterization. OBJECTIVES: This study aimed to evaluate the glucose and mannose profiles among 4 Aeromonas species using Cramoll-QDs conjugates. MATERIALS AND METHODS: CdTe QDs were synthesized and conjugated with Cramoll by adsorption. Optical characterizations of bare QDs and Cramoll-QDs suspensions were performed by UV-Vis absorption and emission spectroscopies. Strains of A. hydrophila, A. caviae, A. dhakensis, and A. jandaei were cultured in tryptic soy broth and kept at 37 ºC for 24 h. Bacterial suspensions were incubated with Cramoll-QDs for 1 h and then analyzed by flow cytometry. The specificity of the interaction was evaluated by inhibiting assays using methyl-α-D-mannopyranoside or mannan. DISCUSSION AND RESULTS: Bare QDs and Cramoll-QDs presented the first absorption maximum peak at 585 nm and an intense emission peak at 621 nm. Different labeling patterns were observed among the tested Aeromonas species. A. hydrophila and A. caviae isolates were efficiently labeled by Cramoll-QDs conjugates, and a decrease in the labeling percentage was observed after incubation with the conjugate inhibited with mannopyranoside. A. dhakensis and A. jandaei showed higher average cell labeling than the other bacteria by the conjugate, even after inhibition with mannopyranoside, but not when mannan was used. These findings suggest a higher availability of glucose/mannose surface structures in A. dhakensis and A. jandaei. CONCLUSION: Cramoll-QDs conjugates showed to be effective nanoprobes for Aeromonas characterization based on carbohydrate surface detection.

Keywords: Bacteria, Fluorescence, Nanocrystals / **Supported by:** FACEPE

M.13 - Establishment of Leishmania major knockout lines to study telomere dynamic through Base J levels and TERRA expression

Luiz Henrique de Castro Assis [1], Beatriz Cristina Dias Oliveira[1], Maria Isabel Nogueira Cano[1]
[1]Genética, Universidade Estadual Paulista "Júlio de Mesquita Filho" (São Paulo, Brasil)

INTRODUCTION: Leishmaniases affects millions nowadays. It's caused by protozoa parasites of the Leishmania genus. Due to the lack of efficient treatment and disease control, many efforts have been made to uncover peculiarities of parasite's biology. Telomeres have been considered antiparasitic drug-targets since they are crucial for genome maintenance and cell survival. In several species telomeres are transcribed in long ncRNA's, such as TERRA, likely to affect telomere regulation. In Leishmania, TERRA transcription seems to be controlled by the modified thymine BaseJ, which is 98% concentrated at telomeres. BaseJ blocks restriction enzymes digestion and is an epigenetic signal for RNA polymerase II transcription termination. Therefore, BaseJ can interfere on TERRA and telomere homeostasis. OBJECTIVES: To test this hypothesis we created knockout lines for JBP1 and JBP2, enzymes involved in BaseJ synthesis in L. major, using a CRIPR-Cas9 system. MATERIALS AND METHODS: L. major promastigotes expressing Cas9/T7 polymerase were individually transfected with specific sgRNAs to create knockout lines for both enzymes. DISCUSSION AND RESULTS: Although JBP1-/- lines appeared to be impracticable, JBP2-/- were successfully generated. JBP2-/- clones were selected by antibiotic resistance and randomly chosen and cultivated for phenotypic analyses at passages 7 and 14. PCR and DNA sequencing confirmed JBP2-/- knockout. Selected clones presented slower growth ratios when compared to wild type parasites in both passages. Qualitative observations showed morphological differences for one clone although none were observed for the others. RT-PCR/qPCR analysis did not show quantitative differences for TERRA expression for JBP2-/-, probably because the effects of losing JBP2 might be cumulative. Finally, quantitative alterations on telomere length were detected by Flow-Fish. Further studies will be carried out using later passages. CONCLUSION: These results will be complemented with cell cycle and southern-blot analyses and the generation of a JBP1 hemi-knockout line using JBP2-/- lines to study telomere/TERRA regulation in a more severe BaseJ depletion scenario.

Keywords: Leishmania, Telomeres, Base J / **Supported by:** FAPESP

M.14 - *In vitro* Evaluation of in silico Selected Drugs with Potential Action Over Enzymes from Lipid Metabolism of Typanosoma cruzi

Caroline Silva Garcia[1], Milena Pereira Batista[1], Cacilda Tezelli Junqueira Padovani[1], Ines Aparecida Tozetti[1], **Alda Maria Teixeira Ferreira[1]**
[1]Instituto De Biociências, Universidade Federal De Mato Grosso Do Sul (Mato Grosso Do Sul, BRAZIL)

INTRODUCTION: Chagas disease, caused by the protozoan *Trypanosoma cruzi*, currently affects about 7 million people worldwide. The etiological treatment of this disease is restricted to the drugs benznidazole and nifurtimox, requiring the search for new treatment options. OBJECTIVES: This study aimed to select *in silico* approved drugs with potential activity against *T. cruzi* strain Dm28. MATERIALS AND METHODS: Online and free databases were used to perform the search and selection of drugs with potential action over homologous enzymes from lipid metabolism of *T. cruzi*, based on the identification of drug targets with high similarity to pathogen genes. Subsequently, *In vitro* tests were performed against epimastigote and trypomastigote forms of *T. cruzi* and cytotoxicity evaluation against Vero cells. DISCUSSION AND RESULTS: In the Tropical Disease Research Targets (TDR) database, 151 genes encoding the parasite's lipid metabolism enzymes were identified. Then, the protein sequence of these enzymes was obtained from TriTrypDB. The sequences were inserted into DrugBank and Therapeutic Targets Databases (TTD) to identify homologous drug targets. The sequences included corresponded to 367 drugs and, of these, 56 were drugs approved for use in humans. Using PUBMED, LILACS and Google Scholar, 25 drugs non-tested against *T. cruzi* were selected. Within this group, the drugs oxiconazole, pitavastatin, pravastatin and azelaic acid were tested *In vitro* against epimastigote forms of the parasite. Only oxiconazole was active, being submitted to the following tests. xiconazole showed low cytotoxicity against Vero cells, however it didn't show activity against trypomastigote forms of *T. cruzi*. CONCLUSION: The *in silico* analyzes indicated that oxiconazole has a potential action on the enzyme lanosterol synthase of *T. cruzi*, therefore, with probable action on the synthesis of ergosterol. This study also listed 21non-tested drugs with potential anti-*T. cruzi* action.

Keywords: Chagas Disease, *Trypanosoma cruzi*, Drug Repositioning / **Supported by:** UFMS, Fundect

M.16 - Phage ZC01 shows potential for phage therapy in infections by Pseudomonas aeruginosa PA14 strain

Layla Farage Martins [1], Ariosvaldo Pereira dos Santos Junior[1]
[1]Bioquímica, Universidade de São Paulo (São Paulo, Brazil), [2]Microbiologia, Universidade de São Paulo (São Paulo, Brazil)

INTRODUCTION: Using *Pseudomonas aeruginosa* PA14 as host, we have previously isolated and characterized phage ZC01 (Siphoviridae; Abidjanvirus; unclassified Abidjanvirus) from São Paulo Zoo Park composting samples. PA14 strain is highly virulent in both animals and plants and is gradually replacing PAO1 strain for pathogenesis research. OBJECTIVES: In the present project we aim at expanded characterization of phage zc01 MATERIALS AND METHODS: Further characterization showed that ZC01 has a latency period of 100 minutes and presents a burst size of ~87 PFU/infected cell upon 120 minutes after infection. ZC01 presents reduced viability upon exposure at acidic and alkaline pHs or temperatures above 60oC. DISCUSSION AND RESULTS: Using proteomics, we estimated that the ZC01 virion is composed of at least 27 proteins, which include proteins with yet unknown function. The ORF ZC01_066, annotated as a tail fiber protein, encodes a predicted depolymerase domain which is line with the ZC01 biofilm degradation activity. Phage susceptibility assays in PA14 mutant strains point to the type-IV pilus pilin (PilA) as the primary determinant for the ZC01 restricted host range. Initial results showed that phage ZC01 improves survival of Galleria mellonella infected with PA14. CONCLUSION: Based on these results, we predict that ZC01 could be used against infections by P. aeruginosa PA14-related strains.

Keywords: Pseudomonas aeruginosa, type-IV pilus, phage
Supported by: CAPES, CNPQ and FAPESP

M.17 - Evaluation of the Occurrence of RSV and Influenza A/B Through Molecular Biology in Foz do Iguaçu-PR

Leonardo Ferreira[1], Aline Cristiane Cechinel Assing Batista[1], Açucena Veleh Rivas[1], Adrieli Barboza de Souza[1], Andressa Faria Rahyn Fitz[1], Francieli Araujo Pegorari Cason[1], Robson Michael Delai[1]
[1]Centro de Medicina Tropical da Tríplice Fronteira, Hospital Ministro Costa Cavalcanti (Paraná, Brasil), [2]Programa de pós-graduação em biociências, Universidade Federal da Integração Latino Americana (Paraná, Brasil)

INTRODUCTION: In addition to SARS-CoV-2, Influenza and Respiratory Syncytial Virus (RSV) infections are also very common. Influenza virus has higher lethality in elderly people while RSV is the main cause of acute respiratory tract infection in children up to 5 years of age and is associated with the development of acute viral bronchiolitis. Besides providing a quick and assertive diagnosis, molecular tests can deliver important data on the evolution of the disease in a region. OBJECTIVES: For this reason, we sought to evaluate the occurrence of cases of Influenza and RSV in patients from a hospital located in Foz do Iguaçu-PR. MATERIALS AND METHODS: For that, we evaluated data from respiratory panels exams performed in the hospital`s lab between 01/04/2022 and 05/17/2022. The exams were performed by RT-PCR using two commercial kits VS-ABR112L and VS-RP0312LRUO (Certest biotec). DISCUSSION AND RESULTS: In total we evaluated data from 261 exams. We observed that the highest incidence of Influenza occurred during January, where 20% of the samples analyzed were positive for Influenza. After this period, the incidence of Influenza has decreased and remains between 4% (April) and 8% (May). RSV infections have increased throughout the year. While in January positive samples for RSV represented 1% of the exams, in May they already represent 33%. The peak of positivity for influenza occurred along with the outbreak of the virus that occurred in January in Brazil. The observed occurrence of RSV agrees with the data from NT 05/2015 of Ministry of Health (MH), which indicates that the highest incidence of RSV in the southern region occurs between April and August. CONCLUSION: Thus, the increase in influenza cases followed its outbreak that occurred in January in Brazil and the increase in RSV infections corroborates the data from the MH on the seasonality of the virus.

Keywords: respiratory syncytial virus, flu, incidence / **Supported by:** Fundação de Saúde Itaguapy

M.18 - Neutrophil extracellular traps (NETs) plays a critical role for the control of SARS-CoV-2 spread

Cícero José Luíz dos Ramos Almeida [1,2], Flavio Protasio Veras[1,2], Isadora Marques Paiva[1,2], Ayda Henriques Schneider [1,2], Juliana Costa Silva[1,2], Victor Ferreira Costa[1,2], Bruna Manuella Souza Silva [1,2], José Carlos Alves-Filho[1,2], Thiago Mattar Cunha [1,2], Fernando de Queiroz Cunha[1,2]
[1]Center of Research in Inflammatory Diseases, Ribeirão Preto Medical School - University of São Paulo (São Paulo, Brasil), [2]Department of Pharmacology, Ribeirão Preto Medical School - University of São Paulo (São Paulo, Brasil)

INTRODUCTION: SARS-CoV-2 virus causes COVID-19, a disease associated with a high mortality rate. In this disease, it was observed an increase in the release of neutrophil extracellular traps (NETs) in moderate and severe patients. However, despite the characterization of NETs release in these patients, little is still elucidated about the mechanisms that regulate the neutrophil activation. OBJECTIVES: Our objective was to assess whether NETs could be involved in inhibition of SARS-CoV-2 replication. MATERIALS AND METHODS: Neutrophils (1x106) were isolated from healthy individuals and infected with SARS-CoV-2 (MOI 1.0) for 4h to analyze the NETs production and viral replication. In parallel, lineage CaCo2 cells (1x105) were infected for 24h with SARS-CoV-2 (MOI 1.0), pre-treated or not, with different concentrations of NETs to analyze viral replication and cell viability. Posteriorly, human neutrophils (1x106), pre-treated or not with Cl-Amidine, an inhibitor of NETs production, were infected with SARS-COV-2 (MOI 1.0) for 4h to analyze virus replication. DISCUSSION AND RESULTS: Through the immunofluorescence analysis it was observed that most of the SARS-CoV-2 particles co-localized with the NETs. To understand if NETs could inhibit viral replication in other cell types, we used as a model the infection of lineage CaCo2 cells. In these cultures we observed that small concentrations of NETs did not induce death cell and inhibited virus replication in CaCo2 cells. When evaluating engagement of PAD4 enzyme in the replication of SARS-CoV-2 in neutrophils, we observed that with consequent inhibition of production of NETs, there was greater replication of SARS-CoV-2 in neutrophils. CONCLUSION: In view of the above, the results indicate that the NETs act in the control of replication and infection of the SARS-CoV-2, mainly at low concentrations due to no induce cell damage. However, we reiterate that more analysis is needed to understand how NETs could inhibit the SARS-CoV-2 virus, thus becoming the goal of future experiments.
Keywords: COVID-19, Innate Immunity , Neutrophils / **Supported by:** CAPES, FAPESP, CNPq

M.19 - Search for inhibitors of Leishmania major and Plasmodium vivax deoxyhypusine synthase (DHS) by screening in silico and in yeast

Suélen Fernandes Silva [1], Angélica Hollunder Klippel[2], Sunniva Sigurdardottir[3], Sayyed Jalil Mahdizadeh[3], Catarina Bourgard[3], Leif Eriksson [3], Ievgeniia Tiukova [4], Ross King[4], Katlin Brauer Massirer[5], Elizabeth Bilsland[6], Per Sunnerhagen[3], Cleslei Fernando Zanelli[2]
[1]Chemistry Institute, São Paulo State University "Júlio de Mesquita Filho" (SP, Brazil), [2]School of Pharmaceutical Sciences, São Paulo State University "Júlio de Mesquita Filho" (SP, Brazil), [3]Department of Chemistry and Molecular Biology, University of Gothenburg (Västra Götaland, Sweden), [4]Department of Biology and Biological Engineering, Chalmers University of Technology (Västra Götaland, Sweden), [5]Molecular Biology and Genetic Engineering Center, University of Campinas (SP, Brazil), [6]Department of Structural and Functional Biology, University of Campinas (SP, Brazil)

The enzyme deoxyhypusine synthase (DHS) catalyzes the first step of the post-translational modification of the eukaryotic translation factor 5A (eIF5A), which is the only known protein to contain the amino acid hypusine. Both eIF5A and DHS are essential for eukaryotic cell viability, and DHS has been suggested as a good candidate target for small molecule-based therapies against eukaryotic pathogens. In this work, we focused on the DHS enzymes from *Leishmania major* and *Plasmodium vivax*, pathogenic eukaryotic organisms that cause cutaneous leishmaniasis and malaria, respectively. We have searched for selective inhibitors for the parasites' DHS in the CHEMBL-NTDs library sets. First, we performed molecular docking of the compound library in the active site of the DHSs, and we tested the stability of the interaction by molecular dynamics simulation (MDS) using the software Maestro (Schrödinger). We generated a *Saccharomyces cerevisiae* platform with the endogenous DHS gene replaced by the orthologous DHS and we measured the inhibition through the differential growth of the strains in the presence and absence of the compounds. Performing the molecular docking we selected nine compounds with stronger interaction between the parasites' DHS when compared to the human protein. The MDS showed that these compounds were stabilized on the active site. The strains replaced by the parasites' DHS had at least 40% of the yield reduced in the presence of 100 µM of three compounds, while no statistically significant reduction was seen for the human DHS. From these candidates, one inhibited the *P. vivax* DHS, one the *L. major* DHS, and one inhibit both *P. vivax* and *L. major*. Here we report novel promising selective inhibitors of the DHS from *L. major* and *P. vivax* that will be tested in a biochemical assay using the purified protein for further elucidations.
Keywords: Deoxyhypusine synthase, Neglected tropical diseases, Drug Discovery / **Supported by:** CNPq, CAPES, FAPESP

M.20 - Distribution, diversity, and dynamics of prokaryotic immune systems in Mobile Genetic Elements of prokaryotic genomes and metagenomes.

Guillermo Uceda Campos [1], João Carlos Setubal[2], Aline Maria da Silva[2]
[1]Programa Interunidades de Pós-graduação em Bioinformática, Instituto de Matemática e Estatística, Universidade de São Paulo (São Paulo, Brazil), [2]Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo (São Paulo, Brazil)

INTRODUCTION: Bacteria and Archaea are in a constant battle with their viruses (phages) in a phenomenon known as “arms race” which led to the evolution of a variety of antiphage defense mechanisms. These mechanisms are collectively called prokaryotic “immune systems”. The most studied systems are Restriction-Modification (RM), CRISPR-Cas, and abortive infection (ABI). Recently, the discovery of new antiphage defense systems has expanded the repertoire of prokaryotic immune systems. OBJECTIVES: In this work we aim to analyze the distribution and diversity of all known immune systems in public prokaryotic genomes and explore the dynamics of those systems in public temporal-scale metagenomics datasets. MATERIALS AND METHODS: With this aim in mind, we established the following analysis workflow: 1) Retrieve the defense system sequences from NCBI and IMG databases; 2) Construct a similarity sequence network (SSN) of the orthologous groups (OGs) belonging to the defense repertoire; 3) Build Hidden Markov Models (HMM) for each OG; 4) Retrieve prokaryotic genomes and metagenomic datasets from the public databases; 5) Predict the Mobile Genetic Elements (MGEs) in the genomes and metagenomes; 6) Identify the defense systems within the MGEs. DISCUSSION AND RESULTS: For this study, 19 types of immune systems were selected, of which 16 have more than one sequence available. A total of 35,211 systems (79,484 sequences) were retrieved. DRUANTIA, one of the selected systems, showed the largest sequences, while a group of RM systems showed the smallest. In the homology analysis, the SSN showed relationships among all the immune system sequences. Sequences belonging to type II RM systems were the most disperse in the network, clustering with other types of immune systems. CONCLUSION: Our initial results allow the conclusion that the immune systems are highly diverse and that type II RM systems could be related to the origin of the other immune systems.

Keywords: antiphage immune system, bacteriophages, genomics / **Supported by:** CAPES e FAPESP

M.21 - Isolation and Characterization of Pseudomonas_Phage_P1_LAFX Against a Carbapenem Resistant Pseudomonas aeruginosa

Ariosvaldo Pereira dos Santos Junior [1], Layla F. Martins[1], Fernando Pacheco Nobre Rossi[1], Deyvid E. Amgarten[2], Aline Maria da Silva[1]
[1]Departamento de Bioquímica, Universidade de São Paulo, Instituto de Química (São Paulo, Brazil), [2]Bioinformática, Hospital Israelita Albert Einstein (São Paulo, Brazil)

INTRODUCTION: Bacteriophages, or phages, are viruses that infect bacteria. Phages can be easily collected from natural sources, using target bacterial strains as hosts. Lytic phages have been proven as useful tools to control bacterial infections (phage therapy), particularly by multidrug-resistant bacteria. OBJECTIVES: In the present project we aim at the isolation and molecular characterization of lytic phages for future application in phage therapy against *Pseudomonas aeruginosa* infections. MATERIALS AND METHODS: Here we describe Pseudomonas_Phage_P1_LAFX, herein LAFX, which was isolated from sewage samples collected at USP- campus Butantã- using *P. aeruginosa* clinical isolates PASM1 and PASM2 as hosts. Phage samples were prepared for visualization under Transmission Electron Microscopy and for whole genome sequencing. DISCUSSION AND RESULTS: These isolates derived from a wound on the left knee of a patient admitted to the University Hospital of São Luís do Maranhão on May-02-2021 and were shown to be resistant to carbapenems (such as meropenem and ertapenem) and to other antibiotics (cephalosporin family, aminopenicillin and tetracyclines). Transmission Electron Microscopy of LAFX showed a morphotype of the order Caudovirales, family Myoviridae. LAFX genome was sequenced and analyzed, confirming it as a Myoviridae phage, genus *Pakpunavirus*. LAFX genome has 94kbp encompassing 190 ORFs, among them several conserved structural virion proteins, putative receptor binding protein (RPB), depolymerase-domain protein, endolysin, DNA polymerase, RNAseH, HNH endonuclease and various conserved domains of unknown function. Based on initial analyses, LAFX genome does not encode toxin genes, virulence factors or antibiotic resistance genes. Phage LAFX presents large lysis plaques which are characteristic of high burst size, with adsorption time around 10min. LAFX host range was verified using several *P. aeruginosa* strains, including the reference strains PA14 and PAO1, and shown that LAFX lytic activity has been restricted to the two strains PASM1 and PASM2.CONCLUSION: These results warrant additional investigation to verify the potential of phage LAFX for application in phage therapy.

Keywords: Caudovirales, Pseudomonas aeruginosa, Genome sequencing / **Supported by:** CAPES, CNPQ and FAPESP

M.22 - Oral Supplementation with Sodium Propionate Revert Depressive-Like Behavior in Rats Exposed to Chronic Unpredictable Mild Stress

Luiza Marques Prates Behrens [1], Juciano Gasparotto[2], José Cláudio Fonseca Moreira[1]
[1]Bioquímica, Universidade Federal do Rio Grande do Sul (Rio Grande do Sul, Brasil), [2]Instituto de Ciências Biomédicas, Universidade Federal de Alfenas (Minas Gerais, Brasil)

INTRODUCTION: Studies about gut microbiota and its importance for the host's mental health and neurological development have grown in the last few years. It is now established that gut microbes and their metabolites can activate signaling pathways in the gut and several human body organs, including the brain. OBJECTIVES: Therefore, the goal of this project was to investigate the effect of Short-Chain Fatty Acids (SCFAs) in Wistar rats exposed to a model of Major Depression, the Chronic Unpredictable Mild Stress (CUMS). MATERIALS AND METHODS: Eighty-eight animals were submitted to a stressful protocol for six weeks, in which were used seven types of mild stressors alternating each week randomly. The sucrose preference test (gold-standard for CUMS model) was performed to check if or to confirm if animals had developed a depressive-like behavior or a resilience-like behavior. The depressive-like group received oral supplementation with sodium acetate (60 mM), sodium butyrate (40 mM) and sodium propionate (50 mM), the three main SCFAs produced in the gut microbiota, or with a mix of the three of them. The SCFAs were diluted in the drinking water for consumption ad libitum. DISCUSSION AND RESULTS: Our results indicate that all treatments attenuated the depressive-like behavior after seven days of supplementation, but the sodium propionate supplementation increased sucrose consumption (p=0,0155). CONCLUSION: In the literature, SCFA supplementation can attenuate depressive-like behavior dose-dependent manner. But the improvement of sucrose consumption after sodium propionates supplementation is a novelty for CUMS model. The impacts of its supplementation can occur due to the activation of signaling pathways via the vagus nerve, via immuno-inflammatory modulation and propionate receptors on blood-brain barrier cells, altering the behavior of the search for reward.
Keywords: depression, microbiota, propionate / **Supported by:** CNPq

M.23 - Investigation of the Participation of Endoplasmic Reticulum Stress in Human Megakaryoblasts during the Infection by Yellow Fever Virus

Guilherme Fonseca Tozatto [1], Marcella Caldeira[1], Beatriz Pedroza[1], Renata de Lima[2], Jerson Silva[1], Andre Gomes[1], Andréa de Oliveira[1]
[1]Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro (RJ, Brasil), [2]Centro Nacional de Biologia Estrutural e Bioimagem, Universidade Federal do Rio de Janeiro (RJ, Brasil)

INTRODUCTION: The Yellow Fever Virus (YFV) is the etiological agent of the Yellow Fever Hemorrhagic Disease. One of the main factors that can aggravate it is the thrombocytopenia. Megakaryoblasts are precursors of megakaryocytes and each one of them can generate up to ten thousand platelets. OBJECTIVES: In this study, our main goal is to investigate the interaction between the YFV and human megakaryoblasts, focusing in the cell's death and endoplasmic reticulum stress (ERS), which can be involved during the infection. MATERIALS AND METHODS: We used the MEG-01 megakaryoblasts cell line and the 17DD YFV viral strain. We evaluated the kinetic of infectious viral particles production by plaque assay in VERO cells. Cell death was evaluated by exclusion counting with Trypan Blue. YFV presence was checked by transmission electron microscopy and its participation in ERS was investigated by Western Blotting assay. MEG-01 cells were submitted to cytotoxicity tests with both an inhibitor and an inductor of ERS. They were, respectively, 4PBA and Tunicamycin. DISCUSSION AND RESULTS: MEG-01 cell line resulted to be permissible and susceptible to YFV. Both were evaluated by plaque assay, which showed production of new infectious particles since day one post infection. We demonstrated GRP78, a general marker of unfolded protein response (UPR) mechanism and therefore a sign of ERS activation, increased expression. We also evaluated other proteins related to UPR, such as ATF-6 and eiF2α / eiF2αP, both with significant results that showed us the activation of this cellular mechanism. At last, we investigated the UPR influence in the production of YFV infectious particles. We treated MEG-01 cells with 4PBA and Tunicamycin and analyzed the kinetic of viral production, which has showed a significant impact in the production of YFV's new infeccious particles. CONCLUSION: With the results above, we assume that ERS has a major key role during infection and production of YFV in human megakaryioblasts.
Keywords: Yellow Fever Virus, Endoplasmic Reticulum, Thrombocytopenia
Supported by: FAPERJ, CNPq, CAPES, INCT-INBEB

M.24 - BK/B2R axis induces caveolae-mediated endocytosis during cerebral malaria

Sarah Aparecida dos Santos Alves [1], Douglas Esteves Teixeira[1], Rodrigo Pacheco da Silva Aguiar[1], Gabriele Costa Cesar[1], Alessandro de Sá Pinheiro[1], Julio Scharfstein[1], Celso Caruso-Neves[1,2,3], Ana Acacia de Sá Pinheiro[1]
[1]Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro (Rio de Janeiro, Brazil), [2], Rio de Janeiro Innovation Network in Nanosystems for Health-NanoSAÚDE/FAPERJ (Rio de Janeiro, Brazil), [3], National Institute of Science and Technology for Regenerative Medicine (, Brazil)

INTRODUCTION: Cerebral malaria (CM) is the major complication of *Plasmodium falciparum* infection. CM could culminate with blood brain barrier (BBB) disruption, however, the molecular mechanisms under this event are unclear. Our group showed that *P. falciparum* infected erythrocytes (*Pf*-iRBC) conditioned medium promotes lost in BBB integrity via activation of the kallikrein kinin system. OBJECTIVES: We aimed to verify the role of bradykinin (BK) on protein transcytosis in brain endothelial cells during malaria infection. MATERIALS AND METHODS: For this, human brain microvascular endothelial cells (hBMEC) were incubated overnight with *Pf*-iRBC conditioned medium (CEP-HUCFF 074/10), or BK. We assessed albumin-FITC uptake by confocal microscopy and fluorimetry. Experimental cerebral malaria (ECM) model was used to analyze the integrity of BBB *In vivo* (008/18). DISCUSSION AND RESULTS: Pf-iRBC conditioned medium increased albumin endocytosis (2.5-fold) in hBMEC (n=2). Cell treatment with BK 10-7 M mimicked the effect of *Pf*-iRBC conditioned medium in albumin endocytosis (n=2). The effect of BK was abolished by treatment with nystatin 25 µg/mL, a lipid raft inhibitor used to block caveolae-mediated endocytosis, but not by treatment with Pit-Stop 2 25 µM, a selective inhibitor of clathrin-mediated endocytosis (n=2). The pre-treatment of hBMEC with HOE-140 10-7 M, a bradykinin B2 receptor antagonist (B2R), prevented the effect of BK, showing a B2R participation in this process. Using ECM, we observed the leakage of BSA-FITC (i.v.) in the brain of infected animals (n=4), before the onset of cognitive impairment, assessed by SHIRPA score (n=4), and BBB breakdown, assessed by Evans Blue (n=4). Interestingly, treatment with Nystatin (8 mg/kg), ameliorates the stability of BBB and cognitive impairment (n=4) in infected mice. CONCLUSION: Altogether, our data showed that BK through B2R induces caveolae-mediated albumin endocytosis in hBMEC. This process seems to precede BBB breakdown according to the findings on the *In vivo* model. These results, open new perspectives for understanding the pathogenesis of cerebral malaria.

Keywords: Blood brain barrier, Bradykinin, Cerebral Malaria / **Supported by:** CAPES, CNPQ and FAPERJ

M.25 - New weapons in biological conflicts: bacterial toxins inducing cell death via dna double-strand breaks

Julia Takuno Hespanhol [1], Daniel Limache[1], Gianlucca Nicastro[1], Edgar Llontop[3], Liam Mead[2], Gustavo Chagas-Santos[1], Chuck Farah[3], Robson Souza[1], Rodrigo Galhardo[1], Andrew Lovering[2], Ethel Bayer-Santos[1]
[1]Depto de Microbiologia, Univ. de São Paulo- Instituto de Ciências Biomédicas (SP, Brazil), [2]Department of Biosciences, University of Birmingham (Birmingham, UK), [3]Depo de Bioquímica, Univ. de São Paulo- Instituto de Química (SP, Brazil)

INTRODUCTION: Bacteria evolved many mechanisms to antagonize competitors. The type VI secretion system (T6SS) is a contractile system that punctures target cell membranes and deliver toxic effectors. This pool of T6SS effectors represents a reservoir of proteins with an unexpected broad range of activities. Antibacterial effectors are natural antimicrobials that bacteria evolved over millions of year and harbor great potential to reveal interesting biological processes and biotechnological applications. *Salmonella* encode several T6SSs acquired via different horizontal transference events, encoded in distinct SPI (*Salmonella* Pathogenicity Islands). These T6SSs were shown to be important for competition with the gut microbiota to overcome colonization resistance and establish a successful infection. There is a lack of knowledge on the effectors secreted by this system, with only two examples identified so far. OBJECTIVES: Characterize new effectors secreted by the SPI-22 T6SS MATERIALS AND METHODS: Bacterial competition assays. Bioinformatic analyzes searching for putative effectors. Cloning and expression of candidates in *E. coli* to test toxicity. Phylogenetic analysis to establish evolutionary relationships. Functional assays to analyze DNA damage. Crystallography to determine protein 3D structure. DISCUSSION AND RESULTS: Interbacterial competitions revealed that SPI-22 T6SS has antibacterial activity. Bioinformatic searches identified putative effectors containing a VRR-Nuc (virus-type replication-repair nuclease) domain (TseV1/TseV2/TseV3/TseV4). *E. coli* toxicity assays revealed that only TseV2 and TseV3 were toxic. Phylogenetic analysis revealed that effectors were related to enzymes involved in interstrand crosslink DNA repair. Point mutations in conserved catalytic residues abrogated toxicity. Expression of TseV2 and TseV3 in *E. coli* leads to nucleoid instability, DNA double-strand breaks and induce SOS response. Two specific immunity proteins were identified (TsiV2 and TsiV3), which prevent self-intoxication. Crystal structure of TseV3-TsiV3 provided insight into the mechanism of toxin neutralization by its immunity, which relays on blockage of substrate binding. CONCLUSION: The study characterized the mechanism of new antibacterial toxins, expanding current knowledge on weapons used in biological conflicts.

Keywords: Biological conflicts, DNase, toxins / **Supported by:** FAPESP

M.26 - Is the tert in *L.major* a pleiotropic gene: genome editing with crispr cas9 reveals the possible involvement of tert in multiple cellular events in the parasite

Mark E. Shiburah [1], Beatriz C. Diaz de Oliveira[1], Veronica S. Fontes[1], Noemi N. Taniwaki[2], Maria Isabel N. Cano[1]
[1]Department of Chemical and Biological Science, Bioscience Institute-UNESP, Botucatu (SP, Brazil), [2]Centro de procedimentos interdisciplinares, Adolfo Lutz Institute (SP, Brazil)

INTRODUCTION: Over the last two decades, telomerase reverse transcriptase (TERT) has been on the radar as a potential drug target for new therapeutics against leishmaniasis, a neglected tropical disease. Precise modification of hard-to-transfect cells like Leishmania parasites has improved tremendously through enhanced CRISPR-Cas tools. OBJECTIVES: Our goal is to perform a functional study of the TERT gene in L.major. MATERIALS AND METHODS: Using flow cytometry, we performed DNA content analyses and estimated the parasites' proliferative capacity, their cell viability as well as their apoptotic rate. The telomere length was evaluated using Southern blot for TRF estimation. Fluorescence and electron microscopy were performed to evaluate parasites' morphological and nuclear status. Determination of DNA damage in the parasites was achieved using the anti-T. brucei ɣH2A immune serum. DISCUSSION AND RESULTS: We highlight a significant growth impairment in cells (P≤0.05) after TERT deletion (LmTERT-/-). A corroborative result was obtained with over 45% disparity between parasite proliferation. We observed prominent morphological aberrations in the knockout cells and found uneven nuclei distribution between control and TERT deficient parasites by a factor of 12.21, in addition to unnucleated cells. Again, a significant (P≤0.05) blockage of the altruistic pathway used by the parasites to evade host immune defenses was recorded. Additionally, we estimated a telomere attrition rate of 3.6-5.2 bp per population doubling compared to WT. We further found about 20% of the LmTERT-/- showed signals for DNA damage as compared to 6% of the WT. Abnormal nucleolar morphology, disruption of parasite organelles, vacuolization of parasite cytoplasm, and nuclear membrane disintegration was also observed in TERT deficient parasites, strongly suggesting that LmTERT-/- experience a type of replicative senescence, which is in agreement with the G0/G1 arrest observed in the cell cycle analysis CONCLUSION: Our results indulge the conversation to use TERT for the development of new drugs against leishmaniasis.
Keywords: CRISPR-Cas9, Telomeres, Cell viability / **Supported by:** FAPESP

M.27 - Effect of ICTI on Yeast Growth and Biofilm Development Of *Candida albicans* And *Cryptococcus gattii*

Ana Paula Ramos Pereira [1], Claudiane Almeida[1], Luís Almeida[1], Camila Gutierrez[1], Caio Oliveira[1], Maria Lígia Macedo[1]
[1]FACFAN, Universidade Federal de Mato Grosso do Sul (MS, Brasil)

Introduction: The discovery of antibiotics and the development of microbial resistance walk close. In the last decades, antimicrobial molecules from plant species have been investigated. Among the plant molecules, enzyme inhibitors display antimicrobial activity due their potential to damage membrane and/or cell wall, altering the cell permeability. **Objectives:** This work aimed to evaluate the antimicrobial and antibiofilm potential of the trypsin inhibitor from *Inga cylindrica* seeds (ICTI), to investigate its mechanism of action against pathogenic yeasts, as well as its toxicity in *Galleria mellonella* model. **Material and Methods:** Followed the isolation, the minimum inhibitory concentration (MIC) and antibiofilm activity were evaluated. The synergic effect of ICTI in association with amphotericin B and the toxicity of ICTI in *G. mellonella* were also analyzed. **Results and Discussion:** ICTI possesses a single polypeptide chain of 20 kDa, with inhibitory activity against trypsin. ICTI showed a MIC of 32.11 µM for *Candida albicans* and *Cryptococcus gattii*. The ICTI showed a synergistic effect with amphotericin B, reducing 535-fold the concentration of ICTI for *C. albicans* and 4-fold for *C. gattii*. The MIC of Amphotericin B was reduced 4-fold for *C. albicans*, and 18-fold for *C. gattii*. At MIC, ICTI inhibited the biofilm formation of *C. albicans* and *C. gattii* by 38.5% and 21.5%, respectively. The ICTI also prompted a partial eradication of mature biofilm by 37.2% and 22.1%, for *C. albicans* and *C. gattii,* respectively. The antifungal activity of ICTI involves binding to ergosterol present in the fungal cell membrane, promoting the leakage of intracellular content. ICTI was no toxic when administered to *G. mellonella* up to 321,1 µM. **Conclusions:** The antifungal activity of ICTI, both alone and in association with Amphotericin B, together with its non-toxicity, make it a potential drug.
Keywords: antimicrobial resistance, pathogenic yeasts, trypsin inhibitor / **Supported by:** Fundect, CNPq, Capes, Propp-Ufms

M.28 - Impact of Different Methods of Inactivation/Lysis of Probiotic Strains on Skin Pathogens for Cosmetic Application

Tuânia Natacha Lopes Silva [1], Adriano Guimarães Parreira[1], Juliana Teixeira Magalhães[1], José Antônio da Silva[1], Daniel Bonoto Gonçalves[2], Paulo Afonso Granjeiro[1]
[1]Laboratory of Biotechnological Processes and Purification of Macromolecules, Federal University of São João del-Rei (Minas Gerais, Brazil), [2]Department of Biosystems Engineering, Federal University of São João del-Rei (Minas Gerais, Brazil)

INTRODUCTION: Disturbances in the skin's microbiota are related to infections and inflammation causing diseases such as acne. Treatment alternatives use probiotic strains such as lactic acid bacteria (LAB), which in adequate amounts confer health benefits. In the cosmetics industry there is a trend in the use of lysates and inactivates, they have advantages such as transport, shelf life, risk of translocation and consumer infection. OBJECTIVES: The aim of this study was to evaluate the inactivation/lysis of five LAB species by different means/methods, to evaluate sub-lethal damage and activity against skin pathogens. MATERIALS AND METHODS: The species LAB UFSJA, UFSJB, UFSJC, UFSJD and UFSJE were cultured in whey or MRS and inactivated by water bath, heat shock and lysozyme. The antimicrobial activity of viable cells and inactivated/lysed cells were tested against *Cutibacterium acnes* ATCC 6919, *Staphylococcus epidermidis* ATCC 12228, *Corynebacterium xerosys* ATCC 373, *Staphylococcus aureus* ATCC 12228 by plate scattering method. DISCUSSION AND RESULTS: All viable LAB cell pellets were able to intermediately inhibit the growth of *C. acnes* and only UFSJA and UFSJD had weak inhibition halos against *C. xerosys*. The other pathogens were not inhibited. All LAB species grown in both media were completely inactivated by water bath and thermal shock. On the other hand, UFSJC and UFSJE demonstrated different levels of sensitivity to lysozyme. Whey provided protection against sublethal damage to the UFSJC strain, unlike MRS medium. The inactivated/lyzed species showed no activity against *C. acnes* and only the UFSJE strain inactivated by water bath in MRS inhibited *C. xerosys*. CONCLUSION: We conclude that different species may be more sensitive to different inactivation or lysis methods and in different cultures. The antimicrobial effect is thermo sensitive, requiring cell viability. Viable probiotic strains have shown a health application in combating Acne.

Keywords: probiotics, inactivation/lysis, antimicrobial
Supported by: CAPES, CNPq, FAPEMIG and UFSJ

M.29 - SOCS3 Genetic Polymorphisms as Biomarkers in Chronic Hepatitis C in Brazilian Patients Under Different Therapeutic Schemes

Thaís de Oliveira Consuli [1], Juliene Antonio Ramos[1], Cristiane Alves Vilella-Nogueira[1], Luísa Hoffmann[1]
[1]Laboratório de Genética Molecular, Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro (RJ, Brasil)

INTRODUCTION: Hepatitis C is a matter of public health worldwide with 58 million people infected. In Brazil, a rate of 11.9 cases per 100.000 inhabitants is estimated. In approximately 80% of cases the infection progresses to chronic hepatitis C (CHC), which can evolve to cirrhosis and hepatocellular carcinoma. It is of clinical interest to use new non-invasive or minimally invasive biomarkers that can identify the susceptibility of a patient to develop more severe liver disease and assist in better monitoring and targeting of treatment. Single nucleotide polymorphisms (SNPs) are promising as biomarkers. Studies in the world demonstrate that the treatment response of CHC is related to SNPs in the gene encoding the SOCS3 protein. It acts by negatively regulating the JAK-STAT signaling pathway, which is activated by interferons and interleukins. OBJECTIVES: This work aims to identify and characterize a Brazilian population with CHC treated with different regimens therapeutics on the A/G rs4969170 and A/G rs4969168 polymorphisms in the SOCS3 gene, in order to determine whether these SNPs influence the effectiveness of treatments and the presence of more advanced liver disease. MATERIALS AND METHODS: The population studied comprises patients from the Hospital of UFRJ, Rio de Janeiro. Clinical and laboratory data will be collected from medical records and genotyping will be done by polymerase chain reaction and gene sequencing by Sanger method using already extracted DNA samples. DISCUSSION AND RESULTS: Until now 74 patients were recruited. Average age are 58 years old. 33,7% of them have mild fibrosis, 40,5% have moderate and 24,3% have severe fibrosis. Only 1,35% have no fibrosis. CONCLUSION: We seek to find an association between SOCS3 genotypes and liver disease, which may contribute to the evaluation prognosis of chronically infected patients. The study of Brazilian population is important to better understand the diversity of different world populations.

Keywords: SOCS3, hepatitis C, biomarker / **Supported by:** Capes

M.30 - Towards an oriented ultrastructural study of the morphological transition of the pathogenic fungi Candida albicans

Rayane Gonçalves Pereira da Silva [1], Marcel Menezes Lyra da Cunha[1]
[1]NUMPEX-BIO, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: Among all fungi species with medical importance, *Candida albicans* stand out as the main fungi pathogenic for humans. *C. albicans* is polymorphic, such a characteristic is considered essential for the pathogenicity of the fungus. The most studied morphotypes are yeast, pseudohyphae and hyphae. OBJECTIVES: To understand how different metabolic sources participate in the morphological transition and impact its cell biology. Thus, we aim to define the ultrastructure of Spitzenkörper components and structures associated with the polarisome under nutritional variation by electron microscopy (EM) techniques with samples processed by cryotechniques. Many obstacles are present during the routine protocols for sample handling for EM that cause sample loss, longer reaction time of the reagents or prevent the orientation of the analyzed samples. Since our main goal is to observe structures located at the tip of the hyphae, we are exploring strategies and materials to be used as supports for EM of fungi. Also, we performed a systematic literature review to understand the advances related to *C. albicans* ultrastructural studies by EM. MATERIALS AND METHODS: *C. albicans* grew for 12 hours at room temperature. To control the orientation of cells, they were adhered to small fragments of a cellulose membrane in different conditions. The fungus was cultivated in YNB medium with 2% (w/v) glucose or fetal bovine serum. Samples were processed and analyzed by scanning EM. The chemical processing of samples were performed as proof of concept to evaluate fungal adhesion and preservation over the cellulose membrane. DISCUSSION AND RESULTS: Among tested membranes, a wet membrane showed better performance for fungal adhesion and dispersion. Till this moment, we inclueded 38 articles that came from search combinations. CONCLUSION: These results are important to refine our protocols that have been implanted in projects that aim to identify ultrastructural changes in *C. albicans* and other fungi.

Keywords: C. albicans, Electron microscopy, Sample orientation
Supported by: FAPERJ

M.31 - Study of the exometabolome of the biofilm of Candida albicans

Vitor Fernando Severino Valverde [1], Beatriz Bastos Fonseca[2], Sonia Rozental[2], Gisele Cardoso de Amorim [1], Marcel Menezes Lyra da Cunha[1]
[1]Campus UFRJ - Duque de Caxias. Núcleo multidisciplinar de pesquisa UFRJ - Xerém , Universidade Federal do Rio de Janeiro (RJ, Brasil), [2]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro (RJ, Brasil)

INTRODUCTION: *Candida albicans* is the most important fungal pathogen for humans because it is the most prevalent in nosocomial infections. Among its main virulence factors is the morphological transition and the formation of biofilms. Yeasts, hyphae and pseudohyphae are characteristic morphologies of this pathogen. While hyphae actively penetrate the tissue of the host, yeasts can be transmitted to other niches of the organism. Biofilms are associated structures of microorganisms that can provide constant infectious cells. OBJECTIVES: The aim of this study is to generate information about the exometabolome of the *C. albicans* biofilm as alternative carbon sources (glucose and lactate) are provided. In addition, we want to observe, in the near future, the effect of antifungals on the metabolome of this pathogen. MATERIALS AND METHODS: For this, the strain SC5314 of *C. albicans* was grown in four different conditions, based on a nitrogenous base medium without amino acids for yeast: (I) supplemented with 2% glucose; (II) supplemented with 2% lactate and (III) supplemented with 1% of both carbon sources. After fungal growth for 24 hours, the supernatant was isolated, lyophilized and stored at -4 °C. To acquire the spectra, the samples were resuspended in 100 mM phosphate buffer, pH 7, and added 10% DSS (4,4-dimethyl-4-silapentane-1-sulfonic acid) as internal standard and 10% D2O. One-dimensional (1H) and two-dimensional (TOCSY and 1H-13C-HSQC) spectra were acquired on a Bruker 500 MHz spectrometer at 298 K. DISCUSSION AND RESULTS: Our results show a higher consumption of glucose when compared to lactate by *C. albicans*. In addition, we also observed the secretion of some metabolism intermediates, such as pyruvate and glycerol, as different carbon sources were provided. CONCLUSION: This study helps to better understand the metabolism of this important pathogen. Furthermore, it may be possible to find possible biomarkers as different carbon sources are assimilated.

Keywords: biofilm, Candida albicans, metabolismo / **Supported by:** FAPERJ

M.32 - Investigation of the trypanocidal activity of naphthoquinone-derived analogues as possible therapeutic agents for leishmaniasis.

Alberto Nogueira Neto [1], Michelle de Oliveira Chain[1], Rubem Figueiredo Sadok Menna Barreto[2], Raphael Salles Ferreira Silva[1], Luiz Dione Barbosa de Melo[1]
[1]Laboratório de Genética Molecular, Instituto Federal de Educação. Ciência e Tecnologia do Rio de Janeiro (Rio de Janeiro, Brasil), [2]IOC/Laboratório de Biologia Celular, Fundação Oswaldo Cruz (Rio de Janeiro, Brasil)

INTRODUCTION: Leishmaniasis is caused by protozoa of the genus *Leishmania spp.* affecting millions of people, with an increasing number of new cases each year, as well as being neglected by health agencies around the world. The drugs used in leishmaniasis can cause adverse effects, which lead to treatment withdrawal and severe liver damage. Thus, the search for new therapeutic agents is essential. In this context, we propose the investigation of naphthoquinone analogues which present the ability to create reactive oxygen species (ROS) in the intracellular environment, inducing oxidative stress and consequent cell death. Previous studies by the group have shown that the analogue 2-Thiocyanyl-1,4-Naphthoquinone (2TIONQ) has an effective trypanocidal action, leading us to test it in *Leishmania amazonensis*. OBJECTIVES: Investigate cellular stress caused in proliferative forms of *L. amazonensis* treated with 2TIONQ quantifying: changes in acidic endocytic profile, production of ROS and alterations in the mitochondrial membrane integrity. Also, investigation of changes in energetic metabolism related gene expression and infection profile upon C57BL mice macrophages. MATERIALS AND METHODS: Cell viability (MTT colorimetric method); Endocytic profile (Acridine Orange assay), ROS production (DHE assay) and Mitochondrial membrane integrity (TMRE assay) by Flow cytometry. Gene expression using quantitative PCR (SYBR Green). Infection analysis (Quick Panoptic method). DISCUSSION AND RESULTS: These results suggest that the 2TIONQ can reduce the viability of the parasitic cells. The naphthoquinones are already known by its relation with ROS creation, and this offers a possible way to stress *L. amazonensis* cells. The drug demonstrates effect in mitochondria integrity, which could be linked to energetic metabolism's enzyme activity. This possibility led us to further pursue these mechanisms. CONCLUSION: The data suggests the increase in oxidative stress caused by the 2TIONQ. The work is still in progress, but shows a promising end towards the drug being used in further tests.
Keywords: leishmania, leishmaniasis, naphthoquinones / **Supported by:** FAPERJ

M.33 - The Novel Antimicrobial Peptide (KWI-19) Displays Antibacterial Activity Through Cytoplasmic Membrane Damages

Luís Henrique De Oliveira Almeida[1], Suellen Rodrigues Ramalho[1], Janaina de Cássia Orlandi Sardi[1], Caio Fernando Ramalho de Oliveira[1], Maria Lígia Rodrigues Macedo[1]
[1]Faculdade de Ciências Farmacêuticas, Alimentos e Nutrição, Univ. Federal de Mato Grosso do Sul (MS, Brazil)

INTRODUCTION: Diseases caused by resistant bacteria are an important cause of morbidity and mortality. Thus, researchers have sought to design new drugs using bioinformatics tools, optimizing the obtainment of active molecules. OBJECTIVES: We aimed to develop a new antimicrobial peptide (AMP) bioinspired in a plant trypsin inhibitor sequence, isolated from *Inga laurina* seeds (ILTI). MATERIALS AND METHODS: Through ILTI *in silico* cleavage and subsequent analysis, we obtained an AMP with 19 amino acid residues, named KWI-19, that was synthesized. The acute toxicity of KWI-19 was performed in *Galleria mellonella* model. The minimum inhibitory concentration (MIC) against bacteria strains, using the broth microdilution technique, was performed according to the CLSI M27-A2. The kinetic of death against *Pseudomonas aeruginosa* and *Staphylococcus saprophyticus* were assayed. To demonstrate that the mechanism of action involves damages on bacteria membrane, the release of nucleic acid was quantified in bacteria treated with KWI-19. DISCUSSION AND RESULTS: No signals of toxicity was noticed in *G. mellonella* larvae that received injections up to 50 μM of KWI-19. The peptide showed antimicrobial activity, showing MIC and MBC ranging from 1.25 μM to 10 μM, against 13 bacteria strains. The time necessary for KWI-19 to kill *P. aeruginosa* and *S. saprophyticus* strains ranged between 30 min and 120 min, respectively. The treatment of *P. aeruginosa* and *S. saprophyticus* at MIC prompted an increase of 74.8% e 32.8%, respectively, of nucleic acid release. Together, the results demonstrate that the target of KWI-19 is the bacteria membrane. CONCLUSION: KWI-19 is a promising antibacterial agent, showing a significant antimicrobial activity and *In vivo* safety.
Keywords: Pseudomonas aeruginosa, Staphylococcus saprophyticus, toxicity / **Supported by:** FUNDECT, CAPES, CNPq, FINEP, and PROPP-UFMS.

N - Neurochemistry

N.01 - Synergistic Effect of Phα1β Combination With Methadone on Rat Synaptosomes by Isobolographic Analysis

Luana Assis Ferreira[1], **Bianka Jaciara dos Santos Gomes** [1], Ísis Katarine Orlandi Leite[1], Marcus Vinícius Gomez[1], Célio José de Castro Júnior[1]
[1]Pós Graduação, Laboratório de Neurofarmacologia, Instituto de Ensino e Pesquisa do Grupo Santa Casa de Belo Horizonte (MG, Brasil)

INTRODUCTION: Multimodal analgesia consists in the concomitant use of two or more drugs to treat pain. This is an even-growing demand in clinic but is usually devoided of rational quantifiable evidence from the basic science. Phα1β, a toxin purified from the venom of the spider *Phoneutria nigriventer*, and metadone, a synthetic opioid, have analgesic potential and interacts synergistically in ameliorating pain phenotypes in rodents. OBJECTIVES: The aim of this study is to evaluate the synaptic mechanisms by which the analgesic interaction of Phα1β and methadone occurs. MATERIALS AND METHODS: We conducted intracelular calcium measurements and glutamate release from rat brain synaptosomes in the presence of Phα1β, methadone or their combination. We performed isobolographic analysis to assess whether the inibition of neurotransmission by these two drugs occurs in synergistic, additive or in a subaditive manner. DISCUSSION AND RESULTS: Rat cortical synaptosomes respond to KCl (30 mM) stimulus with a subtle and sustained calcium increase in the range of 100 nM. Pre incubation of synaptosomes with Phα1β, methadone or their combination dose-dependently inhibited calcium influx with IC50's values of 1.937 nM (1.466 - 2.560), 45.660 nM (11.836 - 176.133) and 0.0006 nM (0.0001 - 0.003), respectively. The IC50 of the combination of Pha1b and methadone was significantly lower than the IC50 theoretically expected for an additive interaction ($p < 0.05$). Glutamate released from synaptosomal preparation was significantly lower when Phα1β + methadone was pre-incubated in comparison with the pre-incubation of the two drugs isolated. CONCLUSION: Together, our data sugests that antinociceptive synergism of these two drugs is associated to synergistic inhibition calcium influx and glutamate release in synaptosomes.
Keywords: Synaptosomes, Phα1β, Methadone / **Supported by:** FAPEMIG, CNPq e CAPES

N.02 - Effect of Static Magnetic Field on the Oxidative Stress of SH-SY5Y Cells

Emilli Caroline Garcia Frachini [1], Jean Bezerra[2], Henning Ulrich[2], Denise Freitas Siqueira Petri[1]
[1]Depto de Química Fundamental, Univ. de São Paulo (SP, Brasil), [2]Depto de Bioquímica, Univ. de São Paulo (SP, Brasil)

INTRODUCTION: A common feature in neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases, is oxidative stress. Administration of levodopa/carbidopa is the most used therapy for Parkinson's disease. However, the development of new non-invasive treatments that avoid cell aging processes is required. Magnetic stimulation has been a promising candidate for the treatment of neuronal disorders because the presence of an external magnetic field (EMF) can improve cell differentiation and decrease cell damage. OBJECTIVES: Herein, the objective is to evaluate the effect of EMF on the hydrogen peroxide (H2O2)-induced oxidative stress of SHSY-5Y cells MATERIALS AND METHODS: human neuroblastomas SHSY-5Y cells were cultured in DMEM/F-12 medium supplemented (1% penicillin/streptomycin and 10% FBS) at 37°C and 5%CO2. The cells were seeded at a density of 1x105cell/mL and after 24h the cells were exposed to 0.3mM of hydrogen peroxide (H2O2) and EMF of 0.4T. MTT analyses were carried out after 16h, 48h and 96h. Magnetic stimulation was designed using NdFeB magnets as shown in Figure 1A. The intracellular reactive oxygen species (ROS) were measured by incubating the cells with 50µM 2',7'-Dichlorodihydrofluorescein diacetate (DCFH-DA) for 1h at 37°C in the dark. Then, the DCFH-DA solution was removed and cells were exposed to 0.3mM of H2O2 and EMF. The intracellular ROS production was measured at excitation/emission wavelengths of 485/525 nm for up to 2h after the exposition. DISCUSSION AND RESULTS: The magnetic stimulus significantly reduced H2O2-induced cell death by about 30% and the statistical analysis indicated that EMF significantly improved the cell viability with $p < 0.005$ (Figure 1B). Besides, EMF also contributed to a significant decrease in the initial stage of intracellular ROS production (Figure 1C). CONCLUSION: The results suggest that exposure to EMF contributes to the protection against H2O2-induced oxidative stress due to a decrease in the ROS level.
Keywords: magnetic field, neurodegenerative diseases, stress oxidative
Supported by: FAPESP, CAPES

N.03 - *In vitro* Studies of New Copper Chelators to Act in the Treatment of Alzheimer's Disease

Mariana Leticia Munin Camargo [1], Rafael Nunes Gomes[1], Giselle Cerchiaro[1]
[1]Centro de Ciências Naturais e Humanas, Universidade Federal do ABC (São Paulo, Brazil)

INTRODUCTION: Alzheimer's disease is a neurodegenerative disease with symptoms such as memory loss and language difficulties. The main characteristic of brains affected by Alzheimer's is the accumulation of the β-amyloid protein fragment, called β-amyloid plaques, outside the neurons. Research indicates that a failure in copper homeostasis can lead to metal overload in the brain, directly linked to these plaques' formation. Several studies have focused on studying chelators for the treatment of the disease, but many are restricted to existing molecules. OBJECTIVES: Thus, this research aims to prepare new selective copper chelators and investigate their efficiency in removing the metal ion from beta-amyloid fibrils so that they can be drug candidates in the treatment of Alzheimer's disease. MATERIALS AND METHODS: For this, five imine compounds were prepared, from the reaction of isatin with different amines, in ethanol or methanol as a solvent and pH adjustment with HCl, when necessary. The solid products were isolated after simple filtration or evaporation of the solvent in a rotary evaporator. DISCUSSION AND RESULTS: The new compounds were characterized by infrared and ultraviolet-visible spectroscopy techniques, NMR, and mass spectrometry, which indicated the formation of the products. For *In vitro* studies, oligomers of beta-amyloid protein with 42 amino acids were prepared, used in competition studies carried out using the EPR technique so that the removal of Cu(II) from the protein was monitored, as well as the formation of Cu(II) complexes with the synthesized ligands. All ligands were able to remove the metal ion from beta-amyloid, and the compound with the best result was able to remove copper from the protein with five equivalents. CONCLUSION: The results indicate that the compounds have the potential to proceed to *In vivo* studies.

Keywords: Alzheimer's disease, Copper chelator, Treatment

N.04 - Genistein Prevents the Decrease in Ganglioside Levels Induced by Amyloid-beta in the Frontal Cortex of Rats

Fernanda dos Santos Petry [1], Juliana Bender Hoppe[1], Caroline Peres Klein[1], Bernardo Gindri dos Santos[1], Régis Mateus Hözer[1], Christianne Gazzana Salbego[1,2], Vera Maria Treis Trindade[1,2]
[1]Programa de Pós-Graduação em Ciências Biológicas: Bioquímica, Universidade Federal do Rio Grande do Sul (Rio Grande do Sul, Brasil), [2]Departamento de Bioquímica, Universidade Federal do Rio Grande do Sul (Rio Grande do Sul, Brasil)

INTRODUCTION: Alzheimer's disease is a neurodegenerative disorder in which amyloid-beta (Aβ) peptide deposition in brain areas can be related to the imbalance of lipid composition of cell membranes, impairing neuronal functions. Genistein, one of the most abundant isoflavones in soy, has been shown to be able to modulate some of the pathogenic processes triggered by the Aβ peptide. However, it is still necessary to deepen the evaluation of its underlying mechanisms. OBJECTIVES: In this study, an *In vivo* model of Aβ toxicity was used to investigate the effects of this peptide and the treatment with genistein on the lipid composition (gangliosides, phospholipids, and cholesterol) in the frontal cortex of rats. MATERIALS AND METHODS: Adult male Wistar rats (aged 90 days) received bilateral intracerebroventricular infusions of Aβ1-42 (2 nmol) and genistein 10 mg/kg orally for 10 days. Frontal cortex was homogenized with chloroform:methanol for lipid extraction and ganglioside, phospholipid and cholesterol levels were evaluated. DISCUSSION AND RESULTS: The Aβ-infused animals showed a significant decrease in ganglioside concentration and relative reduction of GD1b and GQ1b species. Treatment with genistein prevented the decrease in ganglioside levels. Phospholipid and cholesterol contents did not show significant differences. CONCLUSION: Considering the pivotal roles of gangliosides on neuronal function, findings described here can contribute to the knowledge of the potential neuroprotective mechanisms of genistein against Aβ-induced alterations in the frontal cortex of rats and provide a novel view in the multifaceted scenario associated with its beneficial effects.

Keywords: Alzheimer's disease, genistein, lipids
Supported by: CNPq and INCT (EN 465671/2014-4)/CNPq

N.05 - The Effects of Hypercholesterolemia on Astrocytic Activation *In vivo* and *in vitro*

Gabriela Joras Baumart [1], Matheus Scarpatto Rodrigues[1], Hémelin Resende Farias[1], Natália Baltazar do Nascimento[1], Sámili W. da Silva[1], Tainá Schons[1], Sara L. Sulzbach[1], Pedro Rocha de Camargo[1], Jade de Oliveira[1]
[1]Biochemistry Department, Federal University of Rio Grande do Sul (RS, Brazil)

INTRODUCTION: Astrocytes are the main energy supplier in the brain and protect the central nervous system through a controlled inflammatory response. Under pathological conditions, such as neurodegenerative diseases, these glial cells can suffer morphological and functional changes. The increase in inflammatory mediators production in these cells can contribute to the spread of neuroinflammation and neuronal death hallmarks of neurodegenerative diseases. Studies have demonstrated the relationship between hypercholesterolemia and brain dysfunction characterized by hippocampal astrogliosis. OBJECTIVES: This study aims to evaluate the astrocytic activation in the development of brain alterations caused by hypercholesterolemia using *In vivo* and *In vitro* strategies. MATERIALS AND METHODS: We evaluated astrocytic activation in 3- and 14-month-old female and male C57BL/6 wild-type and LDL receptor knockout (LDLr-/-) mice, an experimental model of familial hypercholesterolemia (FH). To analyze the morphology of astrocytic cells from mices, immunofluorescence assay and confocal microscopy were performed on coronal sections of the hippocampus. Furthermore, in the vitro study, C6 glioma cells in high passages, which present characteristics of astrocytes, were exposed to human low-density lipoprotein (LDL). In these cells, we analyzed proliferation, viability, and oxidative stress parameters through MTT, DCFH-DA, and sulforhodamine B assay. DISCUSSION AND RESULTS: Three-month-old LDLr-/- mice presented increased total length and number of processes of astrocytes in comparison to the C57BL/6 wild-type mice of the same age. The 14 months old LDLr-/- displayed more severe astrocyte activation than young LDLr-/- mice. Finally, the exposure to LDL appears to be contributing to cell proliferation *In vitro* but did not cause alterations in the reactive species production. CONCLUSION: We propose that astrocytic activation induced for hypercholesterolemia may contribute to the development of neurodegenerative disease. CNPq and CAPES.
Keywords: Astrocytes, Hypercholesterolemia, Neuroinflammation / **Supported by:** CNPq and CAPES

N.06 - Lipopolysaccharide-induced neuroinflammation alters expression of REST and members of the repressive CoRest complex in the hippocampus

Liebert Bernardes Carvalho[1], Paula Lemes dos Santos Sanna[1], Camila Cristina dos Santos Afonso[1], Rodrigo Augusto da Silva[2], **Alex de Camargo Coque** [2], Alexandra Latini[3]
[1]Dentistry, University of Taubaté (SP, Brasil), [2]Center for Epigenetic Study and Genic Regulation-CEEpiRG, Paulista University (SP, Brasil), [3]Laboratory of Bioenergetics and Oxidative Stress-LABOX, Federal University of Santa Catarina (SC, Brasil)

INTRODUCTION: The transcriptional silencing factor - RE1 (REST) is a transcriptional repressor that acts to control the expression of neural genes by binding to the repressor element 1. Currently, its neuroprotective function has gained special prominence mainly due to its low expression in the brain of adults, increased during normal aging so that it can repress the expression of pro-apoptotic genes, thus reducing the vulnerability of neurons to oxidative stress. OBJECTIVES: Thus, knowing that neuroinflammation has been strongly associated as a risk factor for the development of neurodegenerative diseases, this project aimed to evaluate the effect of neuroinflammation on the epigenetic regulation of REST in the CNS. MATERIALS AND METHODS: : Using a model of neuroinflammation induction by lipopolysaccharide (LPS) (i.p. - 0.33mg/kg) the impact of neuroinflammation on epigenetic regulation of REST was evaluated by qPCR and western blotting. DISCUSSION AND RESULTS: The neuroinflammatory effect of i.p. of LPS was confirmed by increased gene expression of pro-inflammatory cytokines. Increased expression of active and truncated forms of REST and of Hotair and Hottip Long non-coding RNAs (lncRNAs) was also demonstrated. Also the increase in gene expression of members of the repressive complex CoRest, Lsd1, Rcor1 and Rcor2. In this study, no correlation was observed between the expression of REST and members of the CoRest complex with the methylation status of the promoter region. The fact that the increase in the expression of the active and truncated form of Rest by neuroinflammation was not mediated by DNA methylation and the increase in the expression of lncRNAs, Hotair and Hottip and members of the repressive CoREST complex is indicative that several molecular mechanisms may be at play. acting together. CONCLUSION: However, the limited amount of information related to the impact of neuroinflammation on lncRNA expression, especially Hotair and Hottip in the CNS, was a limiting factor for our conclusions.
Keywords: Neuroinflammation, REST, Neuroepigenetic / **Supported by:** CAPES/PROSUP

N.07 - Antioxidant Response of Purple Pitanga Extract (Eugenia Uniflora) on MPTP-Induced Acute Oxidative Stress in Rats.

Eduarda Monteiro Fidelis [1], Eduarda Fidelis[1], Anne Savall[1], Jhuly Mello[1], Caroline Quines[1], Tuane Sampaio[1], Simone Pinton[1]
[1]Pós-Graduação Em Bioquimica, Univesidade Federal Do Pampa (Rio Grande Do Sul, BRASIL)

INTRODUCTION: Parkinson's disease (PD) is characterized by the death of dopaminergic neurons in the substantia nigra pars compacta. The neurotoxin 1- methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is used in experimental models of PD to induced dopaminergic neurons death and oxidative stress in the brain. To mitigate deleterious effects, an alternative is the use of substances rich in polyphenols, such as Eugenia Uniflora, which has an antioxidant and anti-inflammatory effect. OBJECTIVES: Of this study was to evaluated the effect of purple pitanga (fruit of Eugenia Uniflora) hydroalcoholic extract on acute oxidative stress induced by MPTP in rats. MATERIALS AND METHODS: Male Wistar rats (250-300 g) aged 3 months (CEUA 010/2021) were divided into groups: (Control; Pitanga; MPTP and MPTP+Pitanga). The control and MPTP groups received saline vehicle (3ml/kg, oral route) and groups treated received the pitanga (1000mg/kg o.r). After 24 hours, MPTP (0.1mg/nostril) or vehicle was administered via intranasal. After 6 hours, rats were dead and the olfactory bulb (BO), striatum (ES) and substantia nigra (SN) were dissected, oxidative stress markers as reactive oxygen species (ROS); thiobarbituric acid reactive species (TBARS); non-protein thiols (NPSH) and 4-hydroxyl-2-nonenal (4HNE) were analyzed. DISCUSSION AND RESULTS: MPTP increased the ROS and the NPSHlevels in SN, however, MPTP decreased the NPSH levels in BO. Pre-treatment with pitanga was effective in protected against the alteration in NPSH levels in both SN and BO. No difference was observed in TBARS levels in all structures analyzed. The 4HNE levels were altered by MPTP in BO and ES and the pitanga was effective in reduces the 4HNE levels in BO. CONCLUSION: The pitanga was beneficial effects against MPTP toxicity by regulate the levels of NPSH and 4HNE, probably due to the antioxidant properties of the extract. Thus, the pitanga has potential to be useful in the treatment of oxidative stress-related disorder.
Keywords: Parkinson, lipid peroxidation, lipid peroxidation / **Supported by:** CNPq, CAPES, FAPERGS and UNIPAMPA

N.08 - Investigation Of The Neuroprotective And Anti-Neuroinflammatory Potential Of Marine Sponges

Catarina de Jesus Nunes [1], Cinthia Cristina de Oliveira Santos Costa[1], Luciano de Jesus Souza[2], Emilio Lanna[3], Ronan Batista[2], Ravena Pereira do Nascimento[1], Silvia Lima Costa[1]
[1]Department of Biochemistry and Biophysics, Federal University of Bahia (Bahia , Brazil), [2]Department of Organic Chemistry , Federal University of Bahia (Bahia , Brazil), [3]Department of Biology, Federal University of Bahia (Bahia , Brazil)

INTRODUCTION: Neurodegenerative diseases are characterized by the progressive loss of neurons, resulting in disability and death. They have different etiologies, but some show similar components, such as oxidative stress and exacerbated inflammation. Oxidative stress leads to cell death through mitochondrial dysfunction, causing bioenergetic imbalance with subsequent neuronal damage and brain injury, associated with neuroinflammation, orchestrated mainly by glial cells, that contributes to the progression of neurodegeneration. Hence, the search for new therapies from natural compounds aims to discover new neuroprotective substances with neuroprotective and anti-neuroinflammatory effects.OBJECTIVES: To analyze cytotoxicity of extracts from marine sponges to neuronal cells, and to determine the best candidates, considering the viability parameters. MATERIALS AND METHODS: Cultures of neuronal PC12 cells were treated with a total of 23 different extracts (0.1 to 200 µg/mL) obtained with different solvents (methanol, ethyl acetate and chloroform) from 9 species of sponges from the coast of Salvador, Bahia in Brazil, and cell viability was determined after 24 h treatment by MTT test. DISCUSSION AND RESULTS: Most extracts showed toxicity only at the highest concentrations of 100 and 200 µg/mL adopted, but chloroform and methanolic extracts did not show cytotoxicity at concentrations of 0.1, 1 and 10 µg/mL. Moreover, cultures treated with the C1, F1, I1 and J1 chloroform extracts and with the F3 and H3 methanolic extracts, presented the greater amounts of cells that metabolized MTT. Ethyl acetate extracts did not show significant results in the evaluated parameters. These results encourage continuity of studies in neuroinflammation models to determine extracts and its components best candidates and potential application for neurodegenerative diseases.
Keywords: Marine sponge , Neurodegenerative diseases, Neuroprotection
Supported by: National Council for Scientific and Technological Development- Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

N.09 - Behavioral assessment in rats submitted to neurodegeneration and antioxidant therapy

Ilma Regina dos Santos Alves [1], Miguel Tabanez[1], Giselle Cerchiaro[1]
[1]Centro de Ciências Naturais e Humanas, Universidade Federal do ABC (, Brasil)

INTRODUCTION: Introduction: The study of neurodegenerative diseases has grown exponentially, influenced by the growing number of cases of these diseases. Hydroxytyrosol (HT), a polyphenolic compound found in olive oil, and is part of the Mediterranean diet, is associated with reducing the incidence of neurodegenerative diseases. OBJECTIVES: Objectives: This study evaluated the neuroprotective effects of hydroxytyrosol in an animal model of Alzheimer's disease, assessing behavioral measures in male Wistar rats induced to neurodegeneration by Streptozotocin (STZ) with intracerebroventricular injections. MATERIALS AND METHODS: Material and Methods: Control animals received 4 µL of vehicle, pH 4.5 citrate solution. STZ animals received 4 µL of 3 mg/kg STZ solution. HT+ STZ animals received 1 µL of 450 µM HT solution and, after one hour, 3 µL of 3 mg/kg STZ. After 30 days, the animals were submitted to behavioral tests in the Barnes Labyrinth, with 04 days of training and 01 day of the final test. DISCUSSION AND RESULTS: Results and Discussion: In training, escape time in the target hole, distance traveled to the target hole, and average speed in the maze were evaluated. In the distance covered in the maze, the group treated with STZ explored significantly more in the second attempt of the first day, in which the vehicle group and STZ+HT already reduced the distance covered. In the final test, several parameters were analyzed: escape time, time on target, percentage of exploration of the target, rate of time in the quadrant, number of quadrant exits and total distance traveled. The only parameter that obtained a significant difference was the percentage of exploration of the target. The investigation of the STZ group in the target was significantly lower than the vehicle group. CONCLUSION: Conclusion: Based on the results, there was no statistical difference between the groups in the parameters evaluated, and we suggest that the method with HT injections be improved.

Keywords: Neurodegeneration, Streptozotocin, Hydroxytyrosol

N.10 - The Ketamine Metabolite HNK Activates mRNA Translation Signaling Pathways

Felipe Campos Ribeiro[1], Danielle Cozachenco Ferreira[1], João David Calixtro Costa[1], **Rubens Leal Soares Neto** [1], Fernanda Guarino De Felice[1,8,9,10], Mychael Vinícius da Costa Lourenço[1], Sergio Teixeira Ferreira[1,11]
[1]Institute of Medical Biochemistry Leopoldo de Meis, Federal University of Rio de Janeiro (RJ, Brazil), [8]Department of Biomedical and Molecular Sciences, Queen's University (ON, Canada), [9]Department of Psychiatry, Queen's University (ON, Canada), [10]D'Or Institute for Research and Education, D'Or Institute for Research and Education (RJ, Brazil), [11]Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro (RJ, Brazil)

INTRODUCTION: Alzheimer's disease is characterized by impaired synaptic plasticity and progressive memory deficits. Converging evidence indicates that hippocampal mRNA translation, required for memory consolidation, is defective in AD. However, effective pharmacological approaches targeting brain protein synthesis remain elusive. In recent years, ketamine has been characterized as an antidepressant at subanasthetic doses, and it has been shown to engage protein synthesis-related pathways OBJECTIVES: Investigate whether HNK activates signaling pathways that control mRNA translation and dissect the underlying mechanisms MATERIALS AND METHODS: Hippocampal slices were obtained and allowed to recover in artificial cerebrospinal fluid. Mice received i.c.v. infusions of AβOs and hippocampal slices were prepared 7 days later. Newly synthesized polypeptides were detected using surface sensing of translation (SUnSET). Hippocampal slices were dissociated, centrifuged, and the supernatant was collected for Western blot analysis. Protein concentration was determined, and samples were prepared to a final concentration of 3µg/µL. After boiling, 50µg of total protein were loaded per lane and resolved in gels. Proteins were then transferred to a nitrocellulose membrane and incubated with respective primary antibodies. DISCUSSION AND RESULTS: We show that HNK stimulates the activation of hippocampal extracellular signal-regulated kinase 1/2 (ERK 1/2) and p70S6 kinase 1 (S6K1)/S6 signaling, known to contribute to protein synthesis and synaptic plasticity. We further found that HNK-induced S6 phosphorylation was mediated by mechanistic target of rapamycin (mTOR) in hippocampal slices. Moreover, HNK corrects AβO-induced deficits in hippocampal protein synthesis. CONCLUSION: Our findings demonstrate that HNK targets translational control pathways and rescues protein synthesis in the hippocampus of an ex-vivo AD model. Results further raise the prospect that HNK could emerge as a therapeutic approach to correct defective protein synthesis in neurodegenerative diseases.

Keywords: Alzheimer's disease, mRNA translation, HNK

N.11 - PROJECT - Study Of The Cytoprotective Action Of Compounds Present In The Dichloromethane Extract Of Amburana Cearensis Seeds (EDAC) In An Acute Ischemic Stroke Model

Flávia Sanches[2], Jéssica Teles Souza[2], Florisvaldo Ramos[2], Maria de Fátima Dias Costa[2], Silvia Lima Costa[2], Paulo Roberto Ribeiro de Jesus[3], Victor Diogines Amaral da Silva[2]
[2]Departamento de Bioquímica e Biofísica, Universidade Federal da Bahia (BA, Brasil), [3]Departamento de química orgânica, Universidade Federal da Bahia (BA, Brasil)

INTRODUCTION: Glutamatergic excitotoxicity is one of the pathophysiological mechanisms present in chronic and acute neurodegenerative diseases, such as ischemic stroke. Excess glutamate promotes apoptosis in neurons by increasing the entry of Ca2+ into the cell, resulting in DNA damage and cell death. On the other hand, astrocytes actively control the glutamate excess to prevent neuronal death. The improvement of astrocytic function by compounds present in EDAC were recently demonstrated in our previous studies as the potential mechanism involved in neuroprotective action of EDAC against oxygen glucose deprivation. However, more studies need to be done to characterize coumarin, the main compound in EDAC, as the pharmacologically active agent. It is known that cerebral ischemia can lead to the activation of cascades such as MAPK, increasing the expression of proteins such as ERK1/2, which will modulate the expression of enzymes such as glutamine synthetase (GS), an astrogliosis marker, which converts glutamate to glutamine, a non-toxic amino acid. OBJECTIVES: Therefore, this study aims to investigate the effects of EDAC and coumarin in the regulation of astrogliosis markers and proteins of MAPK pathway in an acute ischemic stroke model. MATERIALS AND METHODS: Thus, CNS cells from Wistar rats will be subjected to oxygen and glucose deprivation and/ or treated with EDAC and coumarins from A. cearenses seeds. Subsequently, cell viability will be evaluated by MTT and propidium iodide techniques. Additionally, the expression of astrogliosis markers (GS and GFAP) and MAPK pathway proteins will be investigated by western blot. The analysis of the interaction of coumarin with proteins of the MAPK pathway will be performed through Molecular Docking and Molecular Dynamics. DISCUSSION AND RESULTS: At the end of this project, it is expected to clarify the mechanisms of action related to the neuroprotective effect of compounds from A. cearenses seeds in a study model of cerebral ischemia.

Keywords: Cerebral ischemia, Amburana cearensis, ERK/MAPK pathway
Supported by: Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB)

N.12 - Parâmetros Oxidativos E Membranares Em Hipocampos De Ratos Wistar Com Desenvolvimento Intrauterino Na Ausência De Melatonina Materna Ou Na Presença De Ácido Valproico: Uma Possível Relação Com O Autismo.

Eduardo Henrique de Paula Ribeiro [1]
[1]Laboratório de Fisiologia Animal, Universidade Federal de São João del Rei (Minas Gerais, Brasil)

INTRODUCTION: Autistic spectrum disorders (ASD) are caused by both genetic components and environmental factors. Prenatal exposure to valproic acid (VPA) is a robust model used to study ASD. ASD may be related to insufficient levels of melatonin, a hormone produced by the pineal gland, which regulates the circadian cycle, with antioxidant functions. Elevations in oxidative stress parameters are reported in individuals with ASD. ASD are more prevalent in males. OBJECTIVES: To investigate the relationships between oxidative stress, ASD and melatonin. MATERIALS AND METHODS: Extraction of hippocampi from offspring of (1) rats treated with VPA on the 13th day of pregnancy; (2) rats pinealectomized before mating; (3) rats pinealectomized before mating with oral melatonin supply; (4) SHAM rats, with surgical procedure without removing the pineal gland; (5) naive rats. Data were tested for normality and compared by analysis of variance. Results were expressed as mean ± SEM. Amounts of hydrogen peroxide (H2O2; nmol/ug) and reduced glutathione (GSH; nmol/ug) were determined. DISCUSSION AND RESULTS: H2O2 - Naïve, males (62,09±3,288), females (52,83±4,009); VPA, males (50,23±9,209), females (63,65±5,931); MEL, males (61,60±10,35), females (53,44±1,744); PTX, males (58,88±5,863), females (45,31±2,779); SHAM, males (63,91±11,12), females (65,28±9,278). GSH - Naive, males (51,33± 5,075), females (51,80± 4,212); VPA, males (48,74±3,083), females (55,90± 4,663); MEL, males (47,97± 3,172), females (53,09± 2,920); PTX, males (50,30±5,639), females (45,85± 3,595); SHAM, males (56,52± 5,580), females (45,96± 5,151). The treatments do not seem to cause changes in the hippocampi of young rats, considering the markers and conditions studied here ($p > 0,05$). Additional experiments are being conducted to increase the number of animals in each group, strengthening the statistical analysis.

Keywords: Autismo, Estresse oxidativo, Melatonina

N.13 - Behavioral and Biochemical Evaluation in Cortex and Hippocampus of Cafeteria Diet-Fed Rats Treated with Atorvastatin

Ítalo Leonardo Diogo [1], Alexandre Martins Nascimento[1], Leandro Augusto de Oliveira Barbosa[1], Hérica de Lima Santos[1], Vanessa Faria Cortes[1], Valéria Ernestânia Chaves[1], Luciana Estefani Drumond de Carvalho[1]
[1]Campus Centro-Oeste, Universidade Federa de São João del-Rei (MINAS GERAIS, Brasil)

INTRODUCTION: Obesity is classified as a chronic disease. It causes a mitochondrial dysfunction and an inflammatory process in the body, increasing reactive oxygen species, which can cause damage to the cell and lead to apoptosis. Besides biochemical changes, behavioral changes have also been observed as a result of obesity. Atorvastatin is a drug used to treat hypercholesterolemia and hypertriglyceridemia associated with obesity. OBJECTIVES: To evaluate behavioral and biochemical parameters in the hippocampus and cortex of male Wistar rats fed with cafeteria diet treated with atorvastatin. MATERIALS AND METHODS: Animals were divided into four groups: commercial diet-saline (CoSal), commercial diet-atorvastatin (CoAtor), cafeteria diet-saline (CafSal), and cafeteria diet-atorvastatin (CafAtor). Atorvastatin was administered daily by gavage in doses of 10 mg per mouse body weight (kg), the same applied for saline (0.9%) administration. The cafeteria diet-fed groups received 20% sucrose supplemented water ad libitum. Exploration and memory tests were performed. On the 25th day of the experimental procedure, the animals were euthanized, and cortex and hippocampus was dissected to evaluate the expression of Apoptosis-Inducing Factor (AIF), caspase-8 and caspase-9 by western blot. DISCUSSION AND RESULTS: Cafeteria diet caused hypomobility and spatial memory deficit, while atorvastatin inhibited these effects. The novel object recognition memory was not affected by the diet, but atorvastatin administration alone caused problems in this parameter. There was no difference in AIF expression between groups. Both caspases 8 and 9 expressions were increased by cafeteria diet and were normalized using atorvastatin. CONCLUSION: These results indicates that atorvastatin exerts neuroprotective effect in cafeteria diet-fed groups, and it can be associated with the caspase apoptosis pathway decrease. Furthermore, atorvastatin alone may cause damage in the novel object recognition memory.
Keywords: Neuroprotection, Cafeteria Diet, Atorvastatin / **Supported by:** PROPE/UFSJ and FAPEMIG

N.14 - PROJECT: Investigation of the Neuroprotective and Anti-Inflammatory Potential of Flavonoid Apigenin Conjugated with β-Cyclodextrins

Cinthia Cristina de Oliveira Santos Costa[1], Catarina de Jesus Nunes[1], Camila Miranda Magalhães[1], Victor Diógenes Amaral da Silva[1], Silvia Lima Costa[1], Ravena Pereira do Nascimento[1]
[1]Bioquímica e Biofísica, Universidade Federal da Bahia (Bahia, Brasil)

INTRODUCTION: Spinal cord injury (SCI) is a highly prevalent condition associated with significant morbidity and mortality. Neuroinflammation is one of the main pathophysiological mechanisms for secondary spinal cord injury. However, current therapies for the treatment of spinal cord injury are limited. The literature demonstrates the neuroprotective effects of the flavonoid apigenin in different models of study of the central nervous system, but the role of apigenin in spinal cord injury is still little explored. OBJECTIVES: The aim of this study is to evaluate the neuroprotective effects of the flavonoid apigenin and conjugates of apigenin with β-cyclodextrins in spinal cord injury models. The specific objectives are: to evaluate the cytotoxicity of apigenin and its conjugates to determine the best candidates considering viability parameters in spinal cord cells in the *In vitro* model of spinal cord trauma; to evaluate the effect of apigenin and the best conjugates on the immune response of spinal cord cells in the ex vivo model of spinal cord trauma; and to characterize the morphological response of cells treated with the different conjugates in the *In vitro* and ex vivo model of spinal cord trauma. MATERIALS AND METHODS: At first, the cytotoxicity of a panel of apigenin conjugated to different cyclodextrins will be evaluated to identify the most promising compounds. The *In vitro* and ex vivo spinal cord models will be subjected to injury by chemical and physical methods respectively and, later, will be treated with the best apigenin conjugates. The evaluation of the neuroprotective and anti-inflammatory effect of the flavonoid will occur through rT-PCR for spinal cord cytokines, chemokines and neurotrophins. The analysis of cell morphology and phenotypes will be performed by immunocytochemistry and immunohistochemistry.
Keywords: spinal cord, apigenin, neuroprotection / **Supported by:** FAPESB

O - Omics and Systems Biology

O.01 - A Comprehensive Phenotypic Comparison of The Effects of Putative Dietary Restriction Mimetics in C. elegans

Deisi Lilian Braga Shimo [1], Adriana Leandra Santoro[1], Silas Pinto da Silva[1], Diogo de Moraes[1], Carlos Alberto Vergani Junior[1], Felippe Mousovich Neto[1], Raul Gobato da Costa[1], Augusto Peluso[2], Lars Roed Ingerslev[2], Jonas T. Treebak [2], Alex Inague[3], Sayuri Miyamoto[3], Marco Yoshinaga[3], Marcelo Alves da Silva Mori[1]
[1]Biochemistry and Tissue Biology, University of Campinas (São Paulo, Brazil), [2]Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen (Copenhagen, Denmark), [3]Biochemistry, University of São Paulo (SP, Brazil)

INTRODUCTION: Dietary restriction (DR) extends lifespan and delays the onset of age-related diseases across species. Interventions that act on molecular pathways triggered by DR are good candidates to confer the beneficial effects of the diet without the need to reduce calorie consumption. The complex I inhibitor metformin, the NAD+ donor nicotinamide riboside (NR) and the sirtuin activator pterostilbene (PT) have all been proposed to act on evolutionarily conserved pathways associated with aging and the mechanisms of DR, thereby potentially mimicking the effects of DR. OBJECTIVES: To study the effects of putative DR mimetics on *C. elegans* and identify mechanisms and phenotypic characteristics that are commonly manifested by these interventions. MATERIALS AND METHODS: We measured lifespan, oxidative and heat stress, food intake, lipid accumulation, oxygen consumption, and the transcriptomic and lipidomic profile of *C. elegans* subjected to DR [*eat-2*(ad1116) mutants] or putative DR mimetic interventions [metformin, NR, PT or the combination of NR and PT (NRPT)]. DISCUSSION AND RESULTS: None of the putative DR mimetics entirely recapitulated the effects of DR, although metformin was the intervention that more closely resembled DR, with increased lifespan, reduced lipid accumulation and a transcriptomic and lipidomic profile that overlapped to some extent. NR and NRPT did not alter lifespan and had only mild effects on all the other parameters, although reduced fat accumulation was also observed. PT alone did not affect lifespan or total fat content but resulted in widespread changes in the transcriptome and lipidome which were suppressed by NR supplementation. Metformin, NR and NRPT required S-adenosylmethionine synthase SAMS-1, sirtuin SIR-2.1 and alcohol dehydrogenase SODH-1 to reduce fat accumulation, and acted independently of the bacteria used as the food source. CONCLUSION: In summary, some, but not all, features of DR can be mimicked by interventions that affect metabolism but do not alter caloric intake.
Keywords: Aging , C. elegans; , Dietary restriction; / **Supported by:** FAPESP

O.02 - Nucleic acids are present in Bothrops jararaca snake venom and extracellular vesicles.

Ketlenn Camila Miranda de Barros [1], Larissa Gonçalves Machado[1], Russolina Benedeta Zingali[1]
[1]Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro (Rio de Janeiro , Brasil)
INTRODUCTION: Snake venoms are mainly composed of proteins and other classes of molecules that exist in low concentrations, such as nucleosides, lipids, organic acids, and RNAs. Among those molecules, RNAs are the most unexpected to be found because the venoms are a rich degrading environment for nucleic acids, since they possess a set of enzymes with nuclease activity. On the other hand, it was already shown that venoms contain extracellular vesicles (EVs), which act by transporting bioactive molecules such as lipids, proteins, and nucleic acids for cellular communication. OBJECTIVES: Given the biology of EVs and their conserved nucleic acid content, our hypothesis is that they may be involved in the unusual stability of RNAs in snake venoms. Understanding the constitution of venom-RNAs is the first step to comprehend their possible biological roles in envenoming. MATERIALS AND METHODS: To test our hypothesis, we isolated EVs from Bothrops jararaca venom. Afterwards we purified total RNA from those EVs and from different venom fractions using traditional phenol-chloroform extraction and a miniprep based system (RiboPure). DISCUSSION AND RESULTS: Proteomic data revealed that EVs are enriched with a sort of RNA-binding proteins (for example: RNA-binding protein 3 and HNRPQ). Our results show that isolated EVs are the venom fraction most enriched with RNA, which is in accordance with EVs from other biological sources. In parallel, crude venom is positive for RNAs and the yield of extracted RNA is lower in the venom depleted of vesicles. These results reinforce our initial hypothesis that EVs play a role in the conservation of RNAs in snake venoms. CONCLUSION: In addition to quantify RNA enrichment in the venom fractions, the investigation of qualitative differences of the RNA content is in progress, furthermore it is necessary to distinguish the presence of small RNAs and mRNAs in EVs or venom fractions.
Keywords: Snake venom, extracellular vesicles, RNA / **Supported by:** CNPQ - FAPERJ

O.03 - *Bothrops pauloensis* Snake Venom: An Ontogenetic Approach

Lídia Jorge Tasima [1,2], Daniela Miki Hatakeyama[1,2], Weslei da Silva Aguiar[1,2], Eduardo Oliveira Venâncio de Lima[1,2], Sávio Stefanini Sant'Anna[1], Kathleen F. Grego[1], Karen de Morais-Zani[1,2], Anita Mitico Tanaka-Azevedo[1,2]
[1]Laboratório de Herpetologia, Instituto Butantan (São Paulo, Brazil), [2]Interunidades em Biotecnologia (ICB-IPT-IBut-IB-FMVZ-EP), Universidade de São Paulo (São Paulo, Brazil)

INTRODUCTION: Considerable heterogeneity in venom composition has already been observed in different species of snakes within the Viperidae family. Since the venom of young and adult can cause distinct pathological effects and because the antivenom serum may be less effective in neutralizing envenoming by young snakes compared to adults, it is of paramount importance to understand the ontogenetic variation of snake venom. OBJECTIVES: Thus, the present study aimed to analyze and compare the venom of *Bothrops pauloensis* snakes, searching for possible influences of ontogeny and sex in their biochemical aspects. MATERIALS AND METHODS: *B. pauloensis* venoms were collected and separated into pools according to age and gender: neonates, 1-year-old, 2-years-old, 3-years-old, and adults. SDS-PAGE and HPLC, in addition to PLA_2, LAAO and coagulant activities were performed. DISCUSSION AND RESULTS: The venom of younger individuals was more complex in relation to high molecular mass proteins, with a greater abundance of metalloproteinases, while adults showed a greater abundance of medium and low molecular mass proteins, such as phospholipases A_2 (PLA_2), C-type lectins and serine proteases. Younger snakes showed higher coagulant activity, while adult snakes showed higher L-amino acid oxidase (LAAO) activity. Differences between males and females were observed mainly in the rate of loss of coagulant activity and change in PLA_2 activity. While males presented an increase of PLA_2 activity, females presented a decrease. Furthermore, considering only the adult groups, males showed a higher LAAO, besides the PLA_2 activity. CONCLUSION: With this, it was possible to conclude that there is an ontogenetic variation in the composition and some activities of the *B. pauloensis* snake venom, in addition to sexual differences, reinforcing that there is an intraspecific variation that may result in different symptoms in their envenoming and, consequently, differences in the response to treatment with the antivenom.

Keywords: Biotechnology, Venom variation, Sexual variation
Supported by: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES and FAPESP

O.04 - Quantitative proteomics analysis of the soybean caterpillar Anticarsia gemmatalis midgut in the presence of the Bacillus thuringiensis

Luís Felipe Costa Ramos [1], Yara Martins Silva[1], Gilberto Barbosa Domont[1], Cristiane Dinis Ano Bom[1], Fábio César Souza Nogueira[1], Magno Junqueira[1], Danielle Maria Perpétua Oliveira[1]
[1]Biochemistry department, Chemistry Institute, Federal University of Rio de Janeiro (Rio de Janeiro, Brazil)

INTRODUCTION: *Anticarsia gemmatalis* is one of the main defoliating soybean pests in Brazil, being an important target of pest control. *Bacillus thuringiensis* is a Gram-positive bacterium with wide global dispersion, characterized by the production of Cry toxins during the stationary phase of its growth, to which an entomopathogenic effect is attributed. That is why using this microorganism as a biopesticide to control agricultural pests, including *A. gemmatalis*. However, insects have developed resistance to this control. OBJECTIVES: This work aims to obtain the quantitative proteome of the midgut epithelium of *A. gemmatalis* after being challenged with *B. thuringiensis*. MATERIALS AND METHODS: A bioassay was performed to calculate the lethal concentration (LC50) of *B. thuringiensis*, using 35 caterpillars in six different concentrations of the sporulated bacteria, monitoring the survival rate for 96 hours. After the midgut dissection, protein extraction from epithelial tissue was performed using S-Trap column (Protifi®) protocol. Trypsin (Promega®) was used for the digestion step. After the iTRAQ labeling method, a chromatography fractionation step was applied, and samples were analyzed in Q-exactive Plus (ThermoScientific®) mass spectrometer. Protein identification was performed using Proteome Discoverer 2.4 (ThermoScientific®). DISCUSSION AND RESULTS: Bioassay results showed a LC50 of 0.073 mg/mL using *B. thuringiensis* sporulated bacteria against *A. gemmatalis*. More than 2,900 proteins from 24h exposed caterpillars were identified by the bottom-up proteomics approach using five biological replicates. A deeper proteome analysis will be performed since there is no description for this insect midgut. To overcome this difficulty, we plan to identify proteins using Fragpipe software (Nesvilab), together with de novo sequencing and homology search protocol, used to describe proteomes of unsequenced organisms, comparing up and down-regulated proteins after *B. thuringiensis* infection. In addition, a TMT labeling method will be applied to compare with previous results. CONCLUSION: The description of *A. gemmatalis* proteome during bacteria infection may be helpfull to enhance this bioinseticide effectiveness.

Keywords: Proteomics, Anticarsia gemmatalis, Bacillus thuringiensis infection / **Supported by:** CAPES,CNPq and FAPERJ

O.05 - LACEN: An R Package for lncRNA Functional Annotation Using Co-expression Networks

Leonardo Sanches [1], Gabriel Torres Gomes Pato[2], Diogo Vieira da Silva Pellegrina[1], João Eduardo Ferreira[3], Eduardo Moraes Rego Reis[1]
[1]Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo (SP, Brasil), [2]Programa Interunidades de Pós-Graduação em Bioinformática, Universidade de São Paulo (SP, Brasil), [3]Departamento de Ciência da Computação, Instituo de Matemática e Estatística, Universidade de São Paulo (SP, Brasil)

INTRODUCTION: Long non-coding RNAs (lncRNAS) are a heterogeneous class of transcripts with structural and regulatory roles in several biological processes, such as cellular proliferation, survival and differentiation. Therefore, aberrant lncRNA expression may be associated with cancer biology. Most lncRNAs have no known function, and the lack of knowledge regarding sequence and function relationships in RNA molecules makes it difficult to assign and test their biological roles. Gene co-expression network analysis has been used to identify network modules containing lncRNAs highly correlated with protein-coding genes and molecular pathways deregulated in cancer and based on a "guilt-by-association" approach infer lncRNA function. OBJECTIVES: Here we developed a computational pipeline for functional inference of lncRNAs that unifies several tools in a user-friendly interface, to facilitate hypothesis generation and the selection of candidates for experimental functional analysis. MATERIALS AND METHODS: The package was implemented in R (v4.1.2) and was based on the pipeline developed in our group for the annotation of lncRNAs deregulated in pancreatic tumors (Paixão et al., in press). It takes as input RNA-seq count data (TPM/FPKM) from tumor and adjacent tissue samples, differential expression gene list and the genome annotation. The data is filtered, log-VOOM transformed (limma, v3.50.0) and the obtained co-expression network modules (WGCNA, v1.70.3) are evaluated for gene/pathway enrichment (gprofiler2, v0.2.1) and summarized (rrvgo, v1,6,0). The package and all its dependencies are installed in a container (Docker, v20.10) and the front-end interface can be accessed via an internet browser. DISCUSSION AND RESULTS: The user may select the modules of interest based on the enriched pathways and inspect heatmaps displaying the lncRNAs most correlated to the protein coding genes in these modules. Those lncRNAs may be candidates for experimental functional analysis. CONCLUSION: The package is in the final development stage and will be validated with breast cancer data available in the TCGA consortium.

Keywords: Gene co-expression networks, lncRNA, câncer / **Supported by:** CNPq, CAPES e FAPESP

O.06 - Characterization of Acute Kidney Injury (AKI) Induced by Bothrops jararaca Venom

Taissa Nunes Guerrero [1], Maria de Lourdes S. do Naciemento[1], Mayara Amorim Romanelli Ferreira dos Santos[1], Dayene Santos Gomes[1], Marjolly Caruso Brigido[1], Lucienne da Silva Lara Morcillo[1], Russolina Benedeta Zingali[1]
[1]Instituto de Bioquímica Médica Leopoldo de Meis , Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil), [2]Instituto de Ciências Biomédicas , Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: Every year about 2.5 million people are victims of snake bites. In Brazil, the most medically relevant snake is the *Bothrops jararaca*. The symptoms of envenomation are acute inflammation at the bite site and bleeding disorders, which can lead to kidney failure and death. OBJECTIVES: Despite kidney failure being the main cause of death after poisoning, the kidney damage is not completely understood and needs to be well studied in *In vivo* models. Thus, the objective of this work is to characterize the acute kidney injury induced by *Bothrops jararaca* venom in rats. MATERIALS AND METHODS: Three doses of venom (3.5, 6 and 8 mg/kg) were tested. The control group received 0.9% saline solution. The venom was injected intramuscularly into male Wistar rats (CEUA: n°128/18). After the injection, the animals were kept in metabolic cages and the following parameters were analyzed after 24h: extent of muscle damage and kidney damage (urinary creatinine, proteinuria, plasma creatinine, blood urea nitrogen (BUN) and renal tissue histology). DISCUSSION AND RESULTS: All animals presented a hemorrhagic lesion at the injection site, the extent of the lesion was dose-dependent. Biochemical parameters indicated kidney damage: proteinuria increased 2.7-fold at the dose of 6 mg/kg; BUN increased about 1.5-fold and plasma creatinine increased about 2-fold at the three doses tested. Histological analyzes showed the following changes that occurred in a dose-dependent manner: atrophy and glomerular segmentation; Bowman's capsule space distention; interstitial edema; hemorrhage; collagen deposition in the cortical and medullary region. CONCLUSION: the occurrence of acute kidney injury was observed in a dose-dependent manner as demonstrated by decay in kidney function and confirmed by histological findings. Thus, here an *In vivo* model of kidney injury by B. jararaca venom was established for future work. Acknowledgements: CNPq, FAPERJ and CAPES.

Keywords: AKI, Bothrops jararaca, kidney injury / **Supported by:** CNPq

O.07 - Is there a variation of *B. jararacussu* snake venom from different ages and sex?

Weslei da Silva Aguiar [1,2], Nathália da Costa Galizio[1,2], Kathleen Fernandes Grego[1], Giovanni Perez Machado da Silveira[1], Karen Morais-Zani[1], Anita Mitico Tanaka-Azevedo[1]
[1]Laboratório de Herpetologia, Instituto Butantan (São Paulo, Brazil), [2]Instituto de Ciências Biomédicas, Universidade de São Paulo (São Paulo, Brazil)

INTRODUCTION: The variability in the activity and composition of venoms is a fact described in numerous studies and occurs at various biological levels, such as ontogenetic, geographic, sexual, diet, inter and intra-specific. Ontogenetic changes are observed in different snake species from different genus. The *Bothrops jararacussu (B. jararacussu)* snake belongs to the Viperidae family. These individuals show sexual dimorphism in both size and color. Adult individuals of this species have large amounts of PLA_2 in their venom composition, with most of this exhibiting myotoxic activity on their prey. OBJECTIVES: This present study aims to perform an ontogenetic analysis of *B. jararacussu* snake venom, following the development of newborn individuals in the Laboratory of Herpetology of Butantan Institute until adulthood. MATERIALS AND METHODS: Venom samples were obtained from *B. jararacussu* snakes born from the same litter at the Laboratory of Herpetology of Butantan Institute. Venom samples were subjected to SDS-PAGE, followed by HPLC on a C-18 column. DISCUSSION AND RESULTS: A protein band within the range of 15-10 kDa appears on the male individuals around 9 months of age, while in female ones this same band appears around 15 months. This band increase in size as the animals get older. The HPLC profile shows a peak eluted around the 45-minute mark that also increases over time, while the peaks eluted at the 80-90 region decrease in the same proportion. This peak/band, possibly a PLA_2 with myotoxic activity, increases in intensity with each subsequent extraction CONCLUSION: It was possible to observe that the transition of the protein profile from young to adult occurs around 15 months in the case of females and 9 months in the case of male individuals. We also observed that this change happens gradually. The greatest advantage of our work was the possibility of following the growth and maturing of venom from same individuals, from birth to adulthood.

Keywords: variability, ontogeny, biotechnology
Supported by: FAPESP (2017/01890-0; 2018/25786-0; 2017/16908-2; 2018/20651-0), CAPES and CNPq.

O.08 - *Naja kaouthia* and *micrurus* spp snake venoms similarities: a quest to enrich the antielapid serum venom pool

Beatriz Kopel [1,2], Caroline Serino-Silva[1], Nathália da Costa Galizio[1], Karen de Morais-Zani[1], Sávio Stefanini Sant'anna[1], Kathleen Fernandes Grego[1], Anita Mitico Tanaka-Azevedo[1]
[1]Laboratório de Herpetologia, Instituto Butantan (SP, Brasil), [2]Instituto de Química, Univ. de São Paulo (São Paulo, Brasil)

INTRODUCTION: *Naja kaouthia* is a medically important Asian snake from the *Elapidae* family. In Brazil, the *Elapidae* family is represented by the *Micrurus* genus, and the antielapidic serum is produced using *M. corallinus* and *M. frontalis venoms*. There are still barriers in the antielapidic serum production, such as breeding snakes from the *Micrurus* genus and the small amount of venom extracted from individuals. OBJECTIVES Due to evolutionary and venom composition resemblances between *N. kaouthia* and *Micrurus* genus, like the large amount of three-finger toxins (3FTx) and phospholipases A_2 (PLA_2), it is interesting to search for similar proteins in *N. kaouthia*'s venom as a possible alternative to enrich the pool of *Micrurus*' venoms used in the antielapid serum production. MATERIALS AND METHODS: The venom composition and function of 29 *N. kaouthia* individuals and three different *Micrurus* species, *M. altirostris*, *M. lemniscatus* and *M. spixii*, were evaluated by measurement of enzymatic activities of L-amino acid oxidase (LAAO), PLA_2 and proteolytic activity over azocasein, SDS-PAGE, RP-HPLC, ELISA and Western Blotting (using antielapidic and antinaja serums). DISCUSSION AND RESULTS: Analyzing the results obtained, the *N. kaouthia* venom enzymatic activities were most like the *M. altirostris* venom activities, which suggested similarities in both venom composition. SDS-PAGE profile of the four species had differences in the high molecular weight bands and only quantitative variations in the region correspondent to PLA_2 and 3FTx, between 10 and 15 kDa. RP-HPLC profiles of the four species were severely different, the *Micrurus* species presenting more abundance of peaks. Finally, the Western Blotting showed cross reactivity between a band of approximately 10 kDa from *M. lemniscatus* venom and the antinaja serum and between two bands among 10 and 15 kDa in *N. kaouthia* venom and the antielapidic serum. CONCLUSION: So, the recognized bands in *N. kaouthia*, probably 3FTx and PLA_2, have the potential to enrich the antielapid venom pool.

Keywords: Antivenom, Snake venom, Elapidae / **Supported by:** FAPESP (2018/25786-0 and 2021/05405-5)

O.09 - Metabolomic Profile of Human Breast Milk and Extracellular Micro Vesicles Characterization

Rodolfo Henrique Pereira Sellani [1], Pedro Nobre Azevedo[1], Bianca Teixeira Morais de Oliveira[2], Diana Mota Toro[3,4], Pedro Vieira da Silva Neto[3,4], Adriana Ferreira Lopes Vilela[1], Fausto Brunos dos Reis Almeida[2], Carlos Arterio Sorgi[1,2,4]
[1]Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo (, Brasil), [2]Bioquímica e Imunologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo (, Brasil), [3]Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo (, Brasil), [4]Bioquímica, Instituto de Ciências Biológicas, Universidade Federal do Amazonas, Amazonas (, Brasil)

INTRODUCTION: Breast milk's composition contains a wide variety of macromolecules, immunological agents, micro-extracellular vesicles (MEV) and genetic material. Gestation age, delivery mode and antibiotic use influence its composition. Breastfeeding is a great source of nutrients for babies, as it brings health benefits such as protection against respiratory infections and allergic diseases. Unfortunately, breastfeeding was not always possible, as mothers produce low amounts of milk and, consequently, preterm's health decreases, being hospitalized in an intensive care unit. In this case, feeding is made with formula or pasteurized milk donated by the hospital bank, however sterilization at high temperatures can denature proteins and cause cellular death, leading to activity loss. OBJECTIVES: This study aims to identify human milk metabolites, as well as, the MEVs characterization to performed better feeding supplementation for babies. MATERIALS AND METHODS: We evaluated the humam milk breast metabolite by optimize MS methodologies for metabolomic and lipidomic studies. The characterization and extraction of extracellular MEVs were made using differential ultracentrifugation, 10 kDa membrane filter and Western Blotting test for proof of EMVs. DISCUSSION AND RESULTS: MEVs in breast milk have high intrastructural diversity and components like micro-RNAs, growth factors, lipids and inflammatory mediators. The metabolomics reveals an complex metabolite profile, including carbohydrates, amino acids and complex lipids. CONCLUSION: According to the results, reconstituted pasteurized milk is the best way to feed preterms, causing higher newborn survival rates and less hospitalizations.
Keywords: breast milk, Micro-Extracellular Vesicles, Metabolomics

O.10 - Data-Independent Acquisition (DIA): an Approach to Quantify HDL Proteome with Precision

Douglas Ricardo de Souza Junior[1], Amanda Ribeiro Martins da Silva[1], Graziella Eliza Ronsein[1]
[1]Departamento de Bioquímica, Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: The field of high-density lipoprotein (HDL) research is evolving, and new roles for this particle in health and disease are being constantly unraveled. Mass spectrometry-based proteomics has played an important role in the determination of proteins that are present within HDL in different conditions. Although shotgun proteomics is the most used strategy in proteome research, the data-independent acquisition (DIA) approach is a promising quantification tool, mainly because it does not rely on the stochasticity of shotgun experiments. Different pipelines may be employed to analyze DIA data. Currently, there is no consensus in which proteomic pipeline best suits the HDL proteome. OBJECTIVES: Our goal was to evaluate how different pipelines performed in analyzing DIA data from HDL samples. MATERIALS AND METHODS: We prepared a quality control (QC) HDL sample and injected it multiple times in the same conditions for mass spectrometry analysis. We then analyzed those replicate quality control (QC) samples using different DIA pipelines. The coefficient of variation (CV) was obtained for multiple proteins in HDL and it was used as indicative of precision in the analyses. DISCUSSION AND RESULTS: We compared different pipelines for HDL proteome quantification using DIA data from QC HDL samples (n=11) and showed that CVs vary considerably for the same protein according to the strategy used. CONCLUSION: Our data revealed that majority of HDL proteins can be quantified by several approaches, but choosing the right pipeline may increase the confidence in the quantification used, indicated by lower CVs.
Keywords: high-density lipoprotein, quantitative proteomics, mass spectrometry / **Supported by:** FAPESP

O.11 - Metabolomic, Transcriptomic, Enzymatic and Physiological Profile of Seeds of Ricinus communis L. Submitted to Accelerated Aging

Luzimar Gonzaga Fernandez [1,2,3], Thamires Soares R. Jesus[1,2], Cristiane Dantas de Brito[2], Luiz Eduardo Vieira Del-Bem[4], Paulo R. Ribeiro de Jesus [1,3,2], Renato Delmondez De Castro[2], Henk W. M. Hilhorst[5], Wilco Ligterink[5]
[1]Bioquímica e Biofísica-PMBqBM, [2]Bioquímica e Biofísica-Laboratório de Bioquímica, Biotecnologia e Bioprodutos, [3]Química Orgânica, Metabolomics Research Group, Universidade Federal da Bahia (Bahia, Brasil), [4]Botânica, Universidade Federal de Minas Gerais (Minas Gerais, Brasil), [5]Wageningen Seed Lab, Laboratory of Plant Physiology, Wageningen University (Wageningen , Holanda)

INTRODUCTION: Accelerated ageing (AA) abides by submitting the seeds to high temperatures and humidities. Through the AA, it is possible to evaluate the seed vigour and behaviour at physiological, biochemical and molecular levels. OBJECTIVES: To evaluate the behaviour of BRS 188 Paraguaçu's seeds of Ricinus communis L. regarding the metabolomic, transcriptomic, enzymatic and physiological profiles before and after being submitted to accelerated ageing. MATERIALS AND METHODS: It was the AA standardization, initial characterization and moisture content of seeds, germination test, determination of electrical conductivity and pH exudate, tetrazolium test, lipid peroxidation BY malonaldehyde (MDA) levels, analysis of the activities of antioxidant enzymes (superoxide dismutase–SOD, catalase–CAT, ascorbate peroxidase–APX, monodehydroascorbate reductase–MDHAR, dehydroascorbate reductase–DHAR, glutathione reductase–GR, glutathione peroxidase–GPX and glutathione S-transferase–GST). The RNA-seq test evaluated the transcript production, and the primary and secondary metabolites of seeds were determined by metabolomics. DISCUSSION AND RESULTS: After AA of the seeds, there was a loss of vigour, increased electrical conductivity, alteration in the production of primary metabolites (sucrose, xylose, galactinol, myo-inositol, sorbitol, inositol, ribitol, melibiose and glyoxylic acid). It was a change of terpenes, phenolic compounds and alkaloids and greater gene expression, especially in energy metabolism. In response to AA, the increase in MDA concentration and APX and CAT activity was only significant in the whole seed. At the same time, SOD and DHAR were enzymes that showed a significant increase in activity in the whole seed and the embryo. The activity of GST and GPX were changing significantly only in embryo samples. CONCLUSION: Accelerated ageing is an excellent test to evaluate seeds' physiological, biochemical, and molecular mechanisms. The MDA, SOD, DHAR, and electrolytes increase in the exudate are biochemical biomarkers for evaluating seed quality. The essential genes for energy metabolism are suitable molecular biomarkers for evaluating the quality of seeds of R. communis.
Keywords: genotype BRS 188 Paraguaçu, Energy metabolism, Viability
Supported by: CAPES/PNPD, CNPq, EPAMIG, FAPESB, FINEP, PMBqBM and UFBA

O.12 - Mass Spectrometry application in clinical peptidomics: Identification of potential biomarkers for COVID-19 prognostics

Ana Carolina Silva Batista [1], Cíntia Gomes[2], Luís Maurício Trambaioli da Rocha e Lima[3,4], Adriano de Paula Sabino[2], Mariana Torquato Quezado de Magalhães[1]
[1]Programa de pós-graduação interunidades de Bioinformática, Instituto de Ciências Biológicas (ICB), Departamento de Bioquímica e Imunologia, Laboratório de Biofísica de Macromoléculas (LBM), [2]Programa de Pós-graduação em Ciências Farmacêuticas, Faculdade de Farmácia, Departamento de Análises Clínicas e Toxicológicas, Laboratório Hematologia Clínica, Universidade Federal de Minas Gerais (Minas Gerais, Brasil), [3]Faculdade de Farmácia, Laboratório de Biotecnologia Farmacêutica, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil), [4]Divisão de metrologia aplicada a ciências da vida, Instituto de Metrologia,Qualidade e Tecnologia (RJ, Brasil)

INTRODUCTION: Since 2020, WHO has declared a pandemic state of emergency due to the spread of SARS-CoV-2. The infection caused by this pathogen can manifest differently in each person, and can lead to death in severe cases. Therefore, the prognosis of the disease is a valuable tool for its control and treatment. Among the most important benefits of new biomarkers discovery is that it could lead to earlier assessment of prognosis. In the recent years, sophisticated analytical tools such as mass spectrometry MALDI-based peptidomics have been used to study disease diagnosis and prognosis, identifying and quantifying biomarkers, especially in plasma samples, and has been successfully used in the clinic. OBJECTIVES: In this study, we used MALDI-TOF/MS to identify potential biomarkers for early prognosis. MATERIALS AND METHODS: Knowing that the infection caused by SARS-CoV-2 leads to changes in the plasma proteome, as the disease progresses, we collected samples from 50 individuals with positive PCR results with different outcomes whose plasma was collected at Hospital das Clinicas/BH after the patient's hospital admission (days 1, 3, 7, and 14). Samples were processed using solid phase extraction (SPE), mixtured with alpha-cyano-4-hydroxycinnamic acid and directly applied to the plate. All data were collected using a MALDI-TOF/MS Autoflex III (Bruker) and processed using the MALDIquant, MALDIquantForeign and other R packages. DISCUSSION AND RESULTS: After MS processing, relevant peaks to describe each patient were grouped in feature matrices including 150-260 ions. Logistic regression analyses ($p < 0.05$) was chosen to identify PCA-associated features to distinguished between survival and decease outcomes. Our results demonstrate that 10 out of 36 features presented diminished signals in decease outcomes. The results of proteomic analysis showed that the first days of infection were critical for biomarker identification, suggesting that long-term recovery may be early detected in COVID-19 survivors. CONCLUSION: Thus, it was possible to indicate biomarkers candidates to be further characterized by LC-MS.
Keywords: COVID-19, biomarkes, prognosis / **Supported by:** FAPEMIG, CAPES, CNPq

O.13 - Does White Tailed Jararaca (*Bothrops leucurus*) Snake Venom Undergo Ontogenetic and Sexual Changes?

Victor Koiti Kavazoi [1], Giovanni Perez Machado da Silveira[2], Karen de Morais-Zani[2,1], Kathleen Fernandes Grego[2], Anita Mitico Tanaka Azevedo[1,2]
[1]Interunidades em Biotecnologia, Univ. de São Paulo, (SP, Brazil), [2]Lab. de Herpetologia, Instituto Butantan (SC, Brazil)

INTRODUCTION: The *Bothrops leucurus* species is the main responsible for snake envenomation in Brazil northeast. Several factors, such as geographical origin, climate, sex, age and diet can influence the composition and pathophysiological activities of snake venom. OBJECTIVES: The objective is to understand the ontogenetic variation in the composition of snake venoms and how it can interfere in snake envenomation. MATERIALS AND METHODS: The venom of 37 were pooled according sex and age (newborn, young, adult and senile) and screened by 15% SDS-PAGE (under reducing and non-reducing conditions), Western blot, and LAAO, hyaluronidase, Phospholipase A_2, metalloproteinase and caseinolytic activities. DISCUSSION AND RESULTS: The SDS-PAGE shows that the group of neonates and young have electrophoretic profile similar to each other and that it differs in some bands in relation to the groups of adults and senile. Adult and senile venom showed higher activity when compared with newborn and young in LAAO and hyaluronidase assay. Concerning caseinolytic assay, in females there is little variation between ages, in which young group showed a higher activity when they get older, by contrast, in males newborn group presented greater activity than older ones. In metalloproteinase assay, there was no statistical difference between sexes, but in females there is only statistical difference when senile group are compared with other ones. In the phospholipase A_2 assay, the activity increases up to individuals get adults, however, in the senile group, the activity decreases. There is no sexual difference between the groups. CONCLUSION: Thus, the work showed that senile individuals need more venom spreading factors than newborn, young and adults so that they can be more effective in hunting their prey and that this is more evident in females than in males.

Keywords: Ontogenetic, Snake Venom , Variations

O.14 - Cytotoxic and Proteomic Characterization of the Effects of PA-BJ, a Serine Protease from Bothrops jararaca Venom on Endothelial Cells

Joanderson Pereira Cândido da Silva [1], Alison Felipe Alencar Chaves[1], Bianca Carla Silva Campitelli de Barros[1], Solange Maria de Toledo Serrano[1]
[1]Center of Toxins, Immune-Response and Cell Signaling (CeTICS), Instituto Butantan, Lab. de Toxinologia Aplicada (SP, Brazil)

INTRODUCTION: Snakebite accidents are considered a global public health problem and, in Brazil, most cases are caused by *Bothrops* species. Snake venoms are composed by multiple toxins, which act to disrupt the homeostatic systems of their prey organisms and induce important changes at molecular, cellular, tissue, and systemic levels. PA-BJ is a SVSP (SnakeVenomSerine Protease) isolated from *B. jararaca* venom that induces platelet aggregation. OBJECTIVES: To characterize the effects of PA-BJ on human endothelial cells (HPAECs) to elucidate the role of this toxin in the envenomation process at tissue level. MATERIALS AND METHODS: PA-BJ was purified by cation-exchange chromatography of *B. jararaca* venom. Viability of HPAECs exposed to PA-BJ was evaluated by the MTT assay. Once a sub-cytotoxic dose of PA-BJ was established (50nM), the cells were incubated in the presence or absence of the toxin for 2h, and morphological changes were observed by fluorescence microscopy. The secretome of culture was fractioned by molecular mass filtration, and the fraction containing proteins was subjected to trypsin digestion. Peptides (tryptic and native) were subjected to desalting using stagetips and to LC-MS/MS analysis. DISCUSSION AND RESULTS: Proteomic analysis of secretome resulted in the identification of 324 proteins in PA-BJ-treated cells (including nuclear proteins, proteinases, ECM proteins and others related to regulation of inflammation), 197 proteins in control cells, and 50 common proteins in both secretomes, indicating differential profiles of secretion. Identification of peptides in supernatant of PA-BJ-treated cells revealed proteolysis and suggest potential new substrates of PA-BJ. In the PA-BJ-treated cells, morphological alterations and putative apoptotic bodies were observed. CONCLUSION: These experiments demonstrate effects of a subcytotoxic dose of PA-BJ on the morphology and secretion of endothelial cells and illustrate for the first time the early proteolytic events triggered by the enzyme. Further approaches will be used to explore the mechanism of action of this toxin and its role in the envenomation.

Keywords: Cell Culture, Mass Spectrometry, Snake Venom Serine Protease

O.15 - Alterations in Oxidized Lipids in Blood Plasma Induced by Splenectomy on a LPS Inflammation Model

Larissa Regina Diniz [1], Adriano Chaves-Filho[1], Isis Trzan[2], Eduardo Moretti[2], Alexandre Steiner[2], Sayuri Miyamoto[1]
[1]Bioquímica, Instituto de Química, [2]Departamento de Imunologia, Instituto de Ciências Biomédicas, Universidade de São Paulo (Brazil)

INTRODUCTION: Individuals submitted to splenectomy are shown to be at higher risk of infection that may, in some cases, evolve to overwhelming post-splenectomy infection (OPSI), a syndrome of fulminant sepsis. Although a link between splenectomy and dysregulation of immune response is clear, the role of inflammatory oxylipins formed enzymatically and non-enzymatically (e.g. by reactive oxygen species) in this condition is still understudied. OBJECTIVES: In this work we investigated the effects of splenectomy on the production of oxidized lipids using blood plasma from a model of systemic inflammation induced by LPS. MATERIALS AND METHODS: Male Wistar rats were subjected to splenectomy while control rats were subjected to sham operation. Both groups were treated with different doses of lipopolysaccharide (LPS) ranging from 1 µg/ml to 5000 µg/ml. After 80 min, plasma samples were collected and oxidized lipids were identified through a targeted LC-MS/MS oxylipidomic analysis based on high resolution mass spectrometry that allows the quantification of 148 oxidized lipid species. DISCUSSION AND RESULTS: 40 oxilipin species were identified and quantified. Statistically significant changes were observed in 21 species in the control group, including prostaglandins and mono-hydroperoxides derived from arachidonic and linoleic acid, when compared between LPS doses. Consistent with previous studies, our findings showed that splenectomy contributed to increased levels of inflammatory oxylipin in lower doses of LPS (10 µg/ml and 100 µg/ml) compared to the control group, suggesting that spleen absence is a risk factor for inflammation caused by infection. However, on higher LPS doses (10000 µg/ml), splenectomy was correlated with lower levels of inflammatory oxylipin compared to the control group, pointing towards a role of the spleen as a source of inflammatory molecules. CONCLUSION: Our results demonstrated that the spleen affects oxylipin levels in blood plasma from rats challenged with LPS in a dose dependent manner.
Keywords: oxylipidomics, splenectomy, inflammation
Supported by: CAPES, FAPESP, CEPID-Redoxoma

O.16 - Interactome Analysis Reveal a Broad Regulatory Network Controlled by the PII Protein in Azospirillum brasilense

Edileusa Cristina Marques Gerhardt [1], Erick Parize[1], Fernanda Gravina[1], Adrian Santos[1], Flávia Pontes[1], Gilize Araújo[1], Ana Goedert[1], Alysson Urbanski [1], Franciele Lima[1], Eduardo Campos[1], Maria Berenice Steffens[1], Leda Chubatsu[1], Fabio Pedrosa[1], Emanuel Souza[1], Karl Forchhammer[2], Gustavo Souza[3,4], Luciano Huergo[1,5]
[1]Bioquímica e Biologia Molecular, Universidade Federal do Paraná (PR, Brazil), [2]Interfakultäres Institut für Mikrobiologie und Infektionsmedizin, Eberhard-Karls Universität Tübingen (Germany), [3]Departamento de Bioquímica and [4]Bioinformatics Multidisciplinary Environment, Universidade Federal do Rio Grande do Norte (RN, Brazil), [5]Setor Litoral - Matinhos, Universidade Federal do Paraná (PR, Brazil)

INTRODUCTION: PII proteins are signal transducer proteins ubiquitous in prokaryotes and plant chloroplasts. These proteins are involved in metabolism regulation, acting mostly by direct interaction with a range of target proteins and changing their activities. Such interactions are controlled by binding of effectors ATP, ADP and/or 2-oxoglutarate (2-OG) which are indicators of nitrogen, carbon and energy cell status. In Azospirillum brasilense there are two PII proteins (GlnB and GlnZ) that are involved with transcriptional regulation of nitrogen metabolism genes and nitrogenase post-translational regulation in response to variation of ammonium and 2-OG cellular levels. Since PII proteins are able to sense 2-OG (directly involved with carbon and nitrogen cellular levels) a role in regulation of carbon metabolism by PII proteins has been proposed. OBJECTIVES: Identify new PII targets in A. brasilense and analyze metabolomic changes in response to variation of nitrogen levels. MATERIALS AND METHODS: To identify new PII targets, multiple ligand fishing assays were performed using purified the GlnZ, and fished proteins were identified by LC-MS. To confirm GlnZ-target interactions pulldown assays were used using recombinant proteins. LC/MS was used to analyze untargeted metabolome of A. brasilense wild type and PII mutants to correlate the identified targets with the effect on the bacterial metabolism. DISCUSSION AND RESULTS: 37 novel PII targets were identified in the interactome assays. The functions of these proteins covered a wide range, from nitrogen metabolism to fatty acids metabolism, signaling, coenzyme synthesis, RNA catabolism, and transcription regulation, revealing a broad regulatory network controlled by PII proteins. We confirmed 15 of such interactions using pulldown assays with purified recombinant proteins. Finally, untargeted metabolome analysis showed alteration in the levels of important cellular metabolites in absence of PII. CONCLUSION: We provide evidence that A. brasilense PII protein regulates a broad range of metabolic pathways, thus PII appears to be a pivotal player in A. brasilense metabolism regulation. **Keywords:** PII protein, Azospirillum brasilense, metabolismo / **Supported by:** CAPES, CNPq, Fundação Araucária, CNPq-INCT, and the Alexander von Humboldt Foundation

O.17 - How do *Bothrops erythromelas* and *Bothrops leucurus* venoms act on the coagulation cascade?
Eduardo Oliveira V. de Lima [1,2], Nathália da Costa Galizio[1,2], Victor Koiti Kavazoi[1,2], Daniela Miki Hatakeyama[1,2], Lídia Jorge Tasima[1,2], Caroline Serino-Silva[1], Anita Mitico Tanaka-Azevedo[1,2], Karen de Morais-Zani[1,2]
[1]Laboratório de Herpetologia , Instituto Butantan (SP, Brasil), [2]Interunidades em Biotecnologia, Univ. de São Paulo (SP, Brasil)

INTRODUCTION: In Brazil, approximately 100 deaths occur per year as the result of snakebites, and 40% of these deaths are registered in the Northeast region of the country. Antivenom serum is the only treatment for snakebites, and two snakes from the Brazilian Northeast are not part of the venom pool used for the production of this serum, which are *B. erythromelas* (*Be*) and *B. leucurus* (*Bl*). Bothropic venoms are known to have toxins capable of causing hemostatic disorders, resulting in dysfunction in the coagulation cascade. However, the vast majority of studies only assess the pro and anticoagulant potential of snake venoms with only one method, leaving aside how the toxins act on the coagulation cascade. OBJECTIVES: Therefore, the objective of this work is to characterize the coagulotoxic profile of *Be* and *Bl* venoms and test the *In vitro* neutralizing potential of the anti-bothropic serum (ABS). MATERIALS AND METHODS: To achieve the proposed objectives, 10 *Be* venoms, 13 *Bl* venoms and a pool of *B. jararaca* venoms were collected. Phospholipase A_2 activity (on NOBA substrate), proteolytic activity on azocasein, thrombin-like activity, factor X activation and the neutralization of these activities by ABS were also performed. DISCUSSION AND RESULTS: Phospholipase A_2 activity showed great individual variation in both species studied. The proteolytic activity showed less variability and better neutralization in *Be* samples. The *Be* samples showed little or no thrombin-like activity. Factor X activation showed high individual variation in *Be* and was more homogeneous in *Bl*, and neutralization by ABS was better in *Bl* in this assay. CONCLUSION: here is an imminent difficulty in working with enzymatic cascades, and this work is important to help elucidate how snake venoms may act on the coagulation cascade.
Keywords: Coagulation Cascade, Snake Venom, Enzymatic Activity / **Supported by:** CNPQ

O.18 - Gut microbiota and renin-angiotensin system interaction in the progression of cardiac ischemic injuries
Ana Carolina Lara Ribeiro [1], Thiago Verano Braga[1], Filipe Alex da Silva[1], Lucas Bolais Ramos[1], Diego M. Assis[2]
[1]Physiology and Biophysics, Univ. Federal de Minas Gerais (, Brazil), [2]Bruker Daltonics, Bruker Daltonics (, United States)

INTRODUCTION: The renin-angiotensin system (RAS) is an important humoral modulator of the cardiovascular system. It is formed by the classical and protective axes, whose deregulation can induce cardiac hypertrophy, inflammation, and hypertension. In acute myocardial infarction (AMI), for example, Ang II levels are increased, which can cause cardiac tissue remodeling and consequent loss of contractile function and, eventually, progress to heart failure. Many studies have demonstrated an interaction between gut microbiota and cardiovascular pathologies, including AMI. Recent data have shown that “germ-free” animals treated with Ang II did not develop arterial hypertension, reinforcing the idea that the gut microbiota influences the cardiovascular system homeostasis, but these mechanisms are still unknown. OBJECTIVES: Evaluate the association between gut microbiota and RAS, and their role in cardiac ischemic disease progression.MATERIALS AND METHODS: For dysbiosis induction, male C57Bl6/j mice received anoral single dose of streptomycin (Sigma) together with ampicillin (Sigma) at 1 g/L in drinking water for 7 days. Plasma peptides were extracted and measuredby Multiple Reaction Monitoring (MRM) using a UPLC coupled to a mass spectrometer (Xevo, Waters). Cardiac tissue was processed by shotgun proteomics technique and later analyzed by an LC-MSMS (Tims-TOF, Bruker), using Data-Independent Acquisition (DIA) mode. Finally, the fecal microbiota was analyzed by 16S RNA. DISCUSSION AND RESULTS: The findings suggest an imbalance in RAS endogenous peptides when comparing animals with gut dysbiosis and gut normobiosis, highlighting the increase in angiotensin II and reduction in angiotensin 1-7, and also markedly increased cecal content. Through cardiac proteome, the regulation of proteins and pathways that reinforce the negative impact of the gut microbiota on the cardiovascular system was observed. CONCLUSION: The hypothesis of the work is that gut microbiota dysbiosis, induced by antibiotic therapy, has a deleterious effect on homeostasis of cardiovascular system, specifically on the protective axis of the RAS. This suggests a deleterious effect in the progression of ischemic diseases, such as AMI.
Keywords: cardiac ischemia-reperfusion, gut microbiota, proteomics / **Supported by:** FAPEMIG, CNPq, CAPES

O.19 - Proteomic Analysis of the Venom of the Mollusk Conus regius

Helena Bulhões Fiorotti [1,2], Suely Gomes de Figueiredo[2], Fabiana V. Campos[1,2], Daniel Carvalho Pimenta[1]
[1]Laboratório de Bioquímica, Instituto Butantan (São Paulo, Brasil), [2]Ciências Fisiológicas, Universidade Federal do Espírito Santo (Espírito Santo, Brasil)

INTRODUCTION: Predatory mollusks of the genus *Conus* are distributed in tropical waters across the globe, comprising more than 900 species. These animals produce a venom rich in toxic peptides called conopeptides. Conotoxins – conopeptides rich in disulfide bonds – bind with great specificity to ion channels and receptors. The crucial physiological roles played by these targets, added to the extraordinary structural stability of conotoxins, make the potential of cone snail venoms immeasurable.*Conus regius* is a worm-eating species found in the Brazilian coast whose venom was studied in this work OBJECTIVES: To perform a proteomic analysis of *C. regius* venom found in the Fernando de Noronha Archipelago. MATERIALS AND METHODS: The specimens were captured in the Fernando de Noronha Archipelago and the venom obtained by dissection of the venom duct. Crude venom samples were fractionated by reversed-phase chromatography (Symmetry 300 C18 column), and the eluted fractions were analyzed by MALDI-TOF and ESI-IT-TOF mass spectrometry. The results were analyzed in the Peaks Studio V7.0 software using a *Conus* protein database (Uniprot). DISCUSSION AND RESULTS: A total of 28 molecules were identified in the venom of *C. regius*: 12 conotoxins from the M superfamily (eight of which had been previously described in this species) and one conotoxin from the I1 superfamily that had also been described previously in this species. Both superfamilies target different types of ion channels and receptors. Thirteen showed similarity to conotoxins from different *Conus* species; five showed similarity to conopressins, conopeptides that bind to vasopressin and oxytocin receptors so far unheard of in venoms of vermivorous species; and a protein with similarity to arginine kinase. CONCLUSION: To date, this study represents the first comprehensive characterization of the venom composition of *C. regius*, promoting a better understanding of the diversity of molecules present in this venom, in addition to providing data for future investigations on its biotechnological potential.

Keywords: Conus, Conopeptides, Proteomics / **Supported by:** CAPES, Instituto Butantan, INCTTox

O.20 - Characterization of Polyhydroxybutyrate Mobilization and Expression of phaZ1 and phaZ2 Genes in Herbaspirillum seropedicae

Francisco José Teles Mota [1], Marcelo Müller dos Santos[1], Emanuel Maltempi de Souza[1], Fábio de Oliveira Pedrosa[1], Leda Satie Chubatsu[1]
[1]Department of Biochemistry and Molecular Biology, Federal University of Paraná (Paraná, Brazil)

INTRODUCTION: Polyhydroxybutyrate (PHB) is a biopolymer produced by bacteria under conditions of excess of carbon and unbalanced nutrient availability. This class of biopolymers shows an enormous potential for biotechnological application. The endophytic and diazotrophic bacterium *Herbaspirillum seropedicae* synthesizes this biopolymer and its PHB metabolism is associated with the successful colonization and plant-growth promotion of gramineous plants, as well as stress tolerance such as heat shock. In this bacterium, thirteen genes involved in the PHB metabolism were identified including two coding for PHB depolymerases, *phaZ1* and *phaZ2*. OBJECTIVES: To evaluate PHB mobilization and *phaZ1* and *phaZ2* expression in *H. seropedicae* using wild type and mutants strains. MATERIALS AND METHODS: Intracellular PHB levels were monitored by flow cytometer after Nile red-staining, and *phaZ1* and *phaZ2* expression were monitored using *lacZ* report assays. DISCUSSION AND RESULTS: Our results indicated that PhaZ1 is the main PHB depolymerase to mobilize PHB in *H. seropedicae*, since PHB accumulation was observed in both *ΔphaZ1* and *ΔphaZ12* mutants. *phaZ1* is constitutively expressed, and repression of expression was observed in the mutant *ΔphaC1*, a strain unable to produce PHB. We found evidences that the transcriptional repressor PhaR modulates this process. The PhaZ2 depolymerase, coded by *phaZ2*, seems to mobilize PHB under determined conditions, and it seems unable to complete replace PhaZ1 in *ΔphaZ1* and *ΔphaZ12* mutants. Moreover, *phaZ2* expression is regulated by NtrC and thus dependent on nitrogen levels. CONCLUSION: Our results indicate that PhaZ1 is the main PHB depolymerase in *H. seropedicae*, and although PhaZ2 is able to mobilize PHB its role in PHB metabolism needs further analysis.

Keywords: Herbaspirillum seropedicae, poly-3-hydroxybutyrate (PHB), PHB Depolymerase / **Supported by:** CNPq, and CAPES

O.21 - Bothrops erythromelas snake venom: Ontogenetic and sexual variation in enzymatic activities

Daniela Miki Hatakeyama [1,2], Giovanni Silveira[2], Lídia Tasima[1,2], Caroline Serino-Silva[2], Sávio Sant'Anna[2], Kathleen Grego[2], Karen Morais-Zani[2,1], Anita Tanaka-Azevedo[2,1]
[1]Interunidades em Biotecnologia (USP, IBut e IPT), Universidade de São Paulo (São Paulo, Brazil), [2]Laboratório de Herpetologia, Instituto Butantan (São Paulo, Brazil)

INTRODUCTION: The *Bothrops erythromelas* species is medically relevant in the Brazilian Northeast. Snake venoms are susceptible to intraspecific variations related to numerous factors, including age and sex. These features of the venoms were not completely elucidated in this species and this knowledge contributes to better clinical, ecologic, and evolutional understanding of these animals. OBJECTIVES: Thus, the aim of this work was to characterize and compare the B. erythromelas snake venoms throughout their development. MATERIALS AND METHODS: The venoms of newborn *B. erythromelas*, born and kept at the Laboratory of Herpetology at Instituto Butantan were milked every 6 months up to 30-months-old. Additionally, 13 adults were also milked for comparison. All venoms were lyophilized and submitted to phospholipase A_2 (PLA_2), L-amino acid oxidase (LAAO), and proteolytic activities (with inhibition by adding EDTA or antibothropic serum (ABS)). DISCUSSION AND RESULTS: Proteolytic and PLA_2 activities were higher at older ages. In general, female venoms showed higher proteolytic activity, whereas male venoms presented higher PLA_2 activity. Although LAAO activity is not commonly found in this species, it was detected at higher levels in the venoms of male and older snakes. Both proteolytic activity over casein and collagen were markedly neutralized by the addition of EDTA. ABS inhibited most of collagenolytic activity but weakly neutralized caseinolytic activity. Besides the ontogenetic and sexual variations observed, individual variation was also present in all experiments. CONCLUSION: In conclusion, *B. erythromelas* snake venom varies according to age and sex, and the ontogenetic shift also shows differences among males and females. Furthermore, similar to other species, *B. erythromelas* presents individual variation in the venom.
Keywords: Bothrops erythromelas, Snake venom, Variation / **Supported by:** CNPq (140872/2019-1), CAPES, and FAPESP

O.22 - Optimization of Methodologies for Analyses of Complex Proteomes

Francielle Aguiar Gomes [1], Mariana Pereira Massafera [1], Graziella Eliza Ronsein[1]
[1]Instituto de Química - Departamento de Bioquímica, Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: Successful proteomic analyses rely on reproducible sample preparation workflows and optimized spectra acquisition methods. Both provide higher protein identification and better signal-to-noise ratio on high-resolution mass spectrometers. OBJECTIVES: We aim at optimizing sample preparation protocols (cell lysis, digestion) and spectra acquisition methods for a series of samples (from cell culture, tissues, body fluids) in order to develop a standard workflow for each type of sample. MATERIALS AND METHODS: THP-1 cells were cultured and lysed, then the resulting proteins were quantified via BCA assay and subsequentially digested In Gel, always in quintuplicates. We compared the results of (a) two different peptide elution methods (from the gel), (b) different amounts of digested proteins (1, 5, 10, or 25 µg), and (c) two spectra acquisition methods: Shotgun or BoxCar. DISCUSSION AND RESULTS: In all of the cases analyzed in this work, we obtained over 1500 proteins of the human proteome (ranging from 1636-2292 identified proteins when increasing the amount of digested proteins from 1-25 µg). The number of proteins identified when digesting as low as 1 µg is a promising result, which can be very useful in cases of limited amount of sample (e.g., fractions of biological fluids). Also, for the two different protocols of peptide elution from the gel we tested (in 1 or 2 steps), we observed a clear distinction between the proteins differentially expressed in both cases. Regarding the spectra acquisition methods under investigation, fewer proteins were identified by the BoxCar method, however, the percentage of identification of MS2 spectra was much higher than that obtained via Shotgun analysis. CONCLUSION: The results obtained so far provided guidelines for further optimization and will serve as basis for the development of a sample-oriented processing workflow.
Keywords: Mass Spectrometry, Proteomics, Sample Preparation Protocols / **Supported by:** FAPESP

O.23 - MRGD gene deletion induced a cardiac excitation-contraction coupling impairment in heart mice

Lucas Rodrigues Aguiar Ribeiro [1,2], Itamar Couto Guedes de Jesus[1], Joseph Albert Medeiros Evaristo[3], Fabio Cesar Sousa Nogueira[3], Silvia Carolina Guatimosim Fonseca[2], Thiago Verano Braga[2]
[1]Department of Biochemistry and Immunology and [2]Department of Physiology and Biophysics, Federal University of Minas Gerais (Minas Gerais, Brazil), [3]Department of Biochemistry, Federal University of Rio de Janeiro (Rio de Janeiro, Brazil)

INTRODUCTION: The renin angiotensin system (RAS) plays an essential role in the cardiovascular homeostasis. The deletion of MRGD gene, a RAS component, in mice led to a severe dilated cardiomyopathy (DCM). OBJECTIVES: We aimed to investigate the molecular mechanisms occurring in DCM trigged by MRGD gene deletion using proteomics. MATERIALS AND METHODS: The left ventricle (LV), the ventricular cardiomyocytes (CMV) and the extracellular matrix (ECM) of adult MRGD-knockout (mrgd-ko) and C57Bl6 mice were extracted. After dissected, tissues were submitted to proteomics sample preparation, isotopic labeling, pre-fractionation, phosphopeptide-enrichment and analyzed by liquid chromatography coupled with mass-spectrometry (LC-MS). DISCUSSION AND RESULTS: We have quantified 1,919 proteins and 503 phosphopeptides. Also, 763 proteins were found regulated in LV, CMV and ECM proteome and 80 phosphopeptides were found regulated in CMV phosphoproteome. Functional analysis showed that MRGD gene deletion triggers significant changes in excitation-contraction coupling and sarcomere proteins. Na^+/K^+ ATPase (NKA) and Phospholambam (PLN) dephosphorylation and Na^+/Ca^{2+} exchanger (NCX1) and PLN down-regulation at protein levels suggested alterations in ionic homeostasis. Indeed, calcium transient levels were lower and its reuptake seems to be slower in mrgd-ko cardiomyocytes. Moreover, NKA inhibition by dephosphorylation at Ser-16 may be impairing the homeostasis of Na^+/K^+ ions in these cells leading to an impairment in cardiac action potential. β-myosin heavy chain (MHC), myosin binding protein C (MyBP-C), myosin regulatory light chain 2 (MLC-2) and dystrophin (DMD) were found down-regulated at protein levels in mrgd-ko, suggesting a dysregulation in sarcomere machinery. Also, integrin-linked kinase (ILK) was down-regulated and Titin (TTN) was found dephosphorylated in several sites at “Z disk”, “I Band” and “M Band” portions, which may lead to impaired biochemical force transmission. CONCLUSION: In summary, the MRGD gene deletion seems to induce an impairment in calcium homeostasis and alteration in sarcomere proteins abundance and function, in line with the observed DCM phenotype. **Keywords:** Renin-Angiotensin System, Dilated Cardiomyopathy, Phosphoproteomics / **Supported by:** FAPEMIG, CNPq and CAPES

O.24 - Single-Cell Analysis Reveals Intra- and Intercellular Remodeling of Colonic Epithelium Upon Depletion of Intestinal Microbiota

Vinicius Dias Nirello [1], Nathália Vitória Pereira Araújo[1], Mariane Font Fernandes[1], Marco Aurélio Ramirez Vinolo[1], Patrick Daniel Warga Weisz[1,2]
[1]Department of Genetics, Evolution, Microbiology, and Immunology, University of Campinas (São Paulo, Brazil), [2]School of Life Sciences, University of Essex (Essex, United Kingdom)

INTRODUCTION: Intestinal epithelial cells (IECs) exist in symbiosis with the commensal microbiota, whose products drive metabolism and development in hosts. Colonic epithelium plays a fundamental role in this relationship, building mucosal barriers, secreting immunological mediators, and providing bacterial antigens. However, the contributions of each epithelial cell type to this process are unknown. OBJECTIVES: We aim to investigate cell-type-specific effects upon microbiome depletion. MATERIALS AND METHODS: We performed single-cell transcriptomics in colonic IECs from mice with normal and antibiotic-depleted microbiota. DISCUSSION AND RESULTS: Here we show that microbiota depletion leads to a remodeling of the colonic epithelium profile. In addition, it results in a functional shift in major mature cell types. We identified most of the known IECs types, including progenitor, absorptive, and secretory cells. By comparing control and treated groups, we mapped the microbial impacts on maintaining cellular proportions. We showed that microbiota depletion leads to a reduction in enteroendocrine cell numbers and a decrease in proliferative cells. Furthermore, we reveal the cellular specificity of host response to microbiome. We describe two expression profiles in enterocytes that are relevant in the remodeling caused by microbiota depletion. In the control group, we identified a population of enterocytes mainly related to interaction with the microbiome, presenting genes involved in the metabolism of microbiota-derived metabolites and response to bacteria. In the depleted microbiota group, this enterocyte population was reduced and a population of enterocytes with an energetic profile emerged. We hypothesize that such alterations are directly related to the products of microbiota metabolism, i.e., short-chain fatty acids (SCFA), since there is a regulation of genes involved in the organization of chromatin that are known to be microbiota- and SCFA dependent. CONCLUSION: Our work provides a new overview of the role of microbiota in the maintenance of colonic IECs, exploring the effects on each cell type and linking these changes to epigenetic modifications. **Keywords:** Microbiota, Epigenetic Modifications, Single-Cell Transcriptomics / **Supported by:** FAPESP, CAPES, CNPq

O.25 - Search for Novel Mediators of Adipocyte Size

Diogo de Moraes [1], Letícia de Souza Figueiredo[1], Sarah Santiloni Cury[2], Felippe Mousovich Neto[1], Tereza Cristina Minto Fontes-Cal[1], Robson Francisco Carvalho[2], Henver Brunetta[1], Marcelo Alves da Silva Mori[1,3,4]
[1]Depto de Bioquímica e Biologia Tecidual, Universidade de Campinas (SP, Brasil), [2]Departamento de Biologia Funcional e Estrutural, Univ. Estadual Paulista (Brasil), [3]Obesity and Comorbidities Research Center (OCRC) and [4]Experimental Medicine Research Cluster , Universidade de Campinas (SP, Brasil)

INTRODUCTION: Cardiometabolic diseases are a major public health problem in the elderly or obese individuals. Demographical, anthropometric and histopathological causes are associated with the risk of cardiometabolic diseases. However, patient adipocyte size is determinant, being more associated with the incidence of diabetes than the age or fat percentage. Evidence indicates that large adipocytes may represent a consequence of the loss of pre-adipocytes differentiation capacity. These hypertrophied adipocytes may cause hypoxia in adipose tissue, characteristics found in individuals affected by cardiometabolic diseases, obese or elderly. Hypoxia also causes inflammation, inhibiting pre-adipocyte differentiation, possibly causing a vicious cycle that leads to metabolic dysfunction. Thus, a better understanding adipocyte size regulation can help understand and treat these conditions. OBJECTIVES: Our purpose identifying genes that control the size of adipocytes in humans. MATERIALS AND METHODS: For this, we used public databases containing transcriptome and histological slides of adipose tissue from heterogeneous human cohorts to search for genes that are associated with the size of adipocytes. Next, we will validate these findings using spatial transcriptomics using independent human samples, seek conservation in murines, and test the function of some of these genes in adipocytes through *In vitro* overexpression experiments using CRISPR-Cas9a DISCUSSION AND RESULTS: We found that the adipocyte size of both sexes increase continuously with age, while there was a downward trend in the visceral fat at older ages. We also found that the adipocyte sizes of both deposits are correlated in young males, and this correlation decreased with age, leading to increased fat storage in the visceral fat. In females, however, no correlation was found. We have also elected 27 genes that correlated with adipocyte size within all age and sexes that were also present in other studies. These genes will be biologically validated in the upcoming months. CONCLUSION: these new markers may help understand and treat cardiometabolic diseases. **Keywords:** adipocyte, obesity, bioinformatics

O.26 - Chromosome Level Assembly of the *Bradysia hygida* (Diptera:Sciaridae) Genome and Identification of Enzymes Potentially Involved in the Soil Decomposer System.

Vitor Trinca [1], Sibeli De Carli[2], João Vitor Cardoso Uliana[1], Mariana Mendes [3], VItor Kunes Lopes[2], Luana Parras Meleiro [2], Carolina Victal Garbelotti[2], Guilherme Thomaz Pereira Brancini[3], Luiz Paulo Moura Andrioli[4], Tatiana Teixeira Torres[5], Richard John Ward[2], Nadia Monesi[3]
[1]Programa de Biologia Celular e Molecular, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo (SP, Brasil), [2]Departamento de Química, Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, Universidade de São Paulo (SP, Brasil), [3]Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo (SP, Brasil), [4]EACH, Escola de Artes, Ciências e Humanidades (SP, Brasil), [5]Departamento de Genética e Biologia Evolutiva, Instituto de Biociências, Universidade de São Paulo (SP, Brasil)

INTRODUCTION: Insects, including Sciarids, participate in soil communities feeding on partially decomposed plant matter and make a significant contribution to the carbon cycle in many biomes. Furthermore, some Sciarids feed on living plants and constitute agricultural pests. *Bradysia hygida* (Diptera:Sciaridae) is a non-conventional model organism that has been studied for over 50 years. The laboratory life cycle lasts 36 days at 22 ºC when fed in partially decomposed *Ilex paraguariensis* leaves. OBJECTIVES: To sequence the *B. hygida* genome and to identify candidate Carbohydrate-active enzymes (CAZymes) involved in plant matter degradation. MATERIALS AND METHODS: Illumina and PacBio reads were used to generate a draft assembly. Chromosomes-length scaffolds were obtained using Hi-C reads and the 3D-DNA pipeline. The genome was annotated with MAKER3 using evidence from a *B. hygida* RNA-seq dataset and the SwissProt protein database. Functional annotation of the predicted genes was performed against five databases (NCBI NR, KEGG, SwissProt, CAZy, and InterProScan). DISCUSSION AND RESULTS: The *B. hygida* genome is 609Mb long with 90.3% of the sequence contained in the four longest scaffolds, corresponding to chromosomes A, B, C, and X. The genome contains 18,028 predicted coding genes, of which 16,751 (92.9%) were functionally annotated. Searches against the CAZy database revealed 1028 CAZymes, of which 190 are predicted to be secreted. Proteome analysis of the larval saliva indicates that ~16% of the predicted CAZymes are present in the salivary gland secretome and are glycosyl hydrolases that may participate in the degradation of plant biomass. CONCLUSION: This is the third sciarid genome to be sequenced and the first to be assembled at the chromosome level. The identification of CAZymes in the salivary gland secretome contributes to understanding the role of Sciarids in the soil carbon cycle and will allow the characterization of novel biomass degrading enzymes. **Keywords:** Sciaridae, Chromosome level genome assembly, CAZymes / **Supported by:** FAPESP, CAPES, CNPq, FAC (Fundação de Apoio às Ciências: Humanas, Exatas e Naturais)

O.27 - PROJECT: Use of X-STR Markers in Paternity Tests of Complex Cases: Analysis of the Impact on the Combined Paternity Index

Flvia Anan Saiki[1], Altamir Frederico Guidolin[2], Carla Ivane Ganz Vogel[3]
[1]Programa Multicêntrico de Pós-graduação em Bioquímica e Biologia Molecular, Universidade do Estado de Santa Catarina (Santa Catarina, Brasil), [2]Agronomia, Universidade do Estado de Santa Catarina (Santa Catarina, Brasil), [3]Produção Animal e Alimentos, Universidade do Estado de Santa Catarina (Santa Catarina, Brasil)

INTRODUCTION: Short tandem repeats markers of X Chromosome (X-STR) are on the spotlight in Forensic Genetic field for the resolution of complex paternity cases. X-STR markers can be more informative than autosomal STR depending of the kinship being tested. Regarding Y chromosome and mitochondrial DNA markers, there are advantages because it is not a uniparental analysis. As all markers are on the same chromosome, a population analysis that considers linkage and that also assesses linkage disequilibrium is necessary to use X-STR data in paternity and kinship investigation calculations. OBJECTIVES: This project seeks to evaluate the contribution of X-STR in the analysis of paternity investigation of complex cases, in which the Combined Paternity Index (CPI) obtained with autosomal STRs was not conclusive. MATERIALS AND METHODS: The cases to be evaluated will be post-mortem paternity investigations, in which the alleged father is deceased and biological relatives of him and the proband are collected. All these investigations will be of real cases collected by the Programa DNA em Audiência em Santa Catarina and processed at the Laboratório de Análises Genéticas DNA UDESC - Brazil. A total of 200 unrelated Duos of Mothers and Sons are going to be typed with Investigator® Argus X-12 QS, the Multiplex X-STR kit of Qiagen, for haplotype frequency data. The Arlequin 3.5 software is going to be used for population genetic calculations and FamLinkX for Likelihood Calculations. DISCUSSION AND RESULTS: With a proper X-STR haplotype frequency database, X-STR data will complement autosomal STR CPI of complex cases and potentially more results will become conclusive.
Keywords: X-STR, Complex Paternity Investigation, Combined Paternity Index

P - Membrane-Active Peptides

P.01 - Synthesis and study of the interaction of the bioactive peptides Ocelatin LB1, LB2 and F1 with membrane mimetic media by calorimetric titration calorimetry

Daniel Augusto Guedes Reis Michel [1], Carolina Silva Ferreira[1], Rodrigo Moreira Verly[1]
[1]Departamento de química, Universidade Federal dos Vales do Jequitinhonha e Mucuri (Minas Gerais, Brasil)

INTRODUCTION: Ocelatins are antimicrobial peptides which make part of innate immune system of the anuran Leptodactylus labyrinthicus, being isolated from its cutaneous secretion. Ocelatins, LB2 and F1 were isolated from this secretion, each containing 22, 23 and 25 residues of amino acids, respectively. The study of these peptides became attractive due to the wide spectrum of antimicrobial activity presented. OBJECTIVES: The aim of this work was synthesize, characterize and investigate the interaction of the LB1, LB2 and F1 peptides using phospholipid membranes. MATERIALS AND METHODS: The synthesis of the peptide was carried out in solid-phase method via Fmoc strategy and characterized by mass spectrometry. The study of the interaction peptide-membrane was carried out by isothermal titration calorimetry (ITC) in the presence of large unilamellar vesicles (LUV's) of egg lecithin in order to determine the thermodynamic parameters of the systems peptide-LUVs. DISCUSSION AND RESULTS: The characterization of the solid-phase synthesis was analyzed by mass spectrometry, which showed a mass/charge ratio of 2191.19 Da for LB1, 2305.73 Da for LB2 and 2546.49 Da for F1, in agreement with the respective theorical monoisotopic mass expected for these peptides. The calorimetric curves showed a negative heat flux, demonstrating that the interaction ocurrs in a exothermic process. The adjust of the isotherms revealed the interaction is entropy driven. The higher apparent binding constant and ΔG^{o} observed for Ocelatin F1 suggest that the additional residues contribute to a stronger peptide-membrane interaction, when compared to the LB1 and LB2 peptides. CONCLUSION: Mass spectrometry confirms the synthesis of Ocelatins LB1, LB2 and F1. The ITC experiments show greater entropic contribution, which is concsequence of low net charge of the cationic ocellatins. The higher affinity of F1 peptide is in accordance with its greater antimicrobial activity in comparison LB1 and LB2 ocellatins.

Keywords: Antimicrobial peptides, Biomimetic medium, Peptide solid-phase synthesis

P.02 - Synthesis and Biophysical Studies of Amidated Angiotensin I and II: Peptide Membrane Interaction and Cardiovascular Changes by Systemic Infusion

Amanda Souza Félix[1], Larissa Silveira Matsamura[1], Daniel Campos Villela[2], Fabrício De Oliveira[2], Lucas Franco Ferreira[3], Rodrigo Moreira Verly[1]
[1]Departamento de Química, Universidade Federal Dos Vales Do Jequitinhonha E Mucuri (MG, Brasil),
[2]Departamento de Medicina, Universidade Federal Dos Vales Do Jequitinhonha E Mucuri (MG, Brasil), [3]Instituto de Ciência e Tecnologia, Universidade Federal Dos Vales Do Jequitinhonha E Mucuri (MG, Brasil)

INTRODUCTION: The Renin-Angiotensin-Aldosterone (RAAS) system generates the reactions, which provide balance of blood pressure, amount of sodium and water in the body. The secreted renin cleaves the N-terminal portion of angiotensinogen resulting in the angiotensin I (DRVYIHPFHL), which by the action of angiotensin converting enzyme (ACE) is converted to angiotensin II (DRVYIHPF). OBJECTIVES: The present work aimed to investigate the influence of the amidation of angiotensin I and II in the membrane interaction. MATERIALS AND METHODS: The wide-type and amidated peptides were synthesized by the solid-phase peptide synthesis method via Fmoc strategy, purified by high performance liquid chromatography (HPLC) and characterized by mass spectrometry (Electrospray - ESI). Affinity and interaction were evaluated in the presence of phospholipid membrane models of POPC and POPG by electrochemical impedance spectroscopy, inserting the spacer containing three alanines and one cysteine, in the N- and C-terminal regions. Finally, tests of vascular reactivity and cardiovascular changes were performed by systemic infusion in Wistar rats. DISCUSSION AND RESULTS: The results indicated that the CAAA–Angio I amide sequence promotes higher values of charge transfer resistance when compared to the Angio I–AAAC amide, probably due to the lower impediment found by the cysteine binding to the gold electrode. Resistance had its most pronounced effect on zwitterionic POPC then anionic POPC:POPG LUVs, in agreement with the literature, since angiotensin act in eukaryotic cells. In addition, the CAAA–Angio I carboxy showed vitually null resistance to charge transfer in the presence of the POPC vesicles while CAAA–Angio I amide showed increased resistance to charge transfer in similar conditions. CONCLUSION: Therefore, the decrease of evaluated the vascular activity of Angio I and II amide could be related to the its higher affinity to the zwitterionic membrane.

Keywords: Amidation , Bioactive peptides, Peptide-membrane interaction
Supported by: Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM), CNPq, CAPES, FAPEMIG.

P.03 - Lunatins: A Structure-Function Relationship Investigation of the Broad Spectrum Bioactive peptides

John Alexanders Amaya Parra [1], Bruno de Paula Oliveira Santos[1], Kamila de Sousa Gomes[1], Érica dos Santos Martins Duarte[2], Adriano Monteiro de Castro Pimenta[1], Mariana Torquato Quezado de Magalhães[1]
[1]Bioquímica e Imunologia, Univ. Federal de Minas Gerais (MG, Brazil), [2]Parasitologia, Univ. Federal de Minas Gerais (MG Brazil)

INTRODUCTION: Lunatins are a family of peptides isolated from the venom of the Peruvian scorpion *Hadruroides lunatus*. Several studies have demonstrated the biological activities of lunatins, such as antitumor, antimicrobial, phosphatase inhibitors, and others. Lunatin-1 is a 13mer peptide with antitumor activity against leukemia and breast cancer cells. OBJECTIVES: To evaluate the effects of primary and tertiary structure on the activity of lunatin-1-derived peptides. MATERIALS AND METHODS: The alanine scan assay was performed and thirteen lunatin-1-derived peptides were generated. Using time-lapse imaging experiments of FITC-labeled lunatin-1 peptides and molecular dynamics, we were able to verify the ability of the peptide to enter eukaryotic cells. The structure of the peptides in mimetic environment (TFE-d2 and SDS-d25 micelles) were studied by NMR spectroscopy [1H-1H]- TOCSY, [1H-1H]-NOESY and [1H-13C]-HSQC. DISCUSSION AND RESULTS: Lunatin-1 and its derived peptides, such as lunatin-4A, are amphipathic molecules that represent a coil-to-helix from aqueous to hydrophobic medium. Replacement of each amino acid with alanine led to the identification of a critical residue for cytotoxic activity, Lys-7, which is the only charged residue and its absence resulted in a complete loss of activity. On the other hand, the replacement of Gly-4 leads to an enhancement of bioactivity and increases the cytotoxic activity of lunatin-1. Our data showed that the first half of the lunatin-1 sequence is important for cytotoxic activity and is the first part that initiates the folding process of the peptide, while the second half has potential cell-penetrating activity. CONCLUSION: The lunatin-1-derived peptides are amphipathic bioactive molecules whose sequences have been used as templates for the design of specific or multi-task molecules. Further studies would help us understand the mechanism of action with the possible interacting proteins that also enhance the activities of these molecules.

Keywords: Nuclear Magnetic Resonance, Membrane-active peptides, Molecular Dynamics
Supported by: Conselho Nac. Des. Cient. Tecnologico (CNPq), CAPES and by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

P.04 - The antimicrobial peptide BP100 induces protrusions in planar membranes and vesicles

Peter Park [1,2], Hernan Chaimovich[1], Iolanda Midea Cuccovia[1], Siewert Jan Marrink[2]
[1]Departamento de Bioquímica, Instituto de Química da USP (SP, Brazil), [2]Groningen Biomolecular Sciences and Biotechnology Institute and Zernike Institut, University of Groningen (, the Netherlands)

INTRODUCTION: BP100, an antimicrobial peptide, can permeate cells and act as a carrier. Experiments have not clarified the mechanisms of membrane disruption and penetration. Using all-atom Molecular Dynamics simulations (MD), we described using a single peptide BP100/membrane initial step of interactions. When adsorbed on the membrane, BP100 maintains its alpha-helical conformation and flips, burying its non-polar residues into the membrane, inducing a negative curvature. Here we explored the effect of higher peptide lipid ratios (P/L) on bilayers and vesicles of zwitterionic and negatively charged lipids OBJECTIVES: Analyze the effects of BP100/lipid ratios (P/L) in bilayers and vesicles using MD MATERIALS AND METHODS: We used the recently developed coarse-grained MARTINI3 forcefield. Initial set-ups with BP100, POPC (1-palmitoyl-2-oleoyl-glycero-3-phosphocholine) and POPG (1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) bilayers were assembled using the INSANE script. CHARMM-GUI web server generated vesicles. Simulations and analysis were performed in GROMACS 2020.6. Simulations: NPT ensemble with V-rescale thermostat at 303 K and Parrinello-Rahman Barostat at 1 bar. We used 0.01, 0.10, 0.20, and 0.30 P/L in the simulations following our previous experimental results DISCUSSION AND RESULTS: Peptide binding and flip were observed in our simulations with larger bilayers and vesicles. Peptide adsorption and flip altered bilayers and vesicle shapes in 0.10, 0.20, and 0.30 P/L. Severe membrane protrusions were observed for 0.20 and 0.30 P/L for bilayers and vesicles, indicating that BP100 and possibly other similar antimicrobial peptides disrupt membranes via the carpet model. CONCLUSION: Our previously described peptide flip using all-atom set-ups was reproduced using coarse-grained simulations. Flipped peptides induced membrane shape alterations in larger peptide concentrations, finally inducing membrane protrusions, observed in both bilayers and vesicles. Our findings align with experimental data and indicate the carpet model as the mechanism of action for BP100, and possibly for other similar antimicrobial peptides, at P/L > 0.2

Keywords: antimicrobial peptides, Molecular Dynamics, computer simulations / **Supported by:** FAPESP

P.05 - Structural Studies in Biomimetic Membranes and Cell Death Kinect Assay of the Antimicrobial Peptide ecPiscidin 2

Lúcio Otávio Nunes [1], Kelton R. de Souza[1,4], Talita Lopes dos Santos[1], Victor Hugo de Oliveira Munhoz[2], Jarbas Magalhães Resende[3], Evgeniy Salnikov[4], Christopher Aisenbrey[4], Burkhard Bechinger[4,5], Rodrigo Moreira Verly[1]
[1]Department of Chemistry, Federal University of Jequitinhonha and Mucuri Valleys (, Brazil), [2]Science and Technology Institute, Federal University of Jequitinhonha and Mucuri Valleys (, Brazil), [3]Department of Chemistry, Federal University of Minas Gerais (, Brazil), [4]Chemistry Institute, University of Strasbourg (, France), [5]Chemistry, Institut Universitaire de France (, France)

INTRODUCTION: Bacterial resistance against conventional antibiotics is a important public health problem that culminates in millions deaths annually. Therefore, it is extremely important search for alternatives to existing antibiotic therapies. OBJECTIVES: The present work presents biophysics studies of the peptide ecPiscidin 2 (ecPis-2s) in order to understand the structure-activity relationship. MATERIALS AND METHODS: The ecPis-2s peptide (FFFHIIKGLFHAGRMIHGLV), isolated from *Epinephelus coioides* fish, which presents activity against bacteria and fungi. Circular dichroism (CD) spectra were obtained for solutions of 100 μM ecPis-2s in the presence of different concentrations of sodium dodecyl sulfate (SDS). Solution and solid state NMR experiments were carried out to better understand, in atomic level, the interaction of the peptide with mimetic membranes. Bacterial death kinect assay against *Salmonella typhimurium* also was performed to observe the biological activity of the peptide. DISCUSSION AND RESULTS: CD data showed undefined conformation in water, while the peptide acquires α-helix conformation in the presence of anionic micelles. Solution NMR structures revealed an defined α-helix conformation from the I6 to L19 residue. Accordingly with the solid state NMR data the peptide presents an predominantly parallel orientation to the POPC:POPG bilayers. CONCLUSION: Finally, bacterial death kinect studies demonstrated activity against *Salmonella typhimurium* of the ecPis-2s mainly in the concentrations of peptide equal or superior of 51,12 μM. All the data evidences a predominantly helical structure of the peptide with an unfolded N-terminal region phenylalanine rich. This region can be responsible to anchor the peptide in the membrane allowing high membrane disruption.
Keywords: Peptide, Nuclear Magnetic Resonance, Biomimetic membrane
Supported by: UFVJM, CAPES, CNPq, Fapemig

P.06 - Immobilization of Bioactive Peptide on Alumina Nanoparticles for Biotechnology Applications

Carolina Silva Ferreira[1], **Larissa Silveira Matsamura** [1], Lúcio Otávio Nunes[1], Rodrigo Moreira Verly[1]
[1]Departamento de Química, Universidade Federal dos Vales do Jequitinhonha e Mucuri (MG, Brasil)

INTRODUCTION: The development of multiresistance pathogens has demanded new agents with fungicidal and bactericidal for therapeutic treatments. In this context, peptides rise as a promising class of new antibiotics. In turn, alumina nanoparticles are valuable inorganic material for modification with antimicrobial molecules, in addition to protecting the peptide against proteolytic degradation. Therefore, nanobiostructures of alumina conjugated with antimicrobial peptide extend the perspective for new biotechnological applications. OBJECTIVES: This work proposes the synthesis of alumina nanobiostructures containing two different binding forms of the antimicrobial peptide Lunatin-1: (i) close to the C-terminal region of the peptide (Lun-1-NP) and (ii) close to the N-terminal region (NP-Lun-1), in order to verify if the position of the bond interferes with the activity of the peptide. In addition, the immobilization of the peptide in the nanoparticle can reduce its degradation and potentiate the biological activity. MATERIALS AND METHODS: The obtained nanobiostructures were characterized by transmission microscopy (TEM), infrared spectroscopy (FTIR) and solid phase nuclear magnetic resonance (NMRs). The interaction between nanobiostructures and membrane were investigated through circular dichroism (CD) spectroscopy and isothermal tritation calorimetry (ITC). DISCUSSION AND RESULTS: TEM image revealed nanofiber structures of near 20 nm. The successes of covalently biding obtaining of nanobiostructures containing peptide was proved by NMR and FTIR spectroscopy. CD spectra showed characteristic profile of helix conformation for free peptide and both nanobiostructures in the presence of POPC:POPG LUVs, suggesting the interaction of Lunatin-1 with membrane mimetic media even when binding to the alumina. Finally, using the ITC technique it was possible to obtain the thermodynamic parameters of the interaction of both nanobiostructures with membrane mimetic media, which revealed higher affinity to the anionic membranes for the Lun-NP conjugated when compared to the NP-Lun. CONCLUSION: This results indicate a more effective interaction of Lun-NP, confirming that the N-terminal region is important for the interaction and internalization of the peptide.
Keywords: Nanoparticles, Peptide-membrane interaction, Antimicrobial peptides / **Supported by:** FAPEMIG

P.07 - Application of metallopeptides derived from histatin-5 to fight infections caused by *Candida albicans*.
Nathalia Naliatto[1], Saulo Santesso Garrido[1]
[1]Bioquímica e Química Orgânica, Instituto de Química - UNESP - Araraquara/SP (SP, Brasil)

INTRODUCTION: Fungal infections are often neglected diseases because they are not officially reported. These infections are becoming one of the most important causes of human mortality. They have a high prevalence in Brazil and are more deadly for immunocompromised patients. *Candida albicans* is one of the main pathogens related to prevalent fungal infections. Some studies indicate that incidence of these infections varies according to socioeconomic and individual risk conditions. Therefore, is necessary the development of effective drugs and molecules for the treatment of these patients. In this scenario we have the biologically active peptides found naturally in living organisms, which has been an alternative for this purpose. One of these peptides is the Histatin-5, which is commonly present in human saliva and exhibit a great antifungal activity against *C. albicans*. OBJECTIVES: Improve antifungal activity of the peptide Histatin-5 binding metals copper(II) and zinc(II) in this peptide. MATERIALS AND METHODS: 0WHistatin-5 was synthesized using the solid phase peptide synthesis (SPPS) by the Fmoc/tBu protocol. The formation of a complex between the peptide and the metallic ions Cu^{2+} and Zn^{2+} was verified through measurements of molecular fluorescence spectroscopy and the antifungal potential of the complexes in the inhibition of *C. albicans* strains was evaluated by minimum inhibitory concentration tests (MIC). DISCUSSION AND RESULTS: The results indicate that peptide:metal complexes was formed at ratio of 1:2 molar equivalents. The results showed that concentrations below 80 µM of the complex 0WHistatin-5:Zn^{2+} were able to inhibit more than 95% of the growth of *C. albicans*. Only with 10 µM the WHistatin-5:Zn^{2+} complex was able to inhibit more than 90% of the growth of the ATCC 10231 strain, that is resistant to fluconazole. CONCLUSION: Complexation of Zn with the 0WHistatin-5 peptide was able to increase the antifungal potential of this peptide, being an alternative in the treatment of fungal infections caused by C. albicans
Keywords: Antifungal peptides, Candida albicans, metalopeptides

P.08 - Biophysical Analysis of LyeTxI and LyeTx-b Antimicrobial Peptides
Amanda Neves de Souza [1], Kelton Rodrigues de Souza[1], Rodrigo Moreira Verly[1]
[1]Química, Universidade Federal dos Vales do Jequitinhonha e Mucuri (Minas Gerais, Brasil)

INTRODUCTION: LyeTxI is an antimicrobial and antifungal peptide isolated from the venom of the Lycosa Erythrognatha spider containing 24 amino acid residues. Our research group has developed the LyeTxI-b, a new peptide derived from LyeTxI by deletion of a His residue and acetylation of N-terminus. LyeTxI-b is 10 times more active than native against Escherichia coli. OBJECTIVES: Provide a comparative study of the peptide-membrane interaction by using circular dichoism spectroscopy (CD), isothermal titration calorimetry (ITC) and calcein extravasation techniques. MATERIALS AND METHODS: LyeTxI and LyeTxI-b were synthesized by the solid-phase synthesis. The peptide-membrane interaction of both peptides was characterized by CD, ITC, and calcein extravasation in the presence of 1-palmitoyl-2-oleyl-sn-glycero-3-phosphocholy (POPC), 1-palmitoyl-2-oleyl-sn-glycero-3-phosphoglycerol (POPG) in a 3:1 molar ratio (POPC:POPG) in order to mimicking bacterial and cancer cell membranes. DISCUSSION AND RESULTS: The CD profile of both peptides are in agreement with helical conformation in the presence of mimetic membranes. However, higher helical content is noted to the derivative LyeTxI-b in comparison with native LyeTxI. The extravasation experiments reveals that LyeTxI-b was able to promote greater lytic effect on POPC:POPG vesicles at the same concentration than LyeTxI, resulting in a greater fluorescence intensity. The thermodynamic parameters of peptide-membrane interaction confirm the higher affinity between LyeTxI-b and POPC:POPG when compared to the LyeTxI (twice higher), mainly drove by electrostatic interactions. CONCLUSION: The structural modification of LyeTxI-b provide great changes in the secondary and peptide-membrane interaction, which results in higher lytic activity of the LyeTxI-b when compared to the wyde-type peptide.
Keywords: peptide-membrane interaction, structural modification, pore-forming peptides

P.09 - Combining NMR Spectroscopy and Molecular Dynamics Simulations for Insights into Synoeca-MP Peptide in SDS Micelles

Bruno de Paula Oliveira Santos [1,3], Eliane Santana Fernandes Alves[2], Leticia Valvassori Rodrigues[4], Carlos Daniel Pereira Freitas[2], Lucianna H. S. Santos[8], Simoni C. Dias[5,7], Octávio Luiz Franco[5,6], Luciano Morais Lião[2], Mariana Torquato Quezado de Magalhães[1,3]

[1]Pós-graduação em Bioquímica e Imunologia, Universidade Federal de Minas Gerais (MG, Brazil), [2]Laboratório de RMN, Insituto de Química, Universidade Federal de Goiás (GO, Brazil), [3]Laboratório de Biofísica de Macromoléculas (LBM), Universidade Federal de Minas Gerais (MG, Brazil), [4]IFG, Instituto Federal Goiano (GO, Brazil), [5]Centro de Análises Bioquímicas e Proteômicas, Universidade Católica de Brasília (DF, Brazil), [6]S-Inova Biotech, Pós-Graduação em Biotecnologia, Universidade Católica Dom Bosco (MS, Brazil), [7]Pós-Graduação em Biologia Animal, Universidade de Brasília (DF, Brazil), [8]Biomolecular Simulations Group, Institut Pasteur de Montevideo (, Uruguay)

INTRODUCTION: Synoeca-MP is a 14-residue amidated peptide with antibacterial and antifungal activity already comproved. The peptide belongs to the mastoparan family and it is found in the venom of the wasp Synoeca surinama. The low cytotoxicity of the peptide also makes it an excellent candidate for drug development. OBJECTIVES: To better understand its selectivity and interaction with the anionic membrane, the peptide interaction in membrane-like environments was studied. MATERIALS AND METHODS: The behavior of the peptide in growing hydrophobic moieties and in different pH ranges was studied by CD spectroscopy. The peptide structure in SDS micelles was determined by NMR spectroscopy. The incorporation of residues into the anionic micelles was studied by TOCSY hydrogen-deuterium exchange. The stability, the per-residue interaction, the insertion depth and the orientation of peptide in the micelles was studied by molecular dynamics simulations. DISCUSSION AND RESULTS: Synoeca-MP, bound to SDS micelles, exhibits a partial α-helix conformation, with the first 3-5 residues and the last 2 coiled. H/D exchange showed that the peptide has a slow exchange rate. After 164 hours, four residues had not yet completed H/D substitution (Asn2, Leu6, Ile11, Ala12), suggesting parallel alignment of the peptide with the micelle, mainly due to the hydrophobic interface. The peptide dynamics simulations showed that the residues Trp3, Lys5 and Lys9 are the main responsible for the peptide insertion in SDS micelle. Comparing pre-folded and unfolded peptide insertion, they both seem to interact in a parallel manner, with a preference for the folding beginning in the C-terminal. CONCLUSION; The biophysical analyses can improve the atomic understanding of the mode of action of the peptide and help in future improvements of the peptide for clinical usage.

Keywords: bioactive peptides, peptide structure, AMP / **Supported by:** LMProt/UFMG, CNPq, FAPEG and NMRbox

P.10 - Macrobrachium amazonicum: Acute toxicity to nitrite

Daniela Pereira Garçon [1], Leonardo Milani Fabri[4], Cintya Moraes[4], John C McNamara[3], Francisco de Assis Leone[2]

[1]Campus Iturama, Universidade Federal do Triângulo Mineiro (Minas Gerais, Brazil), [2]Departamento de Química , Universidade de São Paulo (São Paulo, Brazil), [3]Departamento de Biologia, Universidade de São Paulo (São Paulo, Brazil), [4]Departamento de Bioquímica e Imunologia, Universidade de São Paulo (São Paulo, Brazil)

INTRODUCTION: The Amazon River shrimp *Macrobrachium amazonicum* is widely distributed throughout South America, is amply exploited by artesian fisheries, and shows elevated potential for aquaculture investment. OBJECTIVES: To establish the lethal critical concentration of nitrite after 96 h (LC_{50}-96 h). MATERIALS AND METHODS: Adult shrimps were collected from the Rio Grande River, state of Minas Gerais, Brazil, near the Água Vermelha Dam. Individual shrimps (N=10) were exposed to 7 concentrations of sodium nitrite (0, 10, 20, 30, 40, 50 e 60 mg L^{-1}) for 96 h. Six replicate experiments were performed. The LC_{50} after 96 h exposure was calculated using a Probit analysis, and was adjusted employing a linear regression ($P \leq 0.05$). DISCUSSION AND RESULTS: The LC_{50} calculated after 96 h exposure of *M. amazonicum* was 24.9 mg L^{-1} nitrite. Based on these findings we recommend a safe level of 2.49 mg L^{-1} for aquaculture production. CONCLUSION: Intraspecific genetic and physiological variation among *M. amazonicum* populations may explain the difference in LC_{50}-96 h seen between the shrimp population cultivated in the Rio Grande River and those cultivated at other facilities.

Keywords: Macrobrachium amazonicum, Acute toxicity, nitrite / **Supported by:** CNPq, FAPESP, FAPEAM, INCT-ADAPTA, FAPEMIG

P.11 - Evaluation of the insecticide activity of a peptidase inhibitor isolated from Anadenanthera macrocarpa seeds against Anagasta kuehniella

Welington Araújo Leite[1], Ana Cristina Jacobowski[1], **Maria Ligia Rodrigues Macedo [1]**
[1]LPPFB, Universidade Federal Do Mato Grosso Do Sul (Mato Grosso do Sul, Brasil)

INTRODUCTION: Protease inhibitors (PIs) form part of the plant defense system, where they reduce the proteolytic activity of digestive enzymes of insect pests. OBJECTIVES: he aim of this study was to isolate and characterize an inhibitor of trypsin (AmTI) present in the seed of *Anadenanthera macrocarpa* (Benth) (Leguminosae-Mimosoideae) and to assess the defense mechanism developed by the larvae of *Anagasta kuehniella* (Zeller) fed with this inhibitor. MATERIALS AND METHODS: Seed protein extracts were purified on a Sephadex G-50 and trypsin-Sepharose column, and the isolated inhibitor was incorporated into the artificial diet fed to *Anagasta kueniella*. DISCUSSION AND RESULTS: Electrophoresis showed that the inhibitor had a molecular weight of 25 kDa. Stability evaluation showed that the inhibitor was not denatured at temperatures of up to 60 °C, pH 2–10 and concentrations of up to 100 mM DTT for one hour. The inhibitor reacted in a ratio of 1:1 with bovine trypsin and had a Ki = 2.517 x 10^{-8} . Incorporation of the inhibitor in a proportion of 1 mg in 100 mg of artificial diet offered to *A. kuehniella* larvae resulted in no significant difference in the weight or survival of larvae between the third and fifth instars. Trypsin and chymotrypsin activities in the gut of insects revealed that there was an increase in tryptic activity in the fourth instar to overcome this inhibitory activity. CONCLUSION: A new trypsin inhibitor with activity against the digestive enzymes of *A. kuehniella* was discovered; furthermore, trypsin up regulation may represent one of the possible mechanisms of adaptation to insect inhibitors.

Keywords: plant-insect interaction, peptidase inhibitor, Kunitz inhibitor
Supported by: PROPP/UFMS, CAPES, CNPq, FUNDECT

P.12 - Structural Characterization and Antitumor Activity of the Wasp Peptide Protonectin and its Analogue Protonectin-F

Jéssica de Araujo Isaias Muller [1,2], Johannes Koehbach[2], Nicole Lawrence[2], Lai Yue Chan[2], Marcia Renata Mortari[3], David J Craik[2], Monica Cristina Toffoli-Kadri[1]
[1]Laboratory of Pharmacology and Inflammation, Federal University of Mato Grosso do Sul (MS, Brazil), [2]Institute for Molecular Bioscience, The University of Queensland (Brisbane, Australia), [3]Lab. of Neuropharmacology, University of Brasília (FD, Brazil)

INTRODUCTION: Antimicrobial peptides, present in different types of venom are typically 10-40 amino acids in size, C-terminally amidated and can have antitumor properties. However, these peptides can be toxic to healthy cells. Protonectin is a wasp antimicrobial peptide, but its antitumor activity remains unknown. OBJECTIVES: Modify the sequence of protonectin, characterize the structure and evaluate antitumor activity of both peptides. MATERIALS AND METHODS: We changed the second and eighth Leucine of protonectin (ILGTILGLLKGL-NH_2) to Phenylalanine, resulting in the new peptide protonectin-F. Both peptides were synthesized using Fmoc solid-phase peptide synthesis and purified using high-performance liquid chromatography (purity >95%). To characterize the structure, we did circular dichroism and NMR spectroscopy. Both experiments were conducted in water and 30% TFE. NMR assignments were done in CCPNMR and structure prediction using TALOS and CYANA. Resazurin-based cytotoxicity assay was carried out using 5x10^3 cells/well of cancer cell lines MM96L and HCT116 and normal HaCaT and HEK293 cells. The viability was determined 24 hours after addition of peptides in a plate reader. DISCUSSION AND RESULTS: Both peptides were unstructured in water. In 30% TFE, protonectin and protonectin-F are more α-helical between amino acids 2-11 and 4-10, respectively. Cytotoxic concentrations (CC_{50}) of peptides were quite similar against MM96L (protonectin: 14.8±0.87 μM; protonectin-F: 16.0±1.20 μM) and the correlated normal cell HaCaT (protonectin: 18.3±1.15 μM; protonectin-F: 20.2±1.93 μM). The cytotoxicity was higher against HCT116 (protonectin: 8.0±0.46 μM; protonectin-F: 9.8±1.21 μM), however with the correlated normal cell HEK293, protonectin-F was less toxic (18.9±1.14 μM) than protonectin (11.7±0.42 μM). CONCLUSION: The substitution of Leucine for Phenylalanine made the structure of protonectin-F more flexible and reduced the cytotoxicity against HEK293, which indicates that protonectin-F has potential as an antitumor peptide.

Keywords: peptide, α-helix, cytotoxicity / **Supported by:** CAPES, PMBqBM/UFMS

P.13 - New Antimicrobial Peptide (PEPAD) Has Activity Against Resistant Bacteria And Synergistic Effect With Ciprofloxacin

Camila de Oliveira Gutierrez [1], Luís Henrique de Oliveira Almeida[1], Caio Fernando Ramalho de Oliveira[1], Janaína de Cássia Orlandi Sardi[1], Maria Lígia Rodrigues Macedo[1]
[1]LPPFB / FACFAN, Universidade Federal de Mato Grosso do Sul (Mato Grosso do Sul, Brasil)

INTRODUCTION: Bacterial resistance is an overwhelming worldwide public health issue. Therefore, it is necessary to develop new drugs able to treat resistant microorganisms. Studies with antimicrobial peptides have being developed due their antimicrobial potential and several mechanisms of action. OBJECTIVES: We designed a new antimicrobial peptide, named PEPAD, and aimed to assay it antimicrobial activity against bacterial strains, beyond to investigate it synergic effect in association with ciprofloxacin. The toxicity of PEPAD was investigated in *Galleria mellonella* model. MATERIALS AND METHODS: To determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC), the broth microdilution technique was used. The toxicity studies were carried out in 200-300 mg larvae, that received injections of 10 µL of PEPAD, ranging from 4 µM to 50 µM. Control larvae were injected with NaCl 0.9%, and the mortality observed during 72 h. DISCUSSION AND RESULTS: PEPAD is an antimicrobial peptide with 18 amino acids, displays an amphipathic structure and assumes an alpha helix secondary structure in presence of SDS and TFE solutions. The PEPAD showed activity against methicillin resistant *Staphylococcus aureus* (MRSA), with MIC and MBC of 8 µM. The MIC and MBC for *E. coli* KPC+ was10 µM. The combination of PEPAD with ciprofloxacin showed synergism against *Staphylococcus saprophyticus*. The MIC of PEPAD reduced from 4 µM to 0.007 µM; the ciprofloxacin MIC reduced from 1 µM to 0.5 µM. For *Pseudomonas aeruginosa*, the MIC of PEPAD reduced from 5 µM to 0.002 µM; while the MIC for ciprofloxacin remained at 1µM, suggesting an additive effect. No toxicity signals were noticed in *G. mellonella* larvae, supporting the safety for PEPAD administration. CONCLUSION: PEPAD proved to be a peptide with antimicrobial potential, especially when combined with ciprofloxacin, beyond to display no toxic effects in the *In vivo* model.

Keywords: bacterial resistance, Galleria mellonella, *In vivo* assay / **Supported by:** FUNDECT, CAPES, CNPq, Finep and PROPP-UFMS

P.14 - Rational Design Of The Antimicrobial Peptide Ctx(Ile^{21})-Ha Analogs Increases Cationicity And Enhances Antifungal Activities

Acsa Thalita Ferreira Pimenta Santos[1], Caroline Barcelos Costa Orlandi[2], Carolina Orlando Vaso[2], Níura Madalena Bila[2], Maria José Soares Mendes-Giannini[2], **Eduardo Festozo Vicente** [1]
[1]Department of Biosystem Engineering, School of Sciences and Engineering, São Paulo State University (São Paulo, Brazil), [2]Department of Clinical Analysis, School of Pharmaceutical Sciences, São Paulo State University (São Paulo, Brazil)

INTRODUCTION: The antimicrobial resistance is a global concern, which efficient molecules are demanded to tackle enormous problem. Antimicrobial peptides are the most promising candidates to be developed, as they are versatile and shown potent biological activities against bacteria and fungi. Specifically, the antimicrobial peptide Ctx(Ile ^{21})-Ha has been vastly studied by our research group and, by rational design, various analogs were proposed to improve their physicochemical features and increase their biological activities. OBJECTIVES: In this work, it was proposed six peptide analogs of the Ctx(Ile ^{21})-Ha, replacing strategic residues of the primary sequence by lysines, in order to improve the cationicity/hydrophobicity of the molecules. MATERIALS AND METHODS: The peptide analogs were synthesized by solid phase peptide synthesis (SPPS), purified and properly characterized. The minimal inhibitory concentration of the peptide analogs was tested against *Candida albicans* (SC5314), *C. parapsilosis* (ATCC 22019), *Cryptococcus neoformans* (H99), *C. gattii* (ATCC 56990), *Trichophyton rubrum* (ATCC MYA-4438) and *T. mentagrophytes* (ATCC 11481). To evaluate the cytotoxicity, the inhibitory concentration values (IC_{50}) of Ctx(Ile ^{21})-Ha and its analogs were performed in glioblastoma cells (U87), lung fibroblasts (MRC5) and skin fibroblasts (HDFa). The selectivity index (SI) was calculated, obtained by the ratio between the IC_{50} and MIC values. The higher the SI, the less damage is done to host cells. DISCUSSION AND RESULTS: The results showed that the analogs [Pro ^{13}]Ctx(Ile ^{21})-Ha, [Lys ^{4} ,Pro ^{13}]Ctx(Ile ^{21})-Ha, [Lys ^{4}]Ctx(Ile ^{21})-Ha, [Lys ^{4} , ^{15}]Ctx(Ile ^{21})-Ha and [Lys ^{4} , ^{15} , ^{19}]Ctx(Ile ^{21})-Ha were little or no toxic for glioblastoma cells and human skin fibroblasts, with relatively high IC_{50} values, in contrary to the observed in lung fibroblasts. The [Lys ^{4} , ^{15}]Ctx(Ile ^{21})-Ha and [Lys ^{4} , ^{15} , ^{19}]Ctx(Ile ^{21})-Ha analogs presented the best selectivity index in U87 and HDfa cells and for *C. parapsilosis*, *C. neoformans* and *C. gattii* strains, with values between 7.4 and 32. CONCLUSION: In overall, the designed analogs shown promising biological activities and further studies will be performed to understand in details their biophysics and mechanism of actions.

Keywords: Antimicrobial peptides, Structure Activity Relationship, Antifungal activity / **Supported by:** FAPESP

P.15 - Description of Membrane interaction and Proteolytic Resistance on an Antimicrobial Piscidin Peptide

Kelton Rodrigues Souza[1], Lúcio Otávio Nunes[1], **Lucas Rocha Gomes** [1], Elise Glattard [2], Evgeniy Salnikov[2], Talita Lopes dos Santos [1], Victor Hugo de Oliveira Munhoz[1], Jarbas Magalhães Resende[3], Christopher Aisenbrey [2], Burkhard Bechinger[2,4], Rodrigo Moreira Verly[1]
[1]Department of Chemistry, Universidade Federal dos Vales do Jequitinhonha e Mucuri (Minas Gerais, Brazil), [2]Chemistry Institute, University of Strasbourg (, France), [3]Department of Chemistry, Universidade Federal De Minas Gerais (Minas Gerais, Brazil), [4]Chemistry Institute, Institut Universitaire de France (, France)

INTRODUCTION: Multidrug-resistant bacterial infection has become a serious threat in human therapeutics to make raising the development of new antibiotics [1] . Therefore, new approaches have been investigated in order to develop new drug candidates [2] . Antimicrobial peptides represent a promising alternative to the treatment of infections as well as to avoiding bacterial resistance development [3] . OBJECTIVES: In this work, we present biophysical studies of the ecPis-4s peptide previously described as a potent antimicrobial agent against bacterial and fungal strains [4] . MATERIALS AND METHODS: Firstly, ecPis-4s was synthesized by solid-phase synthesis, purified by HPLC, and characterized by mass spectrometry. Circular dichroism spectroscopy shows a high content of helical structure in the presence of POPC:POPG vesicles at different concentrations. The affinity and thermodynamic parameters of peptide-membrane interaction were obtained in a quantitative manner by ITC, revealing significant affinity to negative vesicles (10 3 –10 4 M −1) mainly driven by enthalpic contribution (1.7 Kcal/mol). DISCUSSION AND RESULTS: The results of proteolysis experiments and enzyme kinetics of trypsin showed almost complete degradation after 1h of digestion at all concentrations tested. Interestingly, a fragment at m/z 847.4 was observed which corresponds to the segment encompassing the six first amino acid residues of the ecPis-4s (FFRHIK). These findings suggest that the N-terminal region is not digested by the trypsin enzyme. On the other hand, the degradation kinetics by proteinase K revealed complete degradation after 1 h at 100 µM of peptide concentration and only after 8h of incubation at 300 µM. CONCLUSION: In conclusion, our results indicate the peptide presents a well- known behavior of antimicrobial peptides, which acquires high-defined helical structure when interacting with the anionic membranes. Nevertheless, the concentration-dependent helicity in aqueous solution suggests new structural features which could be related to the N-terminal resistance to the trypsin and the low kinetics degradation from proteinase K. Therefore, these findings reveal the potential of the ecPis-4s as a new antibiotic agent.

Keywords: antimicrobial peptide, peptide-membrane interaction, proteolytic activity
Supported by: Campus France, Université de Strasbourg, CNRS, ANR, UFVJM, CNPq and CAPES

P.16 - PEP-X, a synthetic peptide derived from a spider toxin, is effective against diabetic neuropathic pain in mice and acts synergistically with pregabalin.

Xavier Maia Mariano[1], **Xavier M. Mariano** [1], Luana De Assis Ferreira[1], Célio José de C. Jr[1], Maria Elena De Lima[1]
[1]Programa de Pós-Graduação em Medicina e Biomedicina, Faculdade da Santa Casa de Belo Horizonte (BH, Brazil)

INTRODUCTION: Diabetic neuropathic pain is one of the most painful complications that affect a wide variety of the diabetic population. Pregabalin is one of the most used drugs for the treatment of neuropathic pain, but it has partial efficacy and causes side effects, including drowsiness, dizziness, and peripheral edema. OBJECTIVES: The main objective of our study was to verify the antinociceptive effect of a synthetic peptide (herein called PEP-X*) derived of a toxin from the venom of the spider Phoneutria nigriventer, alone and combined with pregabalin, in diabetic Swiss male mice. MATERIALS AND METHODS: DISCUSSION AND RESULTS: The dose-response curves indicate the ED50 of 0.47± 0.09 nanomoles and 1982.81 ± 0.07 nanomoles for PEP-X and pregabalin, respectively. This result shows that the antinociceptive effect of the peptide was more than 4000 times greater than that of pregabalin. The antinociceptive effect of PEP-X lasted for 60 minutes, while that of pregabalin started after 30 minutes after administration and lasted 120 minutes. The antinociceptive activity of both compounds was dose-dependent and showed synergism, demonstrated by isobolographic analysis. The theoretical (ED50 Zadd) and experimental ED50 values were 1231.25 ± 1.132 nmoles and 274.75 ± 0.85 nmoles, respectively, calculated for two hours of the experiment. The interaction index (I.I.) was 0.22, confirming the synergism of the compounds. Treatment with 0.51 nmoles PnPP-15 caused no spontaneous nor forced motor alterations. CONCLUSION: In conclusion, the combination of PEP-X and pregabalin, compared to the isolated drugs, increased the therapeutic response, significantly reducing the respective doses. The possible mechanism of action of PEP-X isolated and combined with PGB in nociception is being investigated. *PEP-X is a provisional name, considering the requirement of secrecy for patent filing.

Keywords: Neuropathic pain, Hyperglycemia, Pregabalin, PEP-X / **Supported by:** FAPEMIG, CNPq and CAPES

P.17 - Evaluation Of Antifungal Activity Of RQ-18, A Synthetic Peptide

Claudiane Vilharroel Almeida [1,2], Caio Fernando Ramalho de Oliveira[1,2], Janaina de Cássia Orlandi Sardi[2], Maria Ligia Rodrigues Macedo[2]

[1]Instituto de Biociências, Universidade Federal de Mato Grosso do Sul (Mato Grosso do Sul, Brazil), [2]Faculdade de Ciências Farmacêuticas Alimentos e Nutrição, Universidade Federal de Mato Grosso do Sul (Mato Grosso do Sul, Brazil)

INTRODUCTION: The spreading of drug resistant fungi is one of the most threatening worldwide issue. In that view, antimicrobial peptides (AMP) have been considered one of the alternatives for fungal infections control. OBJECTIVES: We aimed to investigate the structural and functional features of a new alpha-helical AMP, named RQ-18, against fungi of clinical importance. MATERIALS AND METHODS: Followed the chemical synthesis, the minimum inhibitory concentration (MIC) and antibiofilm activity were determined. The mechanism of action of RQ-18 on the fungi and time-kill kinetics assays were also evaluated. The toxicity assays were carried, *in vitro*, with murine macrophage (RAW264.7), and *In vivo* , in *Galleria mellonella* larvae, injecting the peptide 10-fold higher than MIC. DISCUSSION AND RESULTS: RQ-18 is a cationic peptide with amphipathic structure, containing 18 amino acid residues. The MIC for *Candida tropicalis* ATCC 22019 and *Cryptococcus gattii* AFLP4 was 5 μM. The mechanism of action of RQ-18 involves damages of plasma membrane, as confirmed by Sytox green fluorescence microscopy and the increase of MIC when the peptide was incubated with *C. tropicalis* in a media enriched with ergosterol, the most abundant sterol in fungal cell membranes. The peptide reduced *C. tropicalis* biofilm formation by 36,7% and 100% at 5 μM and 50 μM respectively and also eradicated mature biofilms by 27,2% and 32,8% at 5 μM and 50 μM respectively. No toxic effect on cell viability was observed in RAW264.7 (up to 64 μM) cells and in *G. mellonella* larvae, up to 100 μM. CONCLUSION: In summary, RQ-18 represents a promising strategy for the development of a new antimicrobial agent to contribute with control of antibiotic-resistant infections.

Keywords: synthetic peptides, antimicrobials, antifungals

Supported by: PROPP-UFMS, CNPq, CAPES, FUNDECT and INCT

Q - Membrane Permeation: Channels and Transporters

Q.01 - Expression profile of zinc channels in human Renal Cell Carcinoma after Temsirolimus treatment

Soraia Barbosa de Oliveira [1], Luana da Silava Ferreira[1], Maria Helena Bellini Marumo[1]
[1]Molecular and Cellular Biology of Cancer Dep, Biotechnology Center, Nuclear and Energy Research Institute (SP, Brasil)

INTRODUCTION: Zinc is an essential trace element for cell proliferation and growth. Cellular Zn is regulated of by ZnT and ZIP family channels but its mechanism still not completely understood. Renal cell carcinoma (RCC) is one of the most malignant renal tumors. The RCC clear cell pathological subtype is associated with the VHL gene mutation, that is responsible for its aggressiveness. Temsirolimus (TEM), an antineoplastic drug used in the treatment of RCC, is a selective inhibitor of mTOR. OBJECTIVES: To evaluate the expression of zinc channels in clear cell renal carcinoma cell line with and without TEM treatment. MATERIALS AND METHODS: The MTS (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide) assay was used define IC50. The expression of ZnT1, ZnT4, ZIP11 and ZIP14 channels from the HK-2, 786-0 and 786-0/TEM cells was evaluated by real-time PCR and Western blot analysis. DISCUSSION AND RESULTS: The IC50 dose was 10µM. Gene expression analysis comparing Hk-2 and 786-0 cell lines revealed decreased levels of ZnT1 of 79.20±3.58% ($P< 0.0001$ vs HK-2) and an increase for the ZIP 11 of 243.3±62.84% ($P< 0.01$ vs HK-2). The comparison between 786-0 with and without TEM treatment showed decreased levels of ZnT1 of 34.03±20.45% ($P< 0.05$ vs 786-0), ZnT4 of 92.82±0.72% ($P< 0.0001$vs 786-0), ZIP14 of 11.24% ($P< 0.01$ vs 786-0) and ZIP11 of 95.96±0.54% ($P< 0.0001$ vs 786-0). Western blot data corroborated the real time results. CONCLUSION: There is a difference in the Zn channel expression profiles between HK-2 and 786-0. The treatment with TEM modulates the expression of these channels

Keywords: RCC, Zn channels, Temsirolimus / **Supported by:** CAPES and IPEN

Q.02 - Effects of $CoCl_2$ on posterior gill K^+-phosphatase activity in the swimming crab *Callinectes danae*

Cintya Mendes Moraes [1], Leonardo Milani Fabri[1], Maria Izabel Cardoso da Costa[3], Daniela Pereira Garçon[4], John Campbell McNamara[2], Francisco de Assis Leone[2]
[1]Departamento de Bioquímica, Faculdade de Medicina de Ribeirão Preto - Universidade de São Paulo (São Paulo, Brasil), [2]Departamento de Biologia, Faculdade de Filosofia Ciências e Letras de Ribeirão Preto - Universidade de São Paulo (São Paulo, Brasil), [3]Departamento de Química, Faculdade de Filosofia Ciências e Letras de Ribeirão Preto - Universidade de São Paulo (São Paulo, Brasil), [4]Departamento de Química, Campus Universitário de Iturama -Universidade Federal do Triângulo Mineiro (Minas Gerais, Brasil)

INTRODUCTION: The aquatic biome is extremely impacted by heavy metals such as cobalt. The swimming crab *Callinects danae* is one of many species affected. Co^{2+} may have an effect on the posterior gill K^+-phosphatase, an enzyme associated with the (Na^+-,K^+-)-ATPase, a transmembrane protein important in crustacean osmoregulation. OBJECTIVES: This study aimed to better understand the effects of Co^{2+} on gill K^+-phosphatase activity. MATERIALS AND METHODS: The kinetic assays were performed continuously at 25 ºC and 410 nm. Total K^+-phosphatase activity was measured in 50 mM Hepes buffer under variable *p*NPP and $CoCl_2$ concentrations, and saturating $MgCl_2$ (7 mM) and KCl (15 mM) concentrations in a final volume of 1 mL. K^+-phosphatase activity is the difference between the total and ouabain (7 mM) insensitive *p*NPPase activities. DISCUSSION AND RESULTS: K^+-phosphatase was inhibited by 72% (K_I = 3 mM) under increasing $CoCl_2$ concentrations (10^{-5} to 2 x 10^{-2} mM) and saturating $MgCl_2$ and *p*NPP (10 mM) concentrations; V_M decreased from 157.5 to 44.3 *p*PNP^- min^{-1} mg^{-1} protein). Interestingly, a K^+-phosphatase stimulated monophasic curve (V_M = 122.5 *p*PNP^- min^{-1} mg^{-1} protein) was seen in the absence of $MgCl_2$. $CoCl_2$ concentrations greater than 10^{-2} mM increasingly inhibits K^+-phosphatase activity, similarly to $MgCl^2$. K^+-phosphatase was stimulated by a single curve (V_M = 128.2 *p*PNP^- min^{-1} mg^{-1} protein) with increasing *p*NPP concentrations, with both saturating $CoCl_2$ (3 mM) and KCl concentrations. Under the same conditions and Mg^{2+}, V_M was 63.1 *p*PNP^- min^{-1} mg^{-1} protein. CONCLUSION: Therefore, it is possible to say that under these conditions Mg^{2+} is able to dislocate up to 2 mM Co^{2+} from its binding site.

Keywords: K^+-phosphatase, $CoCl_2$, Callinectes danae
Supported by: FAPESP, CAPES, CNPq, FAPEAM, INCT-ADAPTA II

Q.03 - Salinity-Dependent Modulation by Protein Kinases and the FXYD2 Peptide of Gill (Na^+, K^+)-ATPase Activity in the Freshwater Shrimp *Macrobrachium amazonicum*

Leonardo Milani Fabri [1], Cintya Mendes Moraes[1], Daniela Pereira Garçon[2], Carlos Frederico Leite Fontes[3], John Campball McNamara[4,5], Francisco Assis Leone[6]

[1]Departamento de Bioquímica e Imunologia, , Faculdade de Medicina de Ribeirão Preto (São Paulo, Brasil), [2]Campus Iturama, Universidade Federal do Triângulo Mineiro (Minas Gerais, Brasil), [3]Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro (RJ, Brasil), [4]Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo (São Paulo, Brasil), [5]Centro de Biologia Marinha, Universidade de São Paulo (São Paulo, Brasil), [6]Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: The (Na^+, K^+)-ATPase is one of the main driving forces for gill based ion uptake in fresh water crustaceans, like *Macrobrachium amazonicum*. One regulatory pathway may involve (Na^+, K^+)-ATPase phosphorylation by PKA. The α- and γ- subunits can be phosphorylated by protein kinases A and C and may be part of a salinity-dependent signaling pathway. OBJECTIVES: Examine regulation of (Na^+, K^+)-ATPase activity using exogenous pig FXYD2 peptide and endogenous protein kinases A and C in *M. amazonicum* on salinity challenge. MATERIALS AND METHODS: Shrimps were acclimated to 21 ‰ salinity for 10 days or used directly after collection from the Tietê River (< 0.5 ‰S) to prepare gill microsomal fractions by differential centrifugation. ATPase activity was assayed at 25 ºC for 20 min through [^{32}P]-Pi release from [γ-^{32}P]-ATP by adding 20 µg of microsomal preparation. DISCUSSION AND RESULTS: Salinity acclimation reduced (Na^+, K^+)-ATPase activity by ≈60% compared to freshwater shrimps. The FXYD2 peptide stimulated activity of the salinity-acclimated shrimps by ≈50%; shrimps in fresh water were unaffected. PKA-mediated phosphorylation inhibited activity by 85% only in the salinity-acclimated shrimps and was fully reversed with H-89. PKC phosphorylation inhibited of enzyme activity by ≈55 and ≈40% in fresh water and salinity-acclimated shrimps, respectively. Regulation of gill (Na^+, K^+)-ATPase activity after salinity acclimation may explain the alterations in enzyme kinetics. CONCLUSION: This is the first demonstration that regulation of gill (Na^+, K^+)-ATPase by protein kinases and FXYD2 is salinity-dependent in freshwater crustaceans. The functional interaction between the gill (Na^+, K^+)-ATPase and the kinases and the FXYD peptides remains unclear but appears to be an important aspect of osmotic regulation in *M. amazonicum*.

Keywords: (Na+, K+)-ATPase regulation, Osmoregulation, Protein kinases / **Supported by:** Capes, FAPESP, CNPq, FAPEAM, INCT-ADAPTA

Q.04 - RND System: A Comparison of the Proteins That Integrate The RND System in Pseudomonas aeruginosa Genome

Vinnícius Machado Schelk Gomes [1], Manuela Leal da Silva[1], Pedro Henrique Monteiro Torres[2]

[1]Instituto de Biodiversidade e Sustentabilidade NUPEM, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil), [2]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: Introduction: Pseudomonas aeruginosa is a Gram-negative opportunistic pathogenic bacteria, which presents a marked antimicrobial resistance, an emerging concern that is predicted to cause 10 million deaths each year by 2050. One of the mechanisms that confers resistance to P. aeruginosa there are the efflux systems such as the RND System. OBJECTIVES: Objectives: Our goal is to analyze the differences between the proteins in the channel, doing comparisons on the proteins with the same functions in the bacterial genome. MATERIALS AND METHODS: Material and Methods: sequences were retrieved from TCDB. BLASTp were performed against proteins derived from the genome of Pseudomonas aeruginosa PAO1 strain. The sequences with an E-value < 0,05 with both, coverage and identity > 85% were taken into count; or were retrieved from NCBI Graphical Sequence Viewer where it was observed for sequences that were present in the same ORF that could complete this channel. Domain searches were performed using the CD-search against NCBI Conserved Domain Database, then the sequences were aligned using the Clustal Omega's EMBL-EBI. DISCUSSION AND RESULTS: Results: TCDB classifies the channel components in three groups, 12 RND pump sequences, 12 MFPs proteins and 8 OMFs were found. About the domains, were found 8 Domains for the RND pumps, 5 to the MFPs and 5 to the OMFs, although the domains from the same group doesn't differ in function. From the alignment we got a top identity for the RND pumps sequences of 60.44%, and average of 31,5%, for the MFPs 43.75% and 27.1% and for the OMFs 39.26% and 26.9% respectively. CONCLUSION: Conclusion: we found 10 complete channels plus 1 incomplete with a single copy, an indication that they could be expressed under different cell requirements, possibly exporting different antibiotics. This is a preliminary analysis, the data here will be the basis for a larger study.

Keywords: Membrane proteins, Antimicrobial resistance, Resistance Nodulation Division

Q.05 - Effect of the Hypoxia on the Expression of Zinc Transporters in Renal Adenocarcinoma Cell Line

Luana da Silva Ferreira [1], Soraia Barbosa de Oliveira [1], Maria Helena Bellini[1]
[1]Molecular and Cellular Biology of Cancer, Nuclear and Energy Research Institute (São Paulo , Brasil)

INTRODUCTION: Renal cell carcinoma (RCC) is a tumor responsible for about 1 to 3 % of all malignancies. The most common histological variant is the clear cell carcinoma (ccRCC), representing about 45% of all cases of RCC in adults. ccRCC is associated with the VHL gene mutation. The loss of the VHL protein prevents the degradation of HIF subunits, which are involved in critical oncogenic pathways. Zinc is an essential trace element and its cellular homeostasis is regulated by zinc transporters such as ZIPs and ZNTs. The profile of their expression in renal tumor is still unknown. OBJECTIVES: Evaluate the expression profile of zinc transporters in ccRCC in normoxia and hypoxia culturing conditions. MATERIALS AND METHODS: 786-0 tumor cells were cultured in hypoxic conditions inside a hypoxia chamber with an oxygen absorber to the atmosphere of 1% O2, 5% CO2, and 94% N2, and placed in an incubator at 37°C for 24 hours. The Altair PRO Single-Gas Detector was used to measure the percentage of O2. For gene expression analysis, RTq-PCR was used and the results were analyzed by the Delta-Delta ct method. DISCUSSION AND RESULTS: VEGF and HIF2a expression in 786-0 cells were evaluated to confirm the efficacy of hypoxia chamber. There was a significant increase in the VEGF expression of 312.8±2.14% (P< 0.0001) and HIF2a of 593.4±57.21% (P< 0.0092). Besides that, the gene expression analysis revealed a downregulation in the hypoxic environment of the channels ZNT9 of 71.41±0.84% (P< 0.0001), ZIP1 of 17.45±3.68% (P< 0.0418), ZIP4 of 76.3±9.75% (P< 0.0054) and ZIP10 of 44.96±4.31% (P< 0.0001). CONCLUSION: The hypoxia modulates the expression of Zn channels in 786-0 cells indicating that such channels play a role in the pathophysiology of ccRCC.

Keywords: Hypoxia, Renal Cell Carcinoma, Zinc Transporters / **Supported by:** CAPES and IPEN (2018.05.IPEN.08)

Q.06 - NCX Expression in Human Glioblastoma Multiforme Cell Lines and Differential Sensitivity to NCX inhibitors

Vyctória dos Santos Ramos [1], Laura Francisca Leite do Prado de Souza[2], Rayssa de Mello Lopes[2], Tiago Rodrigues[2], Ivarne Luis dos Santos Tersariol[3], Valeria Valente[4]
[1]Centro Interdisciplinar de Investigação Bioquímica, Universidade de Mogi das Cruzes (, Brazil), [2]Centro de Ciências Naturais e Humanas, Universidade Federal do ABC (, Brazil), [3]Departamento de Biologia Molecular, Universidade Federal de São Paulo (, Brazil), [4]Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista (, Brazil)

INTRODUCTION: Glioblastoma multiforme (GBM), also called astrocytoma grade IV, is a fast-growing and extremely aggressive type of brain tumor. GBM is very difficult to be treated and, besides surgical removal, the combination of the oral alkylating drug temozolomide and radiotherapy are the current available weapons. Since tumor cells often exhibit altered Ca^{2+} homeostasis to support the high proliferative rate, several calcium pumps and channels have been studied in cancer. However, only few studies have investigated the role of Na^{+}/Ca^{2+} exchanger (NCX). This exchanger has particular importance in GBM due to its high expression in cells from central nervous system OBJECTIVES: To evaluate the expression of the three NCX isoforms (NCX1, NCX2, and NCX3) in two different GBM cell lines comparatively and also to test their sensitivity to NCX inhibitors. MATERIALS AND METHODS: RNA sequencing was performed by Illumina sequencers (Genome Analyzer IIx and NextSeq 500) and NCX expression was obtained. GBM cell lines U-343MG and U-251MG ($3x10^4$ /cm²) were grown in supplemented DMEM high glucose medium. Growth curves were obtained by Neubauer chamber counting. The effects of NCX1 inhibitor KB-R7943 on cell viability were evaluated by the MTT reduction assay DISCUSSION AND RESULTS: 343MG cells exhibited a higher proliferative rate compared to U-251MG and consequently higher sensitivity to temozolomide. Conversely, U-251MG cells exhibited a higher NCX1 expression compared to U-343MG cells, which in turn, expressed more NCX3. In accordance, U-251MG cells were more sensitive to the inhibition of NCX1 by KB-R7943 than U-343MG CONCLUSION: Together, these data show differential expression of NCX in GBM cell lines, which modulates the sensitivity to NCX inhibition. Further studies are required to assess functional aspects of NCX in these GBM models.

Keywords: Glioblastoma, Calcium, NCX
Supported by: FAPESP, CNPq

Q.07 - Tebuconazole Interacts with Human $Na_V1.5$ Channels and Alters the Cellular Excitability of Cardiomyocytes Derived from Human Induced Pluripotent Stem Cells

Leisiane Pereira Marques [1], Artur Santos Miranda[1], Julliane Vasconcelos Joviano dos Santos[1], Elida Adalgisa Neri[2], Jaqueline Oliveira Sarmento[1], Jose Eduardo Krieger[2], Danilo Roman Campos[1]
[1]Biofísica, Universidade Federal de São Paulo (São Paulo, Brasil), [2]Instituto do Coração, Univ. de São Paulo (SP, Brasil)

INTRODUCTION: Pesticides are extensively used in the control and prevention of pests in agriculture, and urban and domestic environments. However, little is known about the cardiotoxicity of these compounds. Tebuconazole is a fungicide widely used in agricultural practice and there is evidence that suggests that it is able to change heart functioning. OBJECTIVES: To investigate the effect of tebuconazole on the biophysical properties of $Na_V1.5$, the voltage-gated sodium channel subtype predominantly expressed in cardiac tissue and the implications for cardiac action potential (AP). MATERIALS AND METHODS: Using a heterologous expression system, the human isoform of $Na_V1.5$ sodium channels were transiently expressed in Human Embryonic Kidney (HEK) 293 cells. Cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs) were used to study the effect of tebuconazole on cardiac AP. Electrophysiological measurements were performed in the whole-cell configuration of the patch-clamp technique, in the voltage-clamp mode for measuring sodium current (I_{Na}) or current-clamp for AP recordings. DISCUSSION AND RESULTS: Tebuconazole was able to reduce the sodium current peak amplitude in a dose-dependent manner and alter the biophysical properties of $Na_V1.5$ channels, such as shifting the steady-state inactivation curve to more negative potentials, delaying recovery from inactivation, and developing a use-dependent block in a voltage-dependent manner. In line with the observed effect on $Na_V1.5$ channels, tebuconazole reduced the amplitude and maximum rate of AP depolarization of hiPSC-CMs. CONCLUSION: The results suggest that tebuconazole may cause a loss of $Na_V1.5$ function and compromise the excitability of cardiac cells. Thus, especially in individuals who have certain hereditary or acquired cardiac pathologies, exposure to fungicide can contribute to the development of arrhythmias.

Keywords: Cardiac Sodium Channel, Cardiotoxicity, Tebuconazole / **Supported by:** CAPES and FAPESP

R - Plants and Synthetic Biology

R.01 - Heterologous Production of Cytokinins in Bacterial Systems through Synthetic Biology in *E. coli*

Ryu Masaki [1], Carem Gledes Vargas Rechia[2], Marcelo Müller dos Santos[1], Emanuel Maltempi de Souza[1], Fábio de Oliveira Pedrosa[1]
[1]Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Paraná (Paraná, Brasil),
[2]Departamento de Ciências Biomoleculares, Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: Cytokinins are phytohormones that currently have applications in plant biotechnology, such as plant micropropagation and production of plant secondary metabolites. In addition, cytokinins can be used as plant growth promoters since they regulate several aspects of agro-industrial interest such as increasing grain, fruit and biomass productivity, legume nodulation and resistance to abiotic and biological stress. However, producing these phytohormones is expensive, either by chemical synthesis or by extraction of plant tissues, hence it is desirable to find a way to produce these phytohormones at a lower cost. OBJECTIVES: The present work proposes the expression of cytokinin biosynthesis pathways genes, such as the IPT4 gene that encodes isopentenyl adenylate transferase 4v from *Arabidopsis thaliana* and the *tmr* gene that encodes isopentenyl or dimethylallyl transferase from *Agrobacterium tumefaciens*, in *E. coli* MG1655 for heterologous cytokinin production. MATERIALS AND METHODS: Synthetic genes for cytokinin production were designed for optimal expression in E. coli from a plasmid platform. A detection system based on plant cytokinin receptor (AHK4) expressed by sensing bacterium (*E. coli* KMI001) that expresses cytokinin-induced β-galactosidase was used. DISCUSSION AND RESULTS: The presence of cytokinins was detected both in culture supernatant and in the cytosol of recombinant bacteria expressing the enzymes Tmr and Ipt4. Callus formation of tomato (*Solanum lycopersicum*) cells was observed when cellular extract of the recombinant bacteria was used as a substitute for synthetic cytokinin BAP, demonstrating that cytokinins produced by recombinant bacteria have biological activity. CONCLUSION: Heterologous cytokinin produced in *E. coli* MG1655 is functional and has biotechnology applications, since both supernatant and bacterial biomass possess functional cytokinins. It is possible to produce cytokinins at a lower cost.
Keywords: synthetic biology, vegetable hormones, biotechnology / **Supported by:** CAPES e CNPq

R.02 - Oxidative stress and accelerated germination of seeds of the wild shrub Calotropis procera submitted to a static magnetic field

Márcio Viana Ramos [1], Emanuel Bezerra[1], Cristina Carvalho[1], Raimundo Costa-Filho[1]
[1]Biochemistry and Molecular Biology, Federal University of Ceará (Ceará, Brazil)

INTRODUCTION: Little is known about magnetic effects on biological systems. Plants exposed to magnetic (MF) field exhibit different responses. MF can promote seed germination. However underlying events are still known. OBJECTIVES: In this study it was investigated metabolic responses of seeds of a non-cultivated species exposed to static MF. MATERIALS AND METHODS: Screening of the MF effect on seed was performed (0; 0.5; 1.0; 2.0 mT) and then the best seed response (2 mT) was fixed in further analyses. Seeds (n = 270) were germinated for five days under 2 mT or seeds were first exposed to 2 mT for five days and then germinated. Treatments were compared to seeds germinated without MF, in terms of anti-oxidative enzyme activity, proteomics and DNA integrity. DISCUSSION AND RESULTS: Exposure to MF reduced the time for seed germination and increased germination rate. This was accompanied by higher fresh and dry matter. Activity of catalase was not altered. Activity of POX increased and that of APX decreased in seeds after MF exposure. The increased contents of hydrogen peroxide and malondialdehyde were indicators of oxidative stress. Cell density and DNA integrity were unaltered, while soluble protein and DNA quantity increased. Proteomics/bioinformatics analysis corroborated these data. However, this did not affect seed germination. Instead, it improved germination performance in terms of time and ratio. CONCLUSION: This study indicates that MF exposure induces an intriguing balance between oxidative stress (potentially harmful) and accelerated germination (desired effect) of seeds. Such effects may be technologically appreciated for seedling production, mainly of recalcitrant seeds of agronomic important species.
Keywords: confocal microscopy, plant responses, ROS / **Supported by:** CNPq; CAPES

R.03 - Potential Effects of Crataeva tapia Bark Lectin (CrataBL) and Its Related Peptides in the Melanoma Model

Kathleen Lie [1], Camila Bonturi[1], Patrícia Paiva[2], Maria Tereza Correia[2], Maria Luiza Oliva[1]
[1]Bioquímica, Universidade Federal de São Paulo (, Brasil), [2]Bioquímica, Universidade Federal de Pernambuco (, Brasil)

INTRODUCTION: Melanoma, a skin cancer developed in melanocytes, is an important pathology due to its degree of metastasis and low chances of cure when diagnosed late. In tumors, proteolysis is exacerbated by tumor cell proteases and protease inhibitors found in legumes are potential candidates to control these events and prevent cancer progression. OBJECTIVES: To investigate the effects of CrataBL and its derived peptides in cell-based assays of melanoma. MATERIALS AND METHODS: CrataBL was extracted from the bark of the tree and purified by ion exchange and size exclusion chromatographies. Cells (SK-MEL-28) were treated with CrataBL or its peptides and viability, proliferation, migration, invasion, and cell death were evaluated. DISCUSSION AND RESULTS: CrataBL (100 and 200 μM) reduced more than 60% of the cell viability. The protein effect was demonstrated to be resistant to proteolysis by the proliferation assay of SK-MEL-28 as its effect is also observed after 72 h of inhibitor-treatment (inhibition greater than 90%). Also, 100 μM of the protein inhibited more than 80% of migration, more than 100% of invasion, and induced cells to enter in late apoptosis at 48 h. The peptide derived (Pep-27), although not effective in inhibiting viability, proliferation, and invasion prevented more than 80% of cell migration at 24 and 48 h and reduced the number of viable cells at 24 h. Pep-27 was also associated with vemurafenib (chemotherapy drug) and caused an increase in early apoptosis after 48 h. Pep-26, on the other hand, interfered with 60% of migration and induced early and late apoptosis within 24 hours of treatment CONCLUSION: The data indicated some promising results for antitumor effects that should continue to be explored in trials on other cellular events related to cancer characteristics such as adhesion and cell signaling.
Keywords: natural products, melanoma, protease inhibitors

R.04 - Analysis of morphological and ultrastructural alterations of Carica papaya laticifers to the Papaya Meleira virus Complex

Lucas Estevão Constantino Nunes [1], Brunno Renato Farias Verçoza[1], Marlonni Maurastoni Araujo[2], Tathiana Ferreira Sá Antunes[2], Juliany Cola F. Rodrigues[1], Patricia Machado Bueno Fernandes[2], Silas Pessini Rodrigues[1]
[1]Núcleo Multidisciplinar de Pesquisa em Biologia, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil), [2]Laboratório de Biotecnologia Aplicada ao Agronegócio, Universidade Federal do Espírito Santo (Espírito Santo, Brasil)

INTRODUCTION: One of the main challenges of papaya production (*Carica papaya*) is an easily spread viral disease called papaya sticky disease (PSD). This disease is caused by the combined infection of two viruses, papaya meleira virus (PMeV) and papaya meleira virus 2 (PMeV2), named PMeV complex. Proteomics experiments revealed that many proteins modulated in C. papaya response to the PMeV complex are related to cell wall remodeling. This suggests that the PMeV complex induces structural changes in the cell walls of papaya laticifers, causing the laticifers to rupture and leading to spontaneous exudation of latex. OBJECTIVES: The objective of this work was to characterize the morphological and ultrastructural responses of *C. papaya* laticifers to PMeV complex. MATERIALS AND METHODS: Thus, symptomatic and asymptomatic post-flowering plants were collected in the field and processed for scanning electron microscopy (SEM) and transmission electron microscopy (TEM). For this, samples were fixed with Karnovsky, post-fixed with a solution containing 1 % osmium tetroxide and 1.25 % potassium ferrocyanide. Then, the samples processed for SEM were dehydrated in a gradual series of ethanol, critical point dried, mounted in stubs and metallized. For TEM, samples were dehydrated in gradual series of acetone, infiltrated with EPOX resin, ultrasectioned and contrasted with uranyl acetate and lead citrate. DISCUSSION AND RESULTS: The results obtained revealed significant differences between asymptomatic and diseased plants. Laticifers from diseased plants presented less intracellular content. Futhermore, cell walls of the diseased plants presented significant alterations such as: (1) swollen; (2) intense disorganization of its structure; and, (3) apparent degradation of its content. Morphometric analysis of the cell wall revealed that its thickness in the disease plants are two-fold larger than the healthy ones. Finally, viral particles were visualized within laticifers. CONCLUSION: Therefore, the results obtained support the hypothesis that the PMeV complex induces structural alterations in the cell walls of *C. papaya* laticifers.
Keywords: Microscopy, Papaya sticky disease, Phytopathology / **Supported by:** CNPq, CAPES, FINEP, FAPERJ, FAPES.

R.05 - Disruption of Hrubri_0242 gene reduces PHB production by Herbaspirillum rubrisubalbicans M1

Roxana Beatriz Ribeiro Chaves [1], Luis Paulo Silveira Alves[2], Emanuel Maltempi de Souza[1], Fabio de Oliveira Pedrosa[1], Rose Adele Monteiro[1]
[1]Bioquímica, Universidade Federal do Paraná (Paraná, Brasil), [2]Bioquímica, Faculdades Estadual de Maringá (Paraná, Brasil)

INTRODUCTION: PHB (polyhydroxybutirate), is the main intracellular reserve of carbon and energy found in some bacteria. It is produced under stress condition, such as nutrient limitation and in the presence of excess carbon source. According to the literature, bacteria that colonize grasses, such as *Herbaspirillum rubrisubalbicans* M1 (*H.r.*M1), have their colonization improved and offer greater protection to the plant when they produce PHB. In this work we disrupted *H.r.*M1 *Hrubri_0242* gene that codify to a non-characterized transcriptional regulator. *Hrubri_0242* strain produces less PHB than the wild type, and it has a lower capacity to colonize sorghum roots during competition with the wild type. OBJECTIVES: Determine the *Hrubri_0242* involvement in PHB production and sorghum roots colonization. MATERIALS AND METHODS: PHB dosage analyses of the two strains were performed in the +N (20mM NH4+) and -N (5mM NH4+) conditions during 24h. The amount of bacterial PHB was determined by acid methanolysis followed by GC coupled to a flame ionization detector and the results were normalized by the weight of the freeze-dried bacteria and expressed as percent PHB / cell dry weight. DISCUSSION AND RESULTS: In the -N condition, higher PHB production was observed by the wild-type and *Hrubri_0242* mutant strain when compared to the +N condition. In the -N condition *Hrubri_0242* mutant strain produced 50% less PHB than the wild type strain. CONCLUSION: We can suggest that the *Hrubri_0242* gene is involved in PHB metabolism regulation which may be one of the factors for the bacterium being impaired in competitive environments.

Keywords: Herbaspirillum rubrisubalbicans M1, Colonization, PHB

R.06 - Combining Fragments of the Natural Protease Inhibitor EcTI with Temozolomide Increases the Antitumor Efficacy in Glioblastoma

Ana Beatriz da Silva Teixeira [1], Camila Ramalho Bonturi[1], Vitória Morais da Rocha[1], Maria Luiza Vilela Oliva[1]
[1]Departamento de Bioquímica, Universidade Federal de São Paulo (São Paulo, Brazil)

INTRODUCTION: The term glioblastoma refers to astrocytomas with the highest level of malignancy. It is the most common and almost always lethal. The standard treatment remains palliative, including surgery, radiotherapy, and chemotherapy with Temozolomide (TMZ). Therefore, alternatives or auxiliaries therapeutic methods are extremely necessary. As the overexpression of proteases in the tumor microenvironment contributes to several aspects of the cancer progression, protease inhibitors are potential therapeutic targets. In glioblastoma, the natural inhibitor EcTI (*Enterolobium contortisiliquum* Trypsin Inhibitor) shows to be effectively antitumorigenic. Therefore, peptides based on the primary sequence of the EcTI were synthesized to establish the smallest structure responsible for the inhibitory function. OBJECTIVES: Evaluation of the antitumoral properties of peptides derived from EcTI primary sequence in glioblastoma cells, U87, and healthy astrocytes. In addition, we will investigate if the fragments are capable of potentializing the chemotherapy treatment. MATERIALS AND METHODS: Three peptides were synthesized from the EcTI structure. The TMZ was initially solubilized in DMSO, to later be diluted in a culture medium. Cell viability was performed using Presto Blue, a reagent capable of quantifying metabolically active cells. DISCUSSION AND RESULTS: Peptides 3 and 7 inhibited the viability of U87 cells (96 h), achieving an inhibition superior to that observed in the isolated treatment with TMZ, even at concentrations lower than the chemotherapy. Furthermore, the combined treatment achieved even greater inhibitory effects on glioblastoma cells. Interestingly, the peptides alone did not interfere with the viability of healthy astrocytes, in any of the periods and concentrations evaluated, while the deleterious effects of the chemotherapy on these cells have already been shown in the 24 h treatment period. CONCLUSION: The peptides related to the EcTI structure show a selective antitumoral effect in glioblastoma cells, similar to the natural protein, being capable of potentializing the chemotherapy effect.

Keywords: glioblastoma, inhibitors, protease / **Supported by:** FAPESP, CAPES and CNPq

R.07 - Effect of the Combination of Humic Acids and Herbaspirillum seropedicae on the Growth of Maize Plants

Rosane de Oliveira Nunes [1], Giselli Abrahão Domiciano[1], Wilber Sousa Alves[2], Jaqueline Aparecida de Oliveira[3], Fábio Cesar Souza Nogueira[1], Luciano Pasqualoto Canellas[3], Fabio Lopes Olivares[3], Russolina Benedeta Zingali[1], Márcia Regina Soares da Silva[1]

[1]Departamento de Bioquímica, Universidade Federal do Rio de Janeiro (RJ, Brasil), [2]Departamento de Ensino Médio e Técnico, Centro Federal de Educação Tecnológica Celso Suckow da Fonseca (RJ, Brasil), [3]Núcleo de Desenvolvimento de Insumos Biológicos para Agricultura, Universidade Estadual do Norte Fluminense Darcy Ribeiro (RJ, Brasil), [4]Laboratório de Proteômica/LADETEC, Universidade Federal do Rio de Janeiro (RJ, Brasil), [5]Unidade de Proteômica, Instituto de Química, [6]Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro (RJ, Brasil)

INTRODUCTION: The use of plant growth-promoting bacteria as agricultural inoculants of plants should be encouraged because of their prominent role in biological nitrogen fixation and abiotic stress mitigation. Humic acids (HA) improve the efficiency of the use of nutrients and act on metabolism of plants. HA have a considerable influence on the productivity of ecosystems due to their effects on the development of the root system. The use of HA with *Herbaspirillum seropedicae* have been associated with an increase in plant productivity. OBJECTIVES: In this study, we evaluate the study of inoculants formed by the combination of *Herbaspirillum* + HA in maize. We investigated changes in maize roots in response to treatment with the *Herbaspirillum* and HA, separately. Protein analyzes on maize roots treated with the combination are in progress. MATERIALS AND METHODS: Root soluble protein profiles were analyzed using the label-free quantitative proteomic approach. Maize plants were cultivated for 7 days after planting in 2mM $CaCl_2$ solution with the bacteria, with HA and *Herbaspirillum* + HA and control (2mM $CaCl_2$), separately. DISCUSSION AND RESULTS: Using this approach, we identified 123 differentially expressed proteins, in plants cultivated with *Herbaspirillum*. The maize root colonization modulated the differential expression of enzymes involved in the stress response, such as peroxidases (POX) and phenylalanine ammonia-lyase (PAL). We identified 34 proteins that were significantly more abundant in the seedlings treated with HA.The results show that the main effect of HA is protective, mainly associated with increased expression of the 2-cys peroxidase and glutathione proteins. The efects on root architecture, such as the induction of lateral roots and biomass increase were observed in maize roots treated with the combination. CONCLUSION: The biomass increase in maize plants co-inoculated with HA and *Herbaspirillum*, suggesting a beneficial effect, which could be, applied in future applications in agricultural production.
Keywords: biostimulation, endophytes, label-free proteomics / **Supported by:** : FAPERJ, CNPq and CAPES

R.08 - Phytoremediation Studies with Sunflower (Helianthus annuus L.), from Lead Contaminated Soil

Elizeu Rosa dos Santos Junior [1], Vinícius da Silva Ferreira[1], Rosane de Oliveira Nunes[1], Marcia R.S. da Silva[1]

[1]Chemistry Institute, Federal University of Rio de Janeiro (Rio de Janeiro, Brazil)

INTRODUCTION: The search for compounds linked to environmental contamination and hazardous to health has increased. The soil has become an area of great contamination, where metals with lead are involved in this contamination. The search for remediation alternatives increased, so phytoremediation stood out for using plants as decontaminant. Among them, sunflower (*Helianthus annuus* L.) has the ability to phytoremediate metals and a high economic potential. OBJECTIVES: Objectives were to analyze the growth/development in soil with different concentrations of lead, identify and classify the expressed proteins, identify possible proteins related to phytoremediation. MATERIALS AND METHODS: The cultivars BRS 323 and 415 were provided by EMBRAPA. We apply doses of $Pb(NO_3)_2$, in mg kg^{-1} : 0, 150, 900, according to CETESB (2020) and CONAMA (2009). We cultivated in greenhouse of Laboratory of Molecular Microbiology and Proteins of the Department of Biochemistry, of the Institute of Chemistry of UFRJ. Germination test (G%), lead tolerance, chlorophyll content, crude protein detection and proteomic analysis were performed DISCUSSION AND RESULTS: In G%, there were distinctions between BRS 323 and 415 in contaminated soil or not, so BRS 323 was chosen. G% in soil on the 10th day were 93.75%, 78.12% and 76.56%, for control , 150 and 900 Pb, after 30 days it reduced to 81.25%, 65.62% and 53.12%. The chlorophyll content reduced in the control and 900 Pb, after 30 days. Total proteins increased at 150 Pb. Plant tissues had high protein concentrations in leaves of 900 Pb. Proteomics detected 381 proteins for control and 409 for 150 Pb, 25.4% exclusive to the control and 30.5% to 150 Pb. Nineteen proteins are marked with different species of genus Helianthus. Some control proteins and 150 Pb were related to bonds with metal ions. CONCLUSION: These results demonstrate that sunflower cultivated in $Pb(NO_3)_2$, present alterations in the development and in the protein profile of the leaf tissues
Keywords: PHYTOREMEDIATION, SUNFLOWER, PROTEOMICS / **Supported by:** CAPES

R.09 - Isolation, partial purification and characterization of a lectin from Mimosa caesalpiniifolia Benth seeds.
Neilton de Lima Oliveira Junior[1], Erika Sa[1], Deize Cruz[1], **Wagner Pereira Felix [1]**
[4]Pós-graduação em Biociências, Universidade Federal do Vale do São Francisco (Pernambuco, Brazil)

INTRODUCTION: Lectins are a class of proteins that recognize carbohydrate structures and bind carbohydrates specifically, and are found in a wide variety of plants, animals, and microorganisms. *Mimosa caesalpiniifolia* is popularly known as "sabiá" and "angiquinho-sabiá" and their extracts have potential for therapeutic indication. OBJECTIVES: Isolate, purify and partially characterize of a lectin from *M. caesalpiniifolia* seeds. MATERIALS AND METHODS: A lectin from *M. caesalpiniifolia* seeds was extracted with Tris-HCl 0.1 M pH 7.8 and NaCl 0.15M. Afterwards, the extract was filtered and centrifuged to get a supernatant as the crude extract. The lectin was further purified by gel chromatography. Then, 50 mL of the filtered liquid was applied to a DEAE-Sephadex A-50 ion exchange gel. The peak fraction prepared for the determination of hemagglutinating activity was collected, dialyzed and applied to an affinity chromatography column in Sephadex G-75. The retained peak was dialyzed, lyophilized to obtain lectin powder for use to SDS-PAGE (12%). 1 $mg \cdot mL^{-1}$ of the purified lectin was used in the bacterial growth inhibition tests of *Pseudomonas aeruginosa*, *Salmonella spp*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli*. DISCUSSION AND RESULTS: The lectin was isolated and named "McB Lectin". Its optimal hemagglutinating activity was in the pH range of 6.5 to 8.5. It´s thermostable up till a maximum temperature of 90 ^{o}C and strongly agglutinates rabbit erythrocytes. For its hemagglutinating activity, McBL requires the presence of divalent cations (Ca^{++}, Mn^{++} and Mg^{++}). This activity was inhibited by Glucose but not by Galactose or Lactose. The McBL concentrations tested were unable to inhibit the growth of the bacterias tested. CONCLUSION: Mimosoideae lectins are heterogeneous in their physical-chemical properties and biological activities. The data presented here are preliminary and therefore require further investigation. However, this study is of great importance for typical species of the Caatinga biome, especially those of the genus *Mimosa*.
Keywords: Mimosa caesalpiniifolia, Lectins, Partial characterization
Supported by: CNPq - CAPES - FACEPE

R.10 - Evaluation of Zinc Resistance and Tolerance in Cultivars of Oryza sativa L.: a Candidate for Phytoremediation
Vinícius da Silva Ferreira [1], Alice Ferrari Oliveira[1], Gabriel de Castro Agner[1], Luana Domingos de Melo[1], Rosane de Oliveira Nunes[1], Elizeu Rosa dos Santos Junior[1], Wilber S. Alves[2], Marcia Regina Soares da Silva[1]
[1]Chemistry Institute, Federal University of Rio de Janeiro (Rio de janeiro, Brazil), [2]Department of Secondary and Technical Education, Federal Center for Technological Education Celso Suckow da Fonseca (Rio de Janeiro, Brazil)
INTRODUCTION: Zinc, an industrial waste residue, is one of the most released heavy metals in nature, causing it to accumulate in the environment, contaminating it and being harmful to existing biodiversity. Plants have played a role in monitoring, phytoremediation and bioaccumulation of these compounds, as the plant used in this study, *Oryza sativa L*. OBJECTIVES: This work aims to evaluate the tolerance and phytoremediation potential of 3 cultivars of *O. sativa L*., to Zn in hydroponic medium and to use the proteomic approach to verify the protein profile of these cultivars. MATERIALS AND METHODS: Three rice cultivars (cv.) were used (Monarca, Caiapó and Sertanejo), supplied by Embrapa. Rice grains of each cultivar were husked and immersed in 70% alcohol and 3 washes with 2% NaCl for decontamination. Seeds were seeded in tubes containing 15 mL of 1.2% agar under aseptic conditions. The cultivation took place in a photoperiod of 12 h at 28 °C. After 5 days of germination, the cultivars were transferred to sterile test tubes with 30 mL of Hoagland medium, and the medium was contaminated with a stock solution of ZnSO4 to a final concentration of 10 ppm. After 21 days of cultivation, the size and mass of the cultivars were measured. DISCUSSION AND RESULTS: The tested cultivars were tolerant to the concentration of 10 ppm of Zn, highlighting the cv. Sertanejo that had a difference in size and mass in the presence of Zn, in addition, presented root hairs of absorption in the roots, becoming a potential phytoremediator. CONCLUSION: The sertaneja cultivar was the most tolerant to Zn, being evaluated in higher concentrations.
Keywords: Oriza sativa L., Phytoremediation, Proteomics / **Supported by:** FAPERJ, CNPq and CAPES.

R.11 - Reduction of Cellular Viability of Triple-Negative Breast Cancer Cells by EcTI-Related Peptides

Vitória Morais da Rocha [1], Camila Ramalho Bonturi[1], Ana Beatriz da Silva Teixeira[1], Maria Luiza Vilela Oliva[1]
[1]Departamento de Bioquímica e Biologia Molecular, Universidade Federal de São Paulo (São Paulo, Brasil)

INTRODUCTION: Triple-negative breast cancer (TBNC) is a subtype that lacks estrogen and progesterone receptors, and does not express HER-2. This subtype is highly aggressive and metastatic, in addition, it has a worse prognosis when compared to the other subtypes. The most commonly used chemotherapeutic agents for the treatment of this subtype are paclitaxel (PTX) and doxorubicin (DOXO), however, they cause side effects such as neuropathy and cardiotoxicity, respectively. In this scenario, protease inhibitors are a good option to propose the establishment of new therapeutic targets. Previously, *Enterolobium contortisiliquum* Trypsin Inhibitor (EcTI), a protease inhibitor, showed antitumor and anti-inflammatory effects in TBNC (MDA-MB-231). Peptides are also strong candidates for therapy because they are often selective for tumor cells. OBJECTIVES: To evaluate the effects of peptides derived from the native inhibitor EcTI, named PEP2, PEP3, and PEP7, isolated and in association with PTX and DOXO, on the viability of MDA-MB-231 cells. In addition, we will verify the toxicity of the peptides on the non-tumorigenic cells, MCF-10A. MATERIALS AND METHODS: The determination of the viability was carried out by fluorescence intensity after the addition of PrestoBlue reagent. DISCUSSION AND RESULTS: The peptides were found to be able to reduce the viability of MDA-MB-231 by approximately 50% after 72 h of treatment. PEP 7 showed more efficiency, being able to reduce viability also after 48 h and 72 h of treatment. PEP 2 and PEP 7 have a similar effect compared to the drug PTX after 72h of treatment. No toxicity was observed after treatment with the two peptides in the non-tumorigenic cell. CONCLUSION: The results suggest that the peptides are selective for tumor cells with potential as a treatment for TBNC. Therefore, further analyses should be performed to elucidate the mechanism of action of the peptides.

Keywords: Cell viability, Protease inhibitors, Triple-negative breast câncer / **Supported by:** FAPESP (, CNPq e CAPES

R.12 - In silico and *In vivo* analysis of microalgae Chlamydomonas reinhardtii secreted proteins

Giovanni Ferreira Montovaneli [1], Fernanda Marcon Barbosa Campos[2], Silas Pessini Rodrigues[1]
[1]Núcleo Multidisciplinar de Pesquisa em Biologia, Universidade Federal do Rio de Janeiro, Campus Professor Geraldo Cidade (Rio de Janeiro, Brasil), [2]Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: Chlamydomonas reinhardtii are unicellular green microalgae, an established model organism for photosynthetic cell studies and biotechnological platform for recombinant protein expression. C. reinhardtii is also an attractive model for the identification of proteins naturally secreted by cells and to understand protein secretion mechanisms. This knowledge is very limited in the literature and could help to establish more effective recombinant protein expression systems in which targeting the expressed protein to secretion may be of interest. OBJECTIVES: This research aimed to predict proteins potentially secreted by C. reinhardtii using bioinformatics and to experimentally identify the secreted proteins. MATERIALS AND METHODS: The databases Phytozome v.5.5 and EVPedia were used for predicting the microalgae potentially secreted proteins and gene ontology was obtained using Blast2GO. For the secretome experiments, C. reinhardtii was grown in TAP-medium followed by centrifugation to collect the culture supernatants. After protein concentration, the proteins were digested and assayed by LC-MS/MS mass spectrometry. DISCUSSION AND RESULTS: In silico, 1,537 protein sequences containing a secretion signal peptide for the classical secretion pathway (CSP) and 3,600 protein sequences homologous to extracellular vesicular proteins (unconventional secretion pathway, USP) were identified. Blast2GO analysis resulted in 795 and 2,614 proteins annotated from CSP and USP, respectively. The analysis of secreted proteins allowed the identification of 85 proteins out of which 59 were among the predicted proteins (42 of CSP and 17 of USP). CONCLUSION: The proteins potentially secreted (1,537 with- and 3,600 without-signal peptide) by C. reinhardtii were identified and annotated using bioinformatics. 85 proteins were experimentally identified from C. reinhardtii culture medium supernatant, out of which 59 were among the predicted proteins.

Keywords: protein secretion, signal peptide, proteomics
Supported by: FAPERJ

R.13 - Project: Toxicity Evaluation and Interaction Mechanisms of TiO2 Nanoparticles in Green Microalgae Chlamydomonas reinhardtii

Júlia Tavares Vieira [1], João Henrique Leite Mendes[1], Marjolly Caruso Brígido[2], Natasha Midori Suguihiro[1], Russolina Benedeta Zingali[2], Silas Pessini Rodrigues[1]
[1]Núcleo multidisciplinar de pesquisa em Biologia, Universidade Federal do Rio de Janeiro (RJ, Brasil), [2]Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro (RJ, Brasil)

INTRODUCTION: Nanoparticles (NPs) are widely used around the world. Due to their variety of structural and elemental compositions. NPs use and production may end-up delivering unwanted materials in the environment which may impact ecosystems at different trophic levels. Previous studies have evaluated the NPs' toxicity using different biological models, but NPs effects and mechanisms of interaction, especially with photosynthetic cells, are largely unknown. OBJECTIVES: This project aims to evaluate the TiO2 NPs and their byproducts' ecotoxicity and mechanisms of interaction with photosynthetic organisms using C. reinhardtii to understand NPs impact on aquatic ecosystems. MATERIALS AND METHODS: Tris acetate phosphate (TAP) culture medium grown C. reinhardtii CC-125 strain will be exposed to different concentrations of TiO2 NPs and their byproducts. The toxicity experiments will include cell counting and optic density-based cell growth measurements. Cell viability will be estimated using fluorescein diacetate reagent and fluorescence microscopy. Experiments of reactive oxygen species (ROS) detection and photosynthetic evaluation will also be conducted. The mechanisms of interaction between TiO2 NPs and C. reinhardtii will be evaluated using scanning and transmission electron microscopy. DISCUSSION AND RESULTS: Growth parameters results will allow the observation of the TiO2 NPs and byproduct's effects on the global behavior of the microalgae population. Together with ROS and photosynthetic results, they ought to provide insights in the direction of the effects of the material on the cells. The subcellular data will allow inferences of the mode of interaction between TiO2 and C. reinhardtii. CONCLUSION: This project is a promising route toward understanding the effects of TiO2 NPs and their byproducts in the first trophic level of aquatic ecosystems.
Keywords: Nanoparticle toxicity, Chlamydomonas reinhardtii toxicity, Toxicity in aquatic ecosystems

R.14 - Gene Characterization of the Antioxidant Enzymes of *Ricinus communis* L.: a Bioinformatics Analyses.

Neide da Hora Conceição [2,1], Luiza Carolina M. Souza[1,2], Amanda Átyla A. A. S. Reis[1,2], Maria Cecília O. S. Santos[1,2], Laisla Cruz Santana[1,2], Catarine Pinheiro Almeida[3], Rentato Delmondez de Castro[1], Paulo Roberto Ribeiro[2,3,1], Luzimar Gonzaga Fernandez[2,1]
[1]Depto de Bioquímica e Biofísica, Laboratório de Bioquímica, Biotecnologia e Bioprodutos, Instituto de Ciências da Saúde, Universidade Federal da Bahia (Bahia, Brasil), [2]Depto de Bioquímica e Biofísica, PMPBM, Salvador (Bahia, Brasil), [3]Depto de Química, Metabolomics Research Group, Instituto de Química, UFBA (Bahia, Brasil)

INTRODUCTION: Antioxidant enzymes are part of the plants' line of defense against different types of stress. In *Ricinus communis* L., the enzymes Superoxide dismutase-SOD, Catalase-CAT, Glutathione peroxidase-GPx, Glutathione reductase-GR, Ascorbate peroxidase-APX, Glutathione S-transferase-GST, and Monodehydro ascorbate peroxidase-MDHAR contribute to the specie adaptation and resistance to abiotic stresses, such as temperature and drought. OBJECTIVES: This study aimed to characterize the gene families of the antioxidant enzymes of *R. communis*. MATERIALS AND METHODS: Initially, the number of members in the gene family of APX, CAT, GPX, GR, GST, and MDHAR was verified using Phytozome. Subsequent analyzes were performed in SMART to verify the conserved domains MEME/Motif Scan, where the most conserved motifs in the enzyme sequences and their functions were verified. In MEGAX, CELLO, ProtParam, PlantCare, Galaxy, and KEEG, we searched for ancestry, location in the cell, physicochemical parameters, cis-regulatory elements, 3D structures, and the functions of these genes, respectively. DISCUSSION AND RESULTS: The genes of APX, CAT, GPX, GR, GST, and MDHAR have, respectively, 08, 04, 06, 02, 17, and 04 members in their gene families, with sequences ranging from 77 (GST2) to 2278 (CAT2) amino acids in the structure. Among the functions of the most conserved motifs, it was found that they were part of the phosphorylation sites of the enzymes with kinase function. The stress-responsive elements that showed greater expressiveness were those responsive to Light. The locations were multiple in the cells, as an example, GPX1 expressed in the nucleus and MDHAR1 in the cytoplasm. The KEEG confirmed the importance of these enzymes in response to abiotic stresses, for example, the action of the GST in response to damage caused by xenobiotics. CONCLUSION: It was concluded that *in silico* research is essential in the characterization of genes and expressed biomolecules, their functions, and interactions in the species, being also useful for better experimental design.
Keywords: Castor bean, Computational biology, Gene family. / **Supported by:** FAPESB

S - Redox Processes

S.01 - Formation of Dinitrosyl Iron Complex (DNIC) Containing Non-Thiol Ligands

Nathália Miranda de Medeiros[1], Daniela Ramos Truzzi[1]
[1]Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo (São Paulo, Brazil)

INTRODUCTION: Dinitrosyl iron complexes (DNICs, $[Fe^{I}(NO)_2(L)_2]^{+}$) are abundant nitric oxide (NO) metabolites consistently detected by Electron Paramagnetic Resonance (EPR) (g ~ 2.03) in NO-producing cells and tissues. The reaction mechanism for the formation of DNIC containing low molecular weight thiols involves the reduction of Fe(II) to Fe(I) promoted by these thiol molecules giving rise to the $\{Fe(NO)_2\}^9$ moiety found in DNICs. In fact, thiols are widely known as reducing agents. However, it has been demonstrated that DNICs can also be formed in absence of thiols through mechanisms in which the Fe(II) to Fe(I) reduction might be promoted by NO or/and disproportionation reaction of $[Fe^{II}(NO)(L)_2]^{2+}$. OBJECTIVES: Thus, the present study aimed to determine the levels of DNIC formed in presence of non-thiol biomolecules to evaluate their relevance to the formation of biological DNIC. MATERIALS AND METHODS: Solutions pH 7.4 containing only Fe(II) and NO, and solutions containing Fe(II), NO, and tyrosine (Tyr), histidine (His), phosphate (HPO_4^{2-}), or glutathione (GSH) were analyzed by room temperature EPR over time. DISCUSSION AND RESULTS: The DNICs formation was observed under all investigated conditions showing that they can be formed in aqueous media in absence of thiols. Despite that, the yields of DNIC formation were from one to two orders of magnitude lower in presence of the non-thiol biomolecules tested than in presence of GSH, exhibiting the followed yield order: His << H_2O/OH^- ~ HPO_4^{2-} < Tyr << GSH. Among the non-thiol biomolecules, Tyr presented the highest DNIC yield possibly due to its reducing character ($E^0_{Tyr•/Tyr}$ = 0.94 V). CONCLUSION: These data show that DNICs can be formed in aqueous media by mechanisms that depend exclusively on Fe(II) and NO, but their yield of formation is considerably increased when reducing agents such as GSH and Tyr are available, attesting the relevance of Fe(II) to Fe(I) reduction reaction promoted by those biomolecules for DNIC formation.

Keywords: Dinitrosyl iron complex, Electron Paramagnetic Resonance, Nitric oxide / **Supported by:** FAPESP

S.02 - Study of the toxicity and antioxidant potential of Myrciaria tenella and Eugenia copacabanensis plant extracts in *Saccharomyces cerevisiae* cells

Rodrigo de Paulo Osorio [1], Jéssica da Silva Cappato[3], Daniele Cabral Pires [2], Víctor de Carvalho Martins[3], Cristiano Jorge Riger[3]
[1]Bioquímica, Universidade Federal Rural do Rio de Janeiro (Rio de Janeiro, Brasil), [2]Bioquímica, Universidade Federal do Rio de janeiro, Rio de Janeiro (Rio de Janeiro, Brasil), [3]Bioquímica , Universidade Federal Rural do Rio de Janeiro (RJ, Brasil)

The condition of oxidative stress is related to an imbalance between the oxidant species and the action of the antioxidant defense system. Several diseases have been associated with the condition of redox imbalance, therefore many natural sources of potential antioxidant substances have been explored. In view of this, the evaluation of the antioxidant potential of plant extracts becomes relevant. Plants of the *Myrtaceae* family have been used in folk medicine for medicinal purposes, and the popular knowledge acquired through generations has directed scientific research to study the biological activities related to these plants. In this context, this work sought to evaluate the toxicity and antioxidant capacity of fractions obtained with *n*-butanol and ethyl acetate from leaves of *Myrciaria tenella* and Eugenia *copacabanensis* plants in *Saccharomyces cerevisiae* cells. A control strain (BY4741) and a mutant strain deficient in the tripeptide glutathione (Δgsh1) were used. The toxicity of the extracts was evaluated in a cell viability assay by plating. The analysis of the antioxidant capacity of the extract was also performed by cell viability, using hydrogen peroxide (2.0 mM) as a stressing agent. The results did not reveal high toxicity of the extracts, being equal to the control assay (cells not exposed to the extracts) in both strains. The analysis of the antioxidant activity showed that the fraction from M. *tenella* obtained with *n*-butanol at a concentration of 17.00 mg.mL-1 showed promising results with the BY4741 strain. In the assay with the Δgsh1 strain, the fraction of M. *tenella* obtained with ethyl acetate in the concentration of 4.00 mg.mL-1 and the fractions of E. *copacabanensis* obtained with ethyl acetate and with *n*-butanol in the concentrations of 4.29 mg.mL-1 and 4.18 mg.mL-1 were antioxidants. In conclusion, both extracts showed promise in terms of antioxidant capacity.

Keywords: antioxidant, plants, *Saccharomyces cerevisiae*
Supported by: CAPES e CNPq

S.03 - Evaluation of the Antioxidant Potential of Acetylcholinesterase Inhibitory Triazole Derivatives in *Saccharomyces cerevisiae* Cells

Danniel Cosme Neves Grillo [1], Daiana de Fátima Portella Franco[2], Arthur Eugen Kümmerle [2], Cristiano J. Riger[1]
[1]Departamento de Bioquímica, Universidade Federal Rural do Rio de Janeiro (RJ, Brasil), [2]Departamento de Química Orgânica, Universidade Federal Rural do Rio de Janeiro (RJ, Brasil)

INTRODUCTION: Alzheimer's Disease (AD) is a progressive neurodegenerative disease known for memory loss and difficulty with the language being formed by a set of pathological factors, among them cholinergic deficit and oxidative stress. Oxidative stress is characterized by the imbalance between the generation of free radicals and/or non-radical reactive species and the action of antioxidant defense systems. Currently, the main treatment for Alzheimer's disease is the use of inhibitors of cholinesterase enzymes. However, due to the multifactorial nature of the disease and the fact that many drug candidates have not been successful, a new approach has emerged, the so-called multi-targets. OBJECTIVES: Therefore, this work sought to evaluate the antioxidant protection of four new compounds containing the 3-amino-1,2,4-triazole-N-1,5-trisubstituted cholinesterase inhibitor core in two strains of *Saccharomyces cerevisiae* BY4147 and Δgsh1 induced by hydrogen peroxide solution. MATERIALS AND METHODS: Two tests were carried out to evaluate the toxicity of the compounds through the tests of resazurin and a growth curve and two tests to evaluate the antioxidant activity through the measurement of lipid peroxidation and intracellular oxidation. DISCUSSION AND RESULTS: In the resazurin assay, the results showed, in both strains, that all compounds are non-toxic at concentrations below 250 mM. Using the concentration of 20 μM the growth curve confirmed the non-cytotoxicity of triazoles in both strains. In strain BY4147 all compounds reduced MDA levels between 30-40% compared to the hydrogen peroxide sample, however in strain Δgsh1 only compounds containing phenolic substituents significantly reduced MDA levels by around 25%. In the evaluation of protection to the intracellular environment, only in the Δgsh1 strain in the yeast fermentation phase, there was a significant reduction of oxidant species promoted by all compounds, with emphasis on the compound with nitro substituent with about 27%. CONCLUSION: In conclusion, triazoles were not toxic and provided antioxidant protection even in the absence of glutathione in yeast cells.
Keywords: Antioxidant, *Saccharomyces cerevisiae*, Triazole Derivatives

S.04 - Antihypertensive and Antioxidant Action of Egg White Hydrolysate in Arteries from Doca-salt Hypertensive rats

Edina da Luz Abreu [1], Camila Moro[1], Samia Kanaan[1], Camila Herrera[1], Pedro Henrique Dornelles[1], Ricardo Bernardino[2], Dalton Vassallo[3], Luciana Rossoni[2], Marta Miguel[4], Giulia Wiggers[1]
[1]Biochemistry, Postgraduate Program in Biochemistry - Federal University of Pampa (Rio Grande do Sul, Brazil), [2]Physiology and Biophysics, Science Institute Biomedical, USP (SP, Brazil), [3]Physiology, Federal University of Espírito Santo (ES, Brazil), [4]Institute of Investigation in Sciences of the Food, Superior Council of Scientific Investigations/Institute of Investigation in Sciences of the Food (Madrid, Spain)

INTRODUCTION: Introduction: Egg white hydrolyzate (EWH) produced by hydrolysis with pepsin has biological properties attributed to bioactive peptides that could act on several cardiovascular diseases like hypertension. OBJECTIVES: Objectives: Investigated the effects of EWH on vascular changes in arteries resulting from the Doca-salt model in rats and the mechanisms involved. MATERIALS AND METHODS: Material and Methods: Male Wistar rats (7 weeks; ± 220 g) were divided and treated for 8 weeks in: a) SHAM (unilateral uninephrectomy + distilled water via gavage); b) SHAM+EWH (unilateral uninephrectomy + EWH – 1kg/day via gavage); c) DOCA (unephrectomy unilateral + acetate and deoxycorticosterone (DOCA) (1st, 2nd - 3rd and 4th - 8th week: 20, 12 and 6 mg/kg respectively); d) DOCA+EWH (unilateral uninephrectomy + DOCA) and EWH – 1kg/day via gavage, 4th-8th week). DOCA and DOCA+EWH animals received daily as drinking water a solution of NaCl (1%) + KCl (0.2%), and the other animals only drink water. Systolic blood pressure (SBP) was recorded. Concentration-response curves to acetylcholine (ACh) and sodium nitroprusside (SNP) were performed and the possible vascular pathways envolved in mesenteric artery and aorta. Were assessed biochemical and immunofluorescence analyses and in situ production of superoxide anion. DISCUSSION AND RESULTS: Results and Discussion: EWH co-treatment: a) reduced the elevated SBP levels produced by the DOCA-salt model, with a 36% drop in SBP values (SHAM: 116.5 ± 1.5; SHAM+EWH: 118.1 ± 0.7 DOCA: 194.9 ± 3.7*; DOCA+EWH: 153.9 ± 5.1*#; in mmHg, *vs. SHAM, #vs. DOCA); b) prevented the vascular dysfunction mediated by ROS reduction (mitochondrial in MRA) ; c) reverted the increased NOX-1, NFkB and TNF-α levels; c) normalized ROS and lipid peroxidation levels in plasma and vessel; and d) reduced the superoxide anion in situ production. CONCLUSION: Conclusions: EWH demonstrated antihypertensive, and antioxidant power in the DOCA-salt model, improving vascular dysfunction, and pointing to therapeutic effects on functional foods in hypertension. **Keywords:** Egg white hydrolysate, Hypertension, Stress Oxidative. / **Supported by:** CAPES, FAPERGS, CNPq

S.05 - Coordination compounds exhibit promising biological antioxidant activity in *Saccharomyces cerevisiae* and *Galleria mellonella* models of study

Larissa Maura de Melo Mattos [1], Hyan Melo Hottum[1], Bruna Barriquel Segat[2], Adolfo Horn Júnior[2], Christiane Fernandes Horn[2], Marcos Dias Pereira[1]
[1]Departamento de Bioquímica, Instituto de Química, Universidade Federal do Rio de Janeiro (, Brasil), [2]Departamento de Química, Universidade Federal de Santa Catarina (, Brasil)

INTRODUCTION: Reactive oxygen species play a key role in cell metabolism. However, ROS overproduction may generate an oxidative stress condition when the antioxidant system is unable to deal with these oxidizing agents. To circumvent ROS toxicity, metal-based compounds with superoxide dismutase (SOD) and catalase (CAT) mimetic properties have been synthesized to act as new synthetic antioxidants. OBJECTIVES: This work aimed to investigate the antioxidant potential of three coordination compounds containing iron (Fe3+), copper (Cu2+) and manganese (Mn2+). MATERIALS AND METHODS: Antioxidant activity was analyzed *In vitro* by DPPH and *In vivo* using *Saccharomyces cerevisiae* and Galleria mellonella models of study. TBARS was used for lipid peroxidation detection. The induction and activation of Hsp104-GFP was determined by fluorescence microscope and the activities of CAT and SOD enzymes were performed by monitoring the decomposition of H2O2 and inhibition of nitrotetrazolium blue (NBT) reduction, respectively. Oxidative stress was assessed by direct exposure of yeast cells to H2O2 (1 mM), menadione (30 mM) and chronological aging. To study the antioxidant protection of the compounds in G. mellonella, larvae were treated with the complexes by direct injection into the hemocoel and then stressed with 5.0 M H2O2 DISCUSSION AND RESULTS: DPPH assay indicated that complexes present moderate antioxidant activity on this model. However, the treatment with metal-based complexes increased yeast tolerance against oxidative stress conditions and extended G. mellonella larvae survival after H2O2 stress. Treatment of cells with complexes reduced lipid peroxidation and was capable to activate SOD activity, whilst no effect in CAT activity was observed. It was observed that the treatments induced the expression of the Hsp104 but did not activate this heat shock protein. CONCLUSION: The antioxidant potential presented by the complexes studied in this work can be further explored to attenuate several oxidative stress-related pathologies.

Keywords: Galleria mellonella, Oxidative stress, *Saccharomyces cerevisiae*

S.06 - Peroxymonocarbonate-mediated Inactivation of Protein Tyrosine Phosphatase 1B Monitored by Enzyme Intrinsic Fluorescence

Matheus Paulino Romano [1], Fernando Rodrigues Coelho[1], Luciana Elena de Souza F. Machado[2], Ohara Augusto[1]
[1]Departamento de Bioquímica, Instituto de Química da USP (SP, Brasil), [2]Departamento de Genética e Biologia Evolutiva, Instituto de Biociências da USP (SP, Brasil)

INTRODUCTION: Usually considered a stable molecule, carbon dioxide (CO_2) reacts with the biologically ubiquitous hydrogen peroxide to produce peroxymonocarbonate (HCO_4^-). This oxidant exists in equilibrated solutions of H_2O_2 and HCO_3^-/CO_2 (bicarbonate buffer), and accelerates relevant biological oxidations, such as that of peroxiredoxins and tyrosine phosphatases. The rate constant for PTP1B inactivation by H_2O_2 in the absence and presence of 25 mM HCO_3^-/CO_2 was previously determined as 24 and 202 $M^{-1} s^{-1}$, respectively, at pH 7.0 and 25 °C. From these data, we calculated the rate constant for PTP1B inactivation by HCO_4^- as $2.5x10^4$ $M^{-1} s^{-1}$, a value three orders of magnitude higher than that of H_2O_2. OBJECTIVES: Such a difference is 10 times higher than that observed for other nucleophiles, arguing for a better understanding of the oxidation of PTP1B by H_2O_2 in the absence and presence of HCO_3^-/CO_2.MATERIALS AND METHODS: To address this problem, we expressed and purified truncated forms (aa 1-301) of recombinant human PTP1B WT and its C121S mutant as previously described and performed kinetics, thiol oxidation and simulations studies. DISCUSSION AND RESULTS: The results showed that the enzymes behaved as previously described with regard to the phosphatase activity and inactivation by peroxides. To obtain mechanistic details, however, it was necessary to measure the rate constants with oxidants directly and not through enzyme inactivation. Considering that PTP1B has a W residue (W^{179}) close to the active site and suffers structural changes upon oxidation, we hypothesized that the intrinsic fluorescence of PTP1B could be used to follow its oxidation. Indeed, upon enzyme oxidation its intrinsic fluorescence decreases in a time- and oxidant concentration-dependent manner and was partially reversed by reductants. CONCLUSION: In conclusion, the developed method permitted to obtain rate constants of the peroxide-mediated oxidations of PTP1B WT and mutant, and opened the way to explore the mechanism by which HCO_4^- accelerates these oxidations (inactivation).

Keywords: bicarbonate buffer, peroxides, tyrosine phosphatases / **Supported by:** FAPESP

S.07 - Capability of Thioredoxin 1 (S. cerevisiae) to Bind to Dinitrosyl Iron Complex.

Arthur Migliatti Vetorazzi Ferreira de Pinho [1], Daniela Truzzi[1]
[1]Instituto de Química, Universidade de São Paulo (, Brasil)

INTRODUCTION: Nitric oxide (NO) is a signaling molecule that plays role in physiological and pathological processes. Among its capabilities, NO can act as an efficient cellular iron sequester forming dinitrosyl iron complexes (DNICs). DNICs can have different types of ligands, but in the biological medium, these ligands are mainly thiols of low or high molecular weight. Although it is well accepted that the cysteine and glutathione are biological ligands of DNIC, the identity of the thiol-proteins involved in DNICs formation remains debatable. Considering that cells exposed to NO show reduction in H2O2 metabolism and DNICs are abundant NO-metabolites, the coordination of enzymes from H2O2 metabolism into DNICs would explain such observation. OBJECTIVES: Therefore, this work aimed to evaluate the capability of the thioredoxin I (Trx1) of S. cerevisiae to form DNIC-Trx1. MATERIALS AND METHODS: Trx1 was cloned into pET-17b, expressed in E. coli strain BL21, and purified by ion exchange and gel filtration chromatography. To evaluate the Trx1 capability to bind to DNIC, first Trx1 was reduced with DTT, then added to a pH=7.4 solution containing DNIC-GS (dinitrosyl iron complexes containing glutathione as a ligand) and monitored by EPR. DISCUSSION AND RESULTS: Different than the symmetrical EPR signal centered at g=2.03 characteristic of DNIC-GS, the solutions containing DNIC-GS and Trx1 exhibited a broad asymmetrical signal, indicating the formation of DNIC-Trx1($g_{\perp}$=2.039 and $g_{\parallel}$ =2.021). The spin quantification performed by EPR indicated that about 50% of Trx1 is coordinated to DNIC-Trx1 and this complex remains stable at pH=7.4 for at least 24h. CONCLUSION: These results show that Trx1 is capable of forming DNIC-Trx1 at a high yield and this complex is stable in aqueous media, suggesting that thioredoxins could be involved in DNIC formation in cells.

Keywords: Nitric oxide, Dinitrosyl Iron Complex, Thioredoxin / **Supported by:** FAPESP

S.08 - Evaluation of the Redox-Active Action of Manganese Porphyrin/Ascorbate System on Promastigote Forms of Cutaneous Leishmaniasis

Tiago Henrique dos Santos Souza [1,4], Jacqueline Bueno-Janice[2], José Sarmento-Neto[2], Júlio Rebouças[2], Beate Santos[3], Regina Figueiredo[4], Adriana Fontes[1]
[1]Departamento de Biofísica e Radiobiologia, Universidade Federal de Pernambuco (PE, Brazil), [2]Departamento de Química, Universidade Federal da Paraíba (PB, Brazil), [3]Departamento de Ciências Farmacêuticas, Universidade Federal de Pernambuco (PE, Brazil), [4]Departamento de microbiologia, Fundação Oswaldo Cruz - Instituto Aggeu Magalhães (PE, Brazil)

INTRODUCTION: Cutaneous leishmaniasis (CL) is an infectious disease caused by protozoans of the genus *Leishmania*. The CL treatment is based on toxic drugs and resistance to the treatment has been reported. Therefore, the development of more effective therapeutical approaches is still needed. *In vivo* studies have demonstrated that Mn porphyrins (MnP) have antitumoral activity and can be used as radioprotectors, due to their capacity to be pro or antioxidant, depending on the redox status of the microenvironment. OBJECTIVES: This study aimed to investigate the redox-active action of MnTE-2-PyP $^{5+}$ in association with sodium ascorbate (Asc.) on *Leishmania amazonensis* and *L. braziliensis* promastigote forms. MATERIALS AND METHODS: The effects on promastigotes were evaluated by cell counting after 48 h of incubation with MnTE-2-PyP $^{5+}$ or Asc., alone, or in combination. MnTE-2-PyP $^{5+}$ was tested at 5 and 10 µmol/L, and Asc. at 2 and 3 mmol/L. The role of H_2O_2, produced in the combined treatment, on the parasite viability was investigated by adding catalase to the system. The cytotoxicity of the treatments on Vero cells was also analyzed. DISCUSSION AND RESULTS: Parasites treated with MnP alone showed similar results as the control (not statistically significant). Only *L. amazonensis* treated with Asc. alone showed a growth reduction of 37%. On the other hand, the treatment with MnTE-2-PyP $^{5+}$ + Asc., caused growth inhibition by 88% and 37%, for *L. amazonensis* and *L. braziliensis*, respectively. The addition of catalase recovered the *L. amazonensis* growth with inhibition of only 14%, compared to the control. These data suggest that the leishmanicidal effect of MnTE-2-PyP $^{5+}$ + Asc. was mediated by the generation of H_2O_2. No considerable toxicity was observed on mammalian cells. CONCLUSION: The association of MnTE-2-PyP $^{5+}$ + Asc was promising for the inactivation of *Leishmania* promastigotes encouraging further pre-clinical studies on CL.

Keywords: Leishmania amazonensis, Leishmania braziliensis, MnTE-2-PyP5+ / **Supported by:** CAPES, CNPq, FACEPE, IAM/FIOCRUZ, FINEP and INCT-INFo.

S.09 - Mechanisms of tryptophan and tyrosine hydroperoxides formation and their role as a source of singlet oxygen

Stella Boutris Jayme [1], Fernanda Manso Prado[1], Mariana P. Massafera[1], Graziella E. Ronsein[1], Paolo Di Mascio[1]
[1]Departamento de Bioquímica, Instituto de Química - Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: Introduction. Proteins are considered potential targets for oxidizing agents due to their high abundance in biological environment and high constants of reaction of amino acid side chains with oxidants. Reactions of singlet oxygen with tryptophan (W) and tyrosine (Y) generate hydroperoxides. Interestingly, hydroperoxides may be also be a source of singlet oxygen ($^1O_2$) when reacting with hypolalous acids such as HOCl and HOBr. OBJECTIVES: Objective. The purpose of this work is to characterize W and Y-derived hydroperoxides, and to evaluate if there are able to generate singlet oxygen upon reaction with HOCl and HOBr. MATERIALS AND METHODS: Materials and Methods. Hydroperoxides derived from W, Y and tripeptides containing these amino acids were synthesized by photooxidation. Photoproducts were analyzed by MS/MS and mechanisms of hydroperoxide formation were proposed. Finally, the reaction between the photooxidation products and the HOCl and HOBr acids were monitored using a photomultiplier in the near infrared (1270 nm) for the detection of $^1O_2$. DISCUSSION AND RESULTS: Results and Discussion. Tryptophan and tyrosine phootoxidation, generated of hydroperoxides and their corresponding alcohols identified by MS/MS from the theoretical masses. Upon reaction of W- and Y- derived peroxides (either isolated or inserted in tripeptides) with HOCl and HOBr, emission of infrared light at 1270 nm (characteristic $^1O_2$ decay) was detected. The reactions were inhibited in the presence of azide (a $^1O_2$ quencher) and more light emission was seen using D_2O as the solvent (that increases $^1O_2$ lifetime). Further studies are underway to unravel the mechanism of $^1O_2$ generating between amino acids hydroperoxides and hypohalous acids.
Keywords: Hydroperoxides, Mass Spectrometry, Singlet Oxygen / **Supported by:** Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)

S.10 - Incorporation of 6-Thioguanine in the DNA of Epithelial Cells

André Luiz Lopes [1], Fernanda Manso Prado[1], Marisa Helena Gennari de Medeiros[1], Graziella Eliza Ronsein[1], Paolo Di Mascio[1]
[1]Departamento de Bioquímica, Instituto de Quimica - Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: The thiopurines azathioprine, 6-thioguanine (6-TG), and 6-mercaptopurine are used as immunosuppressors in transplanted patients, and for treatment of inflammatory diseases. These drugs are metabolized by the purine salvage pathway, converted into thioguanine nucleotides and then incorporated in DNA as 6-TG. Recent studies reveal that thiopurine treatment is related to the incidence of skin cancer (squamous cells carcinoma) on those patients. This side effect arises due to the photosensitization of 6-TG by sunlight, which contains UVA radiation, giving rise to singlet molecular oxygen. OBJECTIVES: The objective of this project was to develop a quantification method of 6-TG incorporated in DNA of cultured epithelial cells. MATERIALS AND METHODS: HaCaT cells were cultivated, treated with 6-TG, and their DNA extracted, hydrolyzed, and submitted to high performance liquid chromatography analysis coupled to absorbance and fluorescence detectors. DISCUSSION AND RESULTS: Our results have shown that 6-TG incorporation in the DNA of HaCat cells was around 2 to 4 %. These values are higher than incorporation measured in the DNA in skin samples of patients treated with Azathioprine, due to the amount of 6-TG that cells were exposed. CONCLUSION: Singlet oxygen is the main contributor to oxidatively generated damage to DNA in cells and human skin upon exposure to the UVA. The generation of singlet oxygen by 6-TG is expected to induce the formation of 8-oxo-7,8-dihydroguanine. The major interest in this work is the elucidation of the oxidation reactions triggered by 1O2 with emphasis on mechanistic aspects and identification of the final degradation products. In addition, the formation of several other base modifications including DNA-protein and interstrand cross-links have been reported in 6-TG containing oligonucleotides upon exposure to UVA. However, no mechanism is available so far to rationalize the generation of complex DNA damage.
Keywords: 6-Thioguanine, Photosensitization, Singlet oxygen
Supported by: FAPESP

S.11 - Neuroprotective Effects of Acrocomia aculeata (Bocaiuva) Pulp Oil Microcapsules on Rats Subjected to Chronic Stress

Ana Cristina Jacobowski [1], Eduardo B. Parisotto[1], Abel Santamaria[2], Maria Ligia Rodrigues Macedo[1]
[1]LPPFB, Universidade Federal Do Mato Grosso Do Sul (MS, Brasil), [2]LAE, Universidad Nacional Del Mexico (, MÉXICO)

INTRODUCTION: *Acrocomia aculeata* fruits are rich in monounsaturated fatty acids, b-carotene, tocopherol and other antioxidant compounds. OBJECTIVE: to investigate the protective effects of microencapsulated *A. aculeata* pulp oil on brain oxidative damage induced by chronic restriction stress (CRS) in rats (cortex, hippocampus and striatum). MATERIAL AND METHODS: Thirty-six Wistar rats were divided into six treatment groups: groups C, P and M received 1 µl/g of body weight of distilled water (control), *in natura* pulp oil and microencapsulated pulp oil by gavage daily, respectively. SC, SP and SM groups received 1 µl/g of body weight of distilled water, pulp oil and microencapsulated pulp oil by gavage daily, respectively, 30min before undergoing 6 h uninterrupted CSR. After 21 days of testing, the mice were euthanized and the brain tissue of the groups was removed for evaluation of markers of oxidative damage and antioxidant enzymes. RESULTS: Stress markers (lipid peroxidation, protein carbonylation and reduced glutathione [GSH]) and antioxidant enzymes (superoxide dismutase and catalase) were determined. Pulp oil and pulp microcapsules induced positive antioxidant responses, mainly by increasing the GSH content, increasing the ability of neural tissues to deal with oxidative stress, thus protecting against neurodegenerative diseases. CONCLUSION: The administration of *A. aculeata pulp oil in natura* and microencapsulated reversed the oxidant parameters, which may protect the brain tissue of rats altered by CRS.

Keywords: antioxidants, brain oxidative damage, chronic restraint stress
Supported by: PROPP/UFMS, CAPES, CNPq, FUNDECT

S.12 - Sulforaphane as an Reactivator of the Nrf2 Antioxidant Pathway in Chronic Kidney Disease

Fernanda Kussi[1], Carmen Lucía Sanz Alarta[1], Angela Cristina Michalichyn[1], Breno Castello Branco Beirão[1], Mikaela Dos Anjos Adur[1], Francisco Filipak Neto[2], Maria Heloisa Massola Shimizu[3], Lia Sumie Nakao[1]
[1]Patologia Básica, Universidade Federal do Paraná (Paraná, Brasil), [2]Biologia Celular, Universidade Federal do Paraná (Paraná, Brasil), [3]Laboratório de Pesquisa Médica, Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: Chronic Kidney Disease (CKD) patients show intense oxidative stress due to uremia. Several studies have reported that Nrf2 antioxidant pathway is repressed in CKD. Sulforaphane (SF) is a known Nrf2 activator found in cruciferous vegetables. OBJECTIVES: To evaluate the potential of SF to activate Nrf2 in CKD context *In vitro* and experimental model. MATERIALS AND METHODS: In a preliminary study, male Wistar rats were nephrectomized or not to characterize the Nx 5/6 model. After 4-10 weeks rats were euthanized, kidney sections stained with PAS, biochemical parameters measured with comercial kits and uremic toxins (IxS, pCS, IAA) by HPLC in sera. In the definitive study, rats were submitted to the same surgeries and after 2 weeks, treatment with SF was started. Body weight was measured weekly until 10 weeks to euthanize. Human sera from CKD and healthy volunteers were also collected for *In vitro* evaluations. DISCUSSION AND RESULTS: On average, the Nx rats showed higher concentrations of urea (92.2 vs 31.5 mg/dL), creatinine (0.88 vs 0.25 mg/dL), uric acid (5.3 vs 2.8 mg/dL), cholesterol (144 vs 41 mg/dL), IxS (24.2 vs 0.5 µM) and pCS (14.5 vs 0.6 µM) than the sham rats. Also, Nx kidneys presented tubular damage, in parallel SF does not improve body weight gain or kidney hypertrophy. Human sera pools were characterized: urea (124,5 vs 35 mg/dL), creatinine (9,2 vs 0.8 mg/dL), uric acid (7,6 vs 5 mg/dL), cholesterol (157,9 vs 166,6 mg/dL), glucose (99 vs 82 mg/dL), PCR (4,1 vs 0,9 mg/dL) IxS (96.34 vs 4.22 µM), pCS (140.04 vs 15.06 µM) and IAA (15.71 vs 1.95 µM). CONCLUSION: We have succesfully validated the Nx model in our laboratory, but SF did not altered the body weight gain and the kydney hipertrophy after 10 weeks. The definitive study is ongoing and biochemical analysis are in progress.

Keywords: Chronic Kidney Disease, Sulforaphane, Oxidative stress
Supported by: Capes

S.13 - PEROXIREDOXIN 2 OLIGOMER ORGANIZATION IN NEUTROPHILS AND MYELOID CELLS

Luiz Felipe de Souza[1], Paul Pace[2], Mark Hampton[2], Christine Winterbourn[2], **Flavia Carla Meotti** [1]
[1]Biochemistry, University of Sao Paulo (SP, Brazil), [2]Pathology, University of Otago (, New Zealand)

INTRODUCTION: Peroxiredoxins (Prxs) are key players in the control of redox homeostasis in the cell, both by its peroxidase activity and its role in redox signaling. We had previously reported that both Prx1 and Prx2 were present as oxidized dimers in human neutrophils under resting conditions (de Souza et al., 2019). However, neutrophils expressed thioredoxin (Trx) and thioredoxin reductase to the same extent as myeloid cells which are efficient in recycling Prx. OBJECTIVES: We hypothesized that this oxidation might be driving protein-protein interactions in the neutrophils. MATERIALS AND METHODS: HL-60 cells were treated with H2O2 and subject to BN-PAGE. Immunoprecipitation (IP) experiments followed by identification by mass spectrometry were then used to identify Prx2 interactors in HL-60 and neutrophils. Proteomic data were validated by Western-Blot. DISCUSSION AND RESULTS: Prx2 in neutrophils are present mainly associated as high molecular weight complexes, while Prx2 in myeloid cells are present mainly as dimers. Upon H2O2 treatment, Prx2 in myeloid cells migrated as high molecular weight complexes with similar sizes as observed for the neutrophils. Interestingly, recombinant Prx2 in BN-PAGE migrated as monomers, dimers or high molecular weight oligomers when reduced, but migrate exclusively as dimers when oxidized, suggesting the oligomers observed in cell lysate are due to interactions with other proteins.We identified several potential interaction partners in myeloid cells. Interestingly, several of the proteins identified were involved in RNA processing and formation of stress granules, such as G3BP1, eIF4a and eEF1A. Furthermore, we confirmed that G3BP1 co-immunoprecipitated with Prx2 in myeloid cells by western blot. CONCLUSION: These results suggest that Prx2 might have a role modulating RNA metabolism and stress granules.

Keywords: PEROXIREDOXIN, NEUTROPHILS, REDOX SIGNALING
Supported by: FAPESP

S.14 - Biological Activity of the Influence of Xanthosoma sagittifolium L. Schott Extract on the Redox Environment in *Saccharomyces cerevisiae* Cells

Nathalia Soares Camargo [1], Aline Teixeira Ferreira Furtado[1], Neide Mara de Menezes Epifânio[2], Douglas Siqueira de Almeida Chaves[2], Cristiano Jorge Riger[1]
[1]Departamento de Bioquímica, Universidade Federal Rural do Rio de Janeiro (RJ, Brasil), [2]Departamento de Ciências Farmacêuticas, Universidade Federal Rural do Rio de Janeiro (RJ, Brasil)

INTRODUCTION: PANC plants (unconventional food plants) are the group of plants that have edible parts that are not part of the daily diet of the population. An example *Xanthosoma sagittifolium* L. Schott. Its chemical characterization describes the presence of flavonoids and other compounds, which can help the organism to maintain cellular redox homeostasis; decreasing the concentration of reactive oxygen species (ROS) and consequently the effects caused by oxidative stress. OBJECTIVES: Evaluation of the antioxidant potential of *Xanthosoma sagittifolium* extract in *Saccharomyces cerevisiae* strains. MATERIALS AND METHODS: The *yap1* strain is deficient in the Yap1 transcription factor associated with the regulation of intracellular redox protection genes, while BY4741 is the control strain. The aqueous extract of the plant leaves was prepared by decoction for 20 minutes, the product was filtered, frozen and dried by lyophilization. A semi-quantitative colorimetric assay with resazurin determined the non-cytotoxic concentrations of the extract. Cell viability in two different culture media determined the toxicity and antioxidant activity of the extract (0.6 mg.mL $^{-1}$) for 2 hours compared to cells under stress with hydrogen peroxide (1.0 mM). The initial chemical profile of the extract was determined by HPLC-DAD. DISCUSSION AND RESULTS: The extract at the concentration evaluated was not toxic to strains BY4741 and <i<yap1, both in fermentation and respiratory media. The antioxidant activity was also evaluated in these same culture media and showed promising results for the 2 strains. According to the initial analysis of the constituents present in the extract, it is estimated that the main compound is diglycosylated apigenin. CONCLUSION: This study suggests so far that the extract of *Xanthosoma sagittifolium* showed considerable antioxidant potential and no toxicity in yeast cells, which may be influenced by the possible presence of flavonoids and other compounds.

Keywords: Antioxidant, PANC, *Saccharomyces cerevisiae*

S.15 - Different responses of wheat genotypes under water shortage and inoculation with the bacterium h. Seropedicae

Hemilly Karini Rezende[1], Brenda Vilseque Fernandes[1], Eliane C. G. Vendruscolo[1], **Marise Fonseca dos Santos** [1]
[1]Biociências, Universidade Federal do Paraná (Paraná, Brazil)

INTRODUCTION: The use of plant growth-promoting bacteria (PGPB) is an alternative to the plant's healthy development and the mitigation of stress biotic and abiotic effects. However, the mechanism of tolerance or its promotion is not completely understood. *H. seropedicae* is a potential bacterium strain that has been shown these abilities in cereals. OBJECTIVES: This study evaluated the glutathione-S-transferase enzymatic activity to confirm the natural tolerant genotype capacity and the *H. seropedicae* inducing tolerance. MATERIALS AND METHODS: For such, in greenhouse conditions, the wheat cultivars CD 104 and CD 120, respectively tolerant and not tolerant to hydric stress, were subjected to water restriction for 15 days during flowering. Four planting conditions were applied: *H. seropedicae* inoculation on sowing, urea fertilization, inoculation with fertilization association, and the control, without inoculation or fertilization. Leaves were collected for analyzing glutathione-S-transferase activity (GST) and stress indicators such as malondialdehyde (MDA), proline (PRO), and relative water content (RWC). One plant per pot was remained for reaching the end of the life cycle to obtain the productivity components. DISCUSSION AND RESULTS: As result, the enzymatic activity of GST was higher in water restricted treatment than in irrigated for all CD 104 conditions and inoculated CD 120. Inoculated CD 120 presented lower MDA and PRO and higher RWC that indicated the mitigation of drought effects. It was not observed increased productivity components in CD 104 but inoculated CD 120 kept similar data between water restricted treatments and normal irrigation. CONCLUSION: It was concluded that GST activity is an answer from tolerant plants and to plant acquired tolerance with *H. seropedicae* inoculation. It was shown plant growth-promoting bacteria are genotype-dependent.

Keywords: Glutathione S-Transferase, water shortage tolerant plant, Plant growth promoting bactéria / **Supported by:** CNPq

S.16 - Ultra-sensitive Quantification of Carnosine-aldehyde Adducts in Rat Muscle by an On-line Liquid Chromatography-Electrospray Tandem Mass Spectrometry Assay

Bianca Scigliano Vargas [1], Reis Pablo Victor Mendes[1], Prado Fernanda Manso[1], Di Mascio Paolo[1], Medeiros Marisa Helena Gennari de[1]

[1]Departamento de Bioquímica, Instituto de Química da Unviersidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: Mutations in the gene encoding cytosolic Cu,Zn-superoxide dismutase (SOD1) have been linked to familial amyotrophic lateral sclerosis. The overexpression of the mutated enzyme (SOD ^{G93A}) in rats leads to the buildup of toxic aggregates, resulting in transgenic animals that mimic the human condition, showing the typical motor neuron degeneration. Cell exposure to redox stress results in the formation of a range of reactive species, including aldehydes as 4-hydroxynonenal (4-HNE) and 4-hydroxy-2-hexenal (HHE). Aldehyde detoxification can occur via reactions catalyzed by alcohol dehydrogenase, aldo-keto reductase, aldehyde dehydrogenase and through conjugation with glutathione. Additionally, carnosine (β-alanyl-L-histidine, CAR), has been shown to detoxify aldehydes *In vivo* . Carnosine is present in millimolar concentrations in skeletal and cardiac muscle. OBJECTIVES: This study was performed in order to quantify carnosine, CAR-HHE and CAR-HNE adducts in rat muscle to further understand the role of this dipeptide in the redox system. MATERIALS AND METHODS: The analytes from skeletal muscles of Sprague Dawley 70 days WT and SOD ^{G93A} (asymptomatic) rats were extracted and analyzed in a HPLC/ESI/MS-MS system, using Scheduled Multiple Reaction Monitoring (sMRM) in the positive mode. DISCUSSION AND RESULTS: A highly sensitive and specific HPLC/ESI/MS-MS method was developed with the use of isotopic dilution. The method allowed quantification of up to 4 and 70 fmol of the CAR-HHE and CAR-HNE adducts, respectively, both present in fmol/mg protein concentrations in rat muscle. CONCLUSION: Supporting the hypothesis that carnosine is an aldehyde quencher, the results showed that the dipeptide is indeed sequestering, *In vivo* , both 4-hydroxy-2-hexenal and 4-hydrohy-2-nonenal produced by lipid peroxidation.

Keywords: Reactive Aldehydes, Carnosine, Mass Spectrometry
Supported by: Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP

S.17 - Investigation of Oxidizing Biological Substrates of AhpC: Basic Features and Applications to Combat Pathogenic Bacteria

Sabrina Vargas Batista [1], Vitoria Isabela Montanhero Cabrera[1], Luis Eduardo Soares Netto[2], Marcos Antonio de Oliveira[1]

[1]Departamento de Ciências Biológicas e Ambientais, Instituto de Biociências, Campus Litoral Paulista, UNESP, SP, Brazil (SP, Brasil), [2]Depto de Genética e Biologia Evolutiva, Instituto de Biociências, Universidade de São Paulo, SP, Brazil (SP, Brasil)

INTRODUCTION: Multiple drug resistance (MDR) bacterial strains are responsible by 1.3 million human deaths all over the world. The pathogens possess efficient enzymes which are able to mitigate the toxicity of reactive oxygen species (ROS) produced by the host immune cells and some antibiotics. Among them, the bacterial peroxiredoxin (Prx) AhpC (Alkyl hydroperoxide reductase C) which is able to decompose hydroperoxides (Hpx) using a highly reactive cysteine (C_P-S^-) together with two polar residues (Thr/Ser and Arg). At high hydroperoxides concentrations the peroxidase activity is loss as a consequence of the C_P hyperoxidation to cysteine sulfinic acid (C_P-SO_2H). Recently it has been shown that synthetic organic hydroperoxides can efficiently hyperoxidize and inactivate these enzymes even at low concentrations, but no work to date has investigated hyperoxidation using biologically relevant organic substrates. OBJECTIVES: The aim of this work was to assess the hyperoxidation susceptibility of *Escherichia coli* AhpC (EcAhpC) by lipid hydroperoxides using computer assisted docking simulations and biochemical approaches. MATERIALS AND METHODS: Several lipid hydroperoxides commonly found in *E. coli* and the host were evaluated by molecular docking approaches and the recombinant EcAhpC expressed and purified. DISCUSSION AND RESULTS: We initially selected the hydroperoxides of cholesterol (8-COL Hpx), oleic (10-OLA Hpx), linolenic (13-LNA Hpx) and linoleic (10,13-LIA Hpx) acids for molecular docking simulations. Our results revealed that long chain fatty acids hydroperoxides (LCFA-Hpx) presented the best hits to EcAhpC with the peroxide function very close to reactive S_γ of C_P (3.44-3.67 Å), ΔG of -5,8 to -4,5 kcal/mol, and are stabilized by hydrophobic and polar interactions. Biochemical approaches are in progress. CONCLUSION: Our results indicate that LCFA-Hpx are better substrates than lipids containing cyclic structures (e.g. cholesterol-Hpx) suggesting that these Hpx are important biological substrates and may act as AhpC inhibitors by hyperoxidation.

Keywords: AhpC , Escherichia coli, Hydroperoxides / **Supported by:** FAPESP and CNPq

S.18 - Evaluation of H2O2-induced oxidative stress in Galleria mellonella larvae.

Thiago Meloni Vasconcellos de Matteo[1], Larissa Maura Melo Mattos[1], Marcos Dias Pereira[1]

[1]Departamento de bioquímica, Instituto de Bioquímica , Universidade federal do Rio de Janeiro (RJ, Brasil)

INTRODUCTION: Galleria mellonella, has emerged as an alternative model for studies of pathogen infection. However, the efficacy of new drugs against pathogen infection as well as the toxicity of chemicals can also be assessed using this invertebrate model. OBJECTIVES: In this work we addressed the potential application of G. mellonella larvae as a model for studying oxidative stress. MATERIALS AND METHODS: To analyze the susceptibility of G. mellonella, the larvae were subjected to increasing concentrations of H2O2, defined from a substance toxicity screening in relation to the species of interest (1, 2, 3, 4 and 5M), which was injected with a syringe into the hemocoel. Larvae survival was monitored at 24h intervals during all insect life stages. Larvae submitted or not to an acute OS with 5M H2O2 for 24h were used to determine OS biomarkers, hemocyte density and total antioxidant capacity of larvae hemolymph (FRAP and TEAC). DISCUSSION AND RESULTS: G. mellonella larvae presented high tolerance to H2O2. However, the increase of H2O2 concentration and time monitoring, enhanced larvae susceptibility to H2O2. Acute H2O2 dose (5M) reduced superoxide dismutase activity and increased the levels of lipid peroxidation and protein carbonylation. Total antioxidant capacity within hemolymph and the activity of catalase increased after H2O2 exposure. It was also observed a reduction in the number of hemocytes after H2O2 exposure. CONCLUSION: We conclude that the larvae of the invertebrate G. mellonella can be used as a model to study the response to oxidative stress as well as to investigate the effect of potential antioxidant substances.

Keywords: Galleria Mellonella, Hemocytes, Oxidative stress
Supported by: FAPERJ/Capes/CNPq

S.19 - Evaluation of the antioxidant activity of ethanolic extract of Cannabis sativa and its potential use against alpha-synuclein toxicity

Daniele Cabral Pires [1], Larissa Maura de Melo Mattos [1], Luiza Boldrini Vasques [1], Neide Mara de Menezes Epifânio[2], Douglas Siqueira de Almeida Chaves[2], Cristiano Jorge Riger[3], Marcos Dias Pereira [1]
[1]Bioquímica, Instituto de Química, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil), [2]Ciências Farmacêuticas, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro (Rio de Janeiro, Brasil), [3]Bioquímica, Instituto de Química, Universidade Federal Rural do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: Synucleinopathies refer to a group of neurodegenerative disorders associated with alpha-synuclein (αSyn) protein aggregation and oxidative stress (OS). To attenuate OS and αSyn toxicities, several studies have been performed to find new antioxidants. In this context, phytocannabinoids has been focused due to its neuropharmacological effects on central nervous system, including the ability to attenuate OS and protein misfolding. OBJECTIVES: In this work, we will investigate the antioxidant capacity of phytocannabinoids present in the ethanolic extract of Cannabis sativa to protect *Saccharomyces cerevisiae* against OS. MATERIALS AND METHODS: Cytotoxicity was assessed by plating the S. cerevisiae BY4741 previously submitted to a 24 h of treatment with different concentrations (25, 50 and 100 µg.mL-1) of phytocannabinoids extract. *In vitro* antioxidant activity was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl). The antioxidant potential was also tested in yeast cells, which were previously treated with phytocannabinoid extract (25, 50, 100, 250 and 500 µg.mL-1) before being exposed to OS caused by hydrogen peroxide (H2O2) at 1mM. DISCUSSION AND RESULTS: The results indicate that the phytocannabinoids extract was well-tolerated by S. cerevisiae after 24 h of exposure. *In vitro*, the phytocannabinoids extract presented antioxidant activity only at concentrations above 60 µg.mL-1 (75% of antioxidant activity). Phytocannabinoids extract also protected yeast cells against OS. CONCLUSION: Phytocannabinoids extract showed promising results against OS; however, further studies are required to understand the antioxidant potential of phytocannabinoids and its ability to attenuate αSyn toxicity.

Keywords: *Saccharomyces cerevisiae*, phytocannabinoids, oxidative stress

S.20 - Chemical Profile, Toxicity and Antioxidant Potential of Different Extracts of *Cannabis sativa* on Strains of *Saccharomyces cerevisiae*

Tamires Cruz [1], João Fonseca[1], Neide Epifanio[2], Marcos Pereira[3], Douglas Chaves[2], Cristiano Riger[1]
[1]Bioquímica, Universidade Federal Rural do Rio de Janeiro (Rio de Janeiro, Brasil), [2]Ciências Farmacêuticas, Universidade Federal Rural do Rio de Janeiro (Rio de Janeiro, Brasil), [3]Biquímica, Univ. Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: *Cannabis sativa* has been widely studied due to its numerous pharmacological aspects. The diversity of its compounds, which includes cannabinoids, flavonoids and alkaloids, has shown great anti-inflammatory, neuroprotective and antioxidant potential. OBJECTIVES: The objective of this work was to determine the chemical profile of four methanolic extracts of *Cannabis sativa* flowers provided by the Association of Cannabinology Canapse through process number 23083.002965/2020-11. Furthermore, to evaluate the toxicity and antioxidant potential of these extracts in two strains of *Saccharomyces cerevisiae*, BY4741 and its isogenic mutant Δ*yap1*, deleted in a transcription factor related to the endogenous antioxidant response in this eukaryotic model. MATERIALS AND METHODS: Chemical profile of four extracts was determined by HPLC. The evaluation of toxicity in *S. cerevisiae* cells was performed qualitatively, in an assay with resazurin, and quantitatively, by growth curves with yeast cells incubated with the extracts (100µg/mL) for 24 hours, as well as cell viability after 2 hours of treatment with the extracts (25µg/mL). Antioxidant potential was evaluated in cells under oxidative stress with hydrogen peroxide (1.0 mM) after pretreatment with the extracts (25µg/mL) for 2 hours. Then, cell suspensions were plated in culture media containing glucose or glycerol and colonies counted. Colony forming units were compared between the different treatments, cells without any treatment and stressed cells. DISCUSSION AND RESULTS: C1 extract was called crude extract. The C2 extract showed a major signal (85%) referring to cannabidiol (CBD). C3 had about 70% THC and C4 presented a mixture of THC/CBN (ratio 1:1). For both strains the extracts showed low or no toxicity at concentrations equal to or lower than 250µg/mL. Cell viability in media with glucose or glycerol suggest antioxidant potential and protection of mitochondrial functionality, respectively. CONCLUSION: Due to these results, marijuana is an interesting candidate in the fight against oxidative stress in yeast cells and it will be more studied.

Keywords: cannabinoids, yeast, oxidative stress / **Supported by:** CNPq

S.21 - Human Peroxiredoxin 2 and Biological Organic Hydroperoxides: Assessing the Affinity and Inactivation Through Biochemical and Computational Approaches

Gabrielle do Nascimento Sividanes [1], Vitoria Isabela Montanhero Cabrera[1], Luiz Eduardo Soares Netto[2], Marcos Antonio de Oliveira [1]

[1]Ciências Biológicas e Ambientais , Universidade Estadual Paulista Júlio de Mesquita Filho, Campus Litoral Paulista (São Paulo , Brasil), [2]Genética e Biologia Evolutiva , Universidade de São Paulo (São Paulo , Brasil)

INTRODUCTION: Typical 2-Cys peroxiredoxins are a group of thiol peroxidases that participate in cellular redox homeostasis using reactive cysteines (C_P) in catalysis. In humans the Prx2 is a very abundant isoform and its relevance is evidenced by its up regulation in several types of cancers. Prx2 is able to decompose hydrogen peroxide (H_2O_2) and peroxynitrite (NOO-) (rates reaching 10^7 M^{-1} s^{-1}) and alkyl hydroperoxides, a group of organic hydroperoxides derived from bases, amino acids and lipids. Under oxidative stress, Prx2 is reversibly inactivated by the hyperoxidation of the C_P to cysteine sulfinic acid (C_P-SO_2H) with implications in cell redox signaling and survival. Despite the sensitivity to hyperoxidation by inorganic hydroperoxides no study assessed the inactivation susceptibility by biological organic hydroperoxides to date, since these molecules have the ability to self propagate. OBJECTIVES: This work aims to study the affinity and hyperoxidation susceptibility of the HsPrx2 to hydroperoxides derivatives from monounsaturated and polyunsaturated fatty acids (MUFAs and PUFAs) by molecular docking and biochemical approaches MATERIALS AND METHODS: Molecular dockings were performed using Autodock and the data analysis by the Ligplot + and PyMol tools. The recombinant human Prx2 was expressed as His-tagged enzyme and purified by IMAC. DISCUSSION AND RESULTS: Our results obtained from docking simulations indicate that MUFAs and PUFAs hydroperoxides can be important substrates for Prx2. Their flexibility allows the accommodation in the active site microenvironment which are stabilized by hydrophobic and polar interactions, presenting free Gibbs energy from -6.5/-4.8 kcal/mol and with the peroxide moieties very close to the C_P (2.993-3.961 Å). Biochemical analyses involving peroxidase activity and inactivation by hyperoxidation are in progress and results will be presented. CONCLUSION: The results presented here indicates that MUFAs and PUFAs hydroperoxides may be important oxidizing substrates to Prx2 with implications in cell signaling and survival.

Keywords: Peroxirredoxin 2, Organic hydroperoxide , Hyperoxidation / **Supported by:** Fapesp e CNPq

S.22 - Investigation of Paracoccidioides brasiliensis Prx1 Inhibitors by computer assisted simulations and biochemichal approaches

Aryeikon Jader de Medeiros Prado[1], Rosana Puccia[2], Luis Eduardo Soares Netto[3], João Henrique Ghilardi Lago[4], Marcos Antonio de Oliveira[1]

[1]Instituto de Biociências, Depto de Ciências Biológicas e ambientais, Universidade Estadual Paulista (SP, Brasil), [2]Depto de Microbiologia, Imunologia e Parasitologia, Univ. Federal de São Paulo (, Brasíl), [3]Instituto de Biociências E [4]Centro de Ciências Naturais e Humanas, Instituto de Biociências, Universidade de São Paulo (Brasíl)

INTRODUCTION: The pathogenic fungus Paracoccidioides brasiliensis is the vector of paracoccidioidomycosis, a systemic and emerging disease in latin america with ~10 million cases annually. The massive production of reactive oxygen species (ROS) by the host's cellular immune system is an essential strategy to restrain the fungal growth. Among the ROS, the hydroperoxides are very toxic antimicrobial compounds and fungal peroxidases are part of the pathogen neutralizing antioxidant arsenal against the host's defense. Recently was characterized a novel peroxiredoxin (PbPrx1), a thiol peroxidase, able to decompose hydroperoxides using a very reactive cysteine residue (peroxidatic cysteine - CP). PbPrx1 very abundant and distributed in several cell (cytosol and mitochondria), in the cell surface and vesicles and is able to decompose efficiently organic hydroperoxides, a group of toxic compounds generated by the immune system. OBJECTIVES: The aims of this work were to assess the inhibitory potential of Brazilian natural compounds and long chain fatty acids hydroperoxides over PbPrx1 peroxidase activity by molecular docking and biochemical approaches. MATERIALS AND METHODS: The theoretical model was build using the coordinates of human Prx6 (PDB: 5B6M) and the Modeller tool. The compounds binding simulations were performed using Autodock Vina. Recombinant PbPrx1 was expressed as his-tagged enzyme and purified by IMAC. DISCUSSION AND RESULTS: The best docking hits were obtained to elongated hydrophobic compounds: the natural compound from NC-PBA1 Piper crassinervium (ΔG = -6.1 to -7.1kcal/mol) and hydroperoxides derivatives from long chain fatty acids as linoleic (ΔG= -4.8 to -5.6kcal/mol) and Oleic hydroperoxides (ΔG= -4.4 to -5.0kcal/mol). Interaction occurs in hydrophobic grooves of the active site microenvironment. Biochemical approaches are in progress. CONCLUSION: The results support that the PbPrx1 substrate affinity is oriented to with long chain hydrophobic compounds a charachteristic that may help to guide the characterization of novel inhibitory molecules.

Keywords: Fungi, Inhibitors, Peroxiredoxin

S.23 - Effects Of Natural Compounds Over The Peroxidase Activity Of Pseudomonas aeruginosa AhpC

Vitória Isabela Montanhero Cabrera [1], Melina Cardoso Santos[1], Deborah Pelosi Sessa[3], João Henrique Ghilardi Lago[3], Luis Eduardo Soares Netto[2], Marcos Antônio de Oliveira[1]
[1]Departamento de Ciências Biológicas e Ambientais, Instituto de Biociências, Campus Litoral Paulista, UNESP (Brazil), [2]Departamento de Genética e Biologia Evolutiva, Instituto de Biociências, Universidade de São Paulo (Brazil), [3]Centro de Ciências Naturais e Humanas, Centro de Ciências Naturais e Humanas, Universidade Federal do ABC (Brazil)

INTRODUCTION: *Pseudomonas aeruginosa* is a pathogenic bacterium that frequently affects immunocompromised individuals. In this organism, the typical 2-Cys peroxiredoxin, named as AhpC (Alkyl hydroperoxide reductase C) is a very reactive thiol peroxidase able to decompose several substrates generated by the immune host cells and is considered a *P. aeruginosa* virulence factor. Despite the importance, no study has aimed at the use of natural compounds as inhibitors of this class of enzymes. To human isoforms, some compounds have already been identified and all of them possess a bulky hydrophobic skeleton, and some compounds have a carbonyl system that can perform a Michael addition chemistry with the catalytic cysteine. OBJECTIVES: The aim of this work was to evaluate the inhibitory properties of natural hydrophobic compounds containing Michael acceptors from the Brazilian biota over the peroxidase activity of PaAhpC. MATERIALS AND METHODS: Recombinant PaAhpC was expressed as His-tagged protein and purified by IMAC. The effects of natural compounds on the activity of PaAhpC were evaluated by the NADPH and DTT oxidation assay. Molecular docking approaches were performed to a better understanding of enzyme-ligand interaction. DISCUSSION AND RESULTS: Only one compound (NC-PBA1) was able to exert inhibitory properties over PaAhpC ($v_{0PaAhpC}$ = 0.22 ± 0.01 μs^{-1} and $v_{0PaAhpC+CNABP1}$ = 0.09 ± 0.02 μs^{-1}) and IC_{50} value = 6.7 (±0.09) μM. By SDS PAGE we showed that the compound was not able to perform Michael addition and molecular docking results revealed that NC-PBA1 can be stabilized in the active site microenvironment of the PaAhpC (ΔG = -6.3 to -7.4kcal/mol) by several hydrophobic and polar interactions. CONCLUSION: Our results reveal that NC-PBA1 is a novel inhibitor of AhpC from *P. aeruginosa* and that the carbonyl system is not essential for a compound to exert inhibitory properties.
Keywords: Peroxiredoxins, Natural compounds, Inhibitor / **Supported by:** FAPESP, CNPq and CAPES

S.24 - Redox Alterations on Melanoma Induced by Melanogenesis Stimulation

Ester Mazepa [1], Elizabeth Sousa da Cunha[1], Hellen Paula Valerio[2], Paolo Di Mascio[3], Glaucia Regina Martinez[1]
[1]Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Paraná (Paraná, Brasil), [2]Centre of Excellence in New Target Discovery, Instituto Butantan (São Paulo, Brasil), [3]Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: Introduction: Our previous work described that melanogenesis-stimulated B16-F10 cells enter in a quiescent state and present inhibited mitochondrial respiration as a protective mechanism against ROS-damage generated by melanogenesis stimulation. These alterations suggest these cells may be under a redox signaling allowing tumor survival. OBJECTIVES: Objectives: The aim of this work was to evaluate which proteins could be redox-regulated in B16-F10 cells after melanogenesis stimulation. MATERIALS AND METHODS: Materials and Methods: A redox proteomics label-free approach based on biotin switch assay technique with biotin-HPDP and N-ethylmaleimide (NEM) was performed to access thiol-oxidated protein profile in B16-F10 cells submitted to melanogenesis stimulation. DISCUSSION AND RESULTS: Results and Discussion: Preliminary results showed that annexin A2 (Anxa2), elongation factor 2 (Eef2), guanine nucleotide-binding protein (Gnb1 and Gnb2l1), isoleucine-tRNA ligase mitochondrial (Iars2), 2-oxoglutarate dehydrogenase mitochondrial (Ogdh, also up-regulated) and calreticulin (Calr) were oxidized after melanogenesis stimulation. On the other hand, heat shock protein HSP90 (Hsp90aa1 and Hsp90ab1) and voltage-dependent anion-selective channel protein 1 (Vdac1) were reduced, and also down-regulated in the same condition. These results suggest that melanogenesis stimulation may be affecting biological processes such as calcium signaling, translation, signal transduction, energetic metabolism, cell cycle control, ion transport and stress response via redox modulation. CONCLUSION: Conclusions: The redox alterations observed after melanogenesis stimulation in murine melanoma cells and the identification of possible target proteins is of great importance to further understand the modulation of tumor resistance process to therapies. Funding: UFPR, CAPES, CNPq, FAPESP, CEPID Redoxoma (#2013/07937-8)
Keywords: Melanoma, Proteomics, Redox

S.25 - A water-soluble bis (1,10 phenantroline) octanodiaoate Mn2+ -complex derivatives protect *Saccharomyces cerevisiae* cells from oxidative stress

Luiza Boldrini Vasques [1], Julliana Muniz Gonçalves[1], Daniela Dias Queiroz[1], Marcos Dias Pereira[1]
[1]Departamento de Bioquímica, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: The oxidative stress (OS) may be caused by the overproduction of reactive oxygen species (ROS), and it can be attenuated by synthetic compounds with antioxidant capacity. We have previously demonstrated the antioxidant activity of a water-soluble bis (1,10 phenantroline) octanodiaoate Mn^{2+} -complex (MD1) on cells under OS. This Mn^{2+} -complex showed a significant antioxidant potential, reducing intracellular oxidation and increasing *S. cerevisiae* resistance to OS. OBJECTIVES: This study aimed to investigate the antioxidant activity of three Mn^{2+} -complex, derivatives of MD1, in *Saccharomyces cerevisiae*. MATERIALS AND METHODS: The antioxidant activity was assessed using the wild type strain BY4741 of *S. cerevisiae*. Yeast cells were treated with each compound for 1 h and then subjected to 2,0 mM of H_2O_2 for 1 h. The TBARS method was used for lipid peroxidation. The activities of CAT and SOD were performed by monitoring the decomposition of H_2O_2 and the inhibition of nitrotetrazolium blue (NBT) reduction, respectively. Intracellular oxidation was estimated by fluorescence with 2,7-dichlorofluorescein diacetate. DISCUSSION AND RESULTS: Treatments with all Mn^{2+} -complex increased survival of yeast cells subjected to H_2O_2 stress. Treating cells with all Mn^{2+} -complex reduced intracellular oxidation and lipid peroxidation, whilst no changes in CAT and SOD activity was observed. CONCLUSION: This study shows that all three Mn^{2+} -complex derivatives of MD1 are capable to protect *S. cerevisiae* from OS increasing survival levels and attenuating intracellular ROS damages.

Keywords: Antioxidant activity, Coordination compounds, Oxidative stress

S.26 - Inhibitor Screening of E. coli AhpC by molecular docking and biochemical approaches

Davi de Mattos R. Lima [1], Luis Eduardo Soares Netto[2], João Henrique Ghilardi Lago[3], Marcos Antonio de Oliveira[1]
[1]Departamento de Ciencias Biológicas e Ambientais, Instituto de Biociências (São Paulo - SP, Brasil),
[2]Departamento de Biociencias, Instituto de Biociências - Universidade de São Paulo (São Paulo - SP, Brasil),
[3]Centro de Ciências Naturais e Humanas, Centro de Ciências Naturais e Humanas - Universidade Federal do ABC (São Paulo - SP, Brasil)

INTRODUCTION: *Escherichia coli* is an emerging pathogen with multi-drug resistant strains leading to the deaths of over 400,000/year worldwide in this century. To combat microbial infections, immune cells will promote oxidative and nitrosative bursts, a rapid increase in the production of reactive oxygen (ROS) and nitrogen species (RNS), which is a crucial mechanism to eliminate infectious agents. Still, pathogens present very efficient antioxidant enzymes which neutralize the ROS and RNS produced by the host's immune cells. Amongst them, the peroxiredoxin AhpC can decompose hydroperoxides (Hpx) using a highly reactive cysteine ($CP\text{-}S^-$). Recently we've shown that a natural compound (NC-PBA1), isolated from Piper crassinervium leaves, exerts inhibitory properties over *E. coli* AhpC (EcAhpC) and death of bacterial cells. The compound NC-PBA1 is composed of a benzene ring containing a carboxylic acid and a long ester which resembles some organic hydroperoxides. OBJECTIVES: Our objective is to assess the structural determinants of EcAhpC inhibition by NC-PBA1 through molecular docking and biochemical approaches using a compound library carrying molecular modifications in the functional groups of NC-PBA1. MATERIALS AND METHODS: The theoretical model of EcAhpC was built using the molecular coordinates of Salmonella typhimurium AhpC (PDB: 1N8J) and the Modeller tool. Compound binding simulations were performed using Autodock Vina. Recombinant EcAhpC was expressed as his-tagged enzyme and purified by IMAC. DISCUSSION AND RESULTS: We tested NC-PBA1 similar compounds containing modifications in the benzene ring and hydrocarbon tail. The best docking hits were obtained to more elongated hydrophobic compounds C2 and C7 of EAPC, EACN and EAFR subgroups (ΔG = -6.0 to -6.3 kcal/mol). Interaction occurs in hydrophobic grooves within EcAhpC active site microenvironment. Biochemical approaches are ongoing. CONCLUSION: The results support that both functional groups of the compounds are involved in EcAhpC interaction, and the long tail must be a factor of importance of the enzyme-ligand complex stability.

Keywords: Inhibitor screening, Molecular docking, Peroxiredoxin

S.27 - Nitrite Reduction by Copper-Containing Nitrite Reductases: Relevance of the Aspartic Acid of the Sensing Loop in the Catalysis of Nitrite Reduction

Guevara Cuasapud Lorieth Alejandra[1], **Maria Gabriela Rivas** [1], Ferroni Felix Martin[1], Rizzi Alberto Claudio[1], Gonzalez Pablo Javier[1], Carlos Dante Brondino[1]
[1]Physics, Depto de Física, Facultad de Bioquímica y Ciencias Biológicas, Univ. Nacional del Litoral/CONICET (SF Argentina)

INTRODUCTION: Nitrite reductases (NirKs) play a key role in the denitrification pathway catalyzing the proton-coupled one-electron reduction of nitrite to nitric oxide ($NO_2^- + 2\ H^+ + e^- \rightarrow NO + H_2O$). Most of NirKs are heterotrimeric enzymes containing two Cu atoms per protein monomer. The electron transfer center is a type 1 (T1) copper whereas the catalytic center is a type 2 (T2) copper. Two pathways link T1 and T2: the Cys-His bridge and the substrate sensing loop. The substrate sensing loop is thought to work as a relay triggering the T1→T2 electron flow through the Cys-His bridge when the substrate is bound to the catalytic center. An aspartic acid (D134, *Sinhorizobium meliloti* NirK – *Sm*NirK- numbering) present in the sensing loop was proposed to be essential in the catalysis process. However, D134 is not fully conserved and NirK from *Thermus scotoductus* presents a serine in this position. OBJECTIVES: Study the relevance of the aspartic acid residue at the substrate sensing loop in the catalytic mechanism. MATERIALS AND METHODS: Site directed mutagenesis, continuous kinetic assays monitored spectroscopically and UV-visible and EPR techniques. DISCUSSION AND RESULTS: The absorption spectrum of the as purified variant showed absorption maxima at 585nm and 459 nm with extinction coefficient values similar to the wild-type *Sm*NirK(ε_{585nm}=3.0(4) mM^{-1} cm^{-1} and $\varepsilon_{459\ nm}$=3.4(6) mM^{-1} cm^{-1}). Metal incorporation was a more difficult process when compared with the wild type *Sm*NirK and, in agreement with this, metal quantification showed 1.6(1) Cu/monomer. Although D134S is active using the physiological electron donor (pseudoazurin), the kcat and Km values decrease 240-fold and 10-fold, respectively, when compared with the wild-type *Sm*NirK. D134S EPR signal of the as isolated enzyme showed two overlapped spectral components associated with T1 and T2. As expected, the g-values of the T2 copper show differences when compared with the wild-type enzyme.

Keywords: nitrite reductase, metalloenzymes, enzyme catalysis / **Supported by:** FONCyT (PICT-2017-2186), CONICET (PIP 112 20150100550), and CAI-D-UNL. M.G.R, P.J.G., F.M.F and C.D.B. are member of CONICET.

S.28 - QUANTIFICATION OF GLUTATHIONE-2,4-HEXADIENAL AND GLUTATHIONE-4-HYDROXYNONENAL ADDUCTS IN LIVER AND SKELETAL MUSCLE OF ALS RAT MODELS BY MASS SPECTROMETRY

Pablo Victor Mendes dos Reis [1], Bianca Scigliano Vargas[1], Adriano de Britto Chaves Filho[1], Graziella Eliza Ronsein[1], Mariana Pereira Massafera[1], Fernanda Manso Prado[1], Sayuri Miyamoto[1], Henrique Velasquez de Oliveira[1], Paolo Di Mascio[1], Marisa Helena Gennari de Medeiros[1]
[1]Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo (São Paulo, Brazil)

INTRODUCTION: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that specifically affects motor neurons, leading to progressive paralysis. Mutant forms of cooper/zinc superoxide dismutase (SOD1) have been identified in a subset of individuals with familial ALS (FALS). Transgenic rats carrying the SOD1G93A mutant develop a phenotype that resembles the human condition. FALS-linked SOD1 mutants tend to form toxic aggregates and cause oxidative molecular damage. Glutathione (GSH) is a well-known antioxidant and detoxifying agent in cells leading to GSH-aldehyde adducts formation during redox stress. *Trans, trans*-2,4-hexadienal (HDE) is an important breakdown-product of lipid peroxidation. This aldehyde is cytotoxic to mammalian cells and is known to be implicated in protein damage. One of the mostly likely candidates for the consumption of HDE in cytosol is GSH. Conjugation of GSH with aldehydes to give Michael adducts is a recognized detoxification pathway of reactive aldehydes in cells. OBJECTIVES: The aim of the present work is to assess biomolecule damage associated with aldehyde exposure. MATERIALS AND METHODS: Structural characterization of GSH-HDE adduct was performed by mass spectrometry. A very sensitive method using reverse phase high performance liquid chromatography coupled to electrospray ion trap tandem mass spectrometry in the selected reaction monitoring mode for the simultaneous quantification of the GSHDE and glutathione-4-hydroxynonenal (GSHNE) adducts. Stable isotope-labeled GSHNE adduct (GSHNEm) was used as internal standard. DISCUSSION AND RESULTS: The structural characterization of the GSHDE adduct was performed. GSHDE and GSHNE adduct concentrations were found in in liver and skeletal muscle of ALS and control animals at fmol/mg protein. CONCLUSION: Our data confirm that aldehydes are been detoxifying by GSH but we did not observe statistical differences between ALS and the control group.

Keywords: amyotrophic lateral sclerosis, gluthatione, aldehydes / **Supported by:** FAPESP - CEPID Redoxoma (#2013/07937-8) ; CNPq and Pró-Reitoria de Pesquisa USP.

S.29 - Singlet Molecular Oxygen and Hypohalous Acids Reactions with Peptides and Proteins containing Methionine generate Dehydromethionine

Rafaela Oliveira Nascimento [1], Fernanda Manso Prado[1], Mariana Pereira Massafera[1], Paolo Di Mascio[1], Graziella Eliza Ronsein[1]
[1]Departamento de Bioquímica, Instituto de Química - USP (São Paulo, Brasil)

INTRODUCTION: Methionine (Met) is an amino acid highly susceptible to oxidation. Met reacts with most biological oxidants to generate exclusively Met sulfoxide. However, its oxidation by hypohalous acids (HOCl and HOBr) can also generate dehydromethionine (DHM), and this product was suggested as a biomarker of oxidative stress induced by these oxidants. DHM can also be generated when Met is oxidized by singlet oxygen ($^1O_2$), in a reaction involving pH-dependent mechanisms. OBJECTIVES: This work aims to compare the oxidation products of peptides and proteins containing Met by $^1O_2$ and hypohalous acids, as well as to understand methionine oxidation mechanisms by $^1O_2$. MATERIALS AND METHODS: Peptides and proteins containing Met were reacted with $^1O_2$ or hypohalous acids at different pHs (5, 7.4, or 9.2). For the oxidative mechanisms, we performed Met photooxidation in $H_2{}^{18}O$. The samples were analyzed by HPLC/MS/MS. DISCUSSION AND RESULTS: The analysis indicated the formation of DHM after oxidation of peptides and proteins by hypohalous acids and $^1O_2$. In general, we observed that DHM yields increased with increasing pH. Reactions with HOCl showed lower DHM yields, while reactions with HOBr and $^1O_2$ showed higher DHM yields and similar product distribution. Met photooxidation in $H_2{}^{18}O$ generated labeled and unlabeled Met sulfoxide indicating that different pathways can form this molecule. Unlabeled Met sulfoxide formation was favored in lower pHs, while labeled Met sulfoxide generation was favored in higher pHs. CONCLUSION: We concluded that $^1O_2$ contribution to DHM generation in the organism may be significant since it was able to generate DHM with yields closer to those observed with HOBr and higher than observed with HOCl. Also, Met oxidation by $^1O_2$ can go through different mechanisms depending on the reaction pH.

Keywords: Hypohalous acids, Methionine, Singlet oxygen / **Supported by:** CAPES

S.30 - NRF2-Related Gene Signature Downregulation Predicts Sensitivity To Auranofin In Cancer

Helena Souza Zancanaro [1], Marcelo Luiz Brunatto Falchetti[1], Marina Delgobo[1], Karoline Almeida Lima[1], Raquel Nascimento das Neves[1], Alfeu Zanotto Filho[1]
[1]Farmacologia, Universidade Federal de Santa Catarina (Santa Catarina, Brasil)

INTRODUCTION: Auranofin is a thioredoxin reductase-1 inhibitor originally approved for the treatment of rheumatoid arthritis. Auranofin has been repurposed as a potential anti-tumor drug against different malignancies and, because it intervenes with key components involved in redox control of protein-thiol homeostasis, it is expected that different tumor types should present distinct susceptibility patterns in response to auranofin. OBJECTIVES: In this study, we aimed to identify a gene expression signature associated with auranofin resistance in different types of tumors. MATERIALS AND METHODS: We integrated data from a previously published auranofin cytotoxicity screen with publicly available transcriptome data from lung cancer cell lines. Gene mutation and copy number alterations associated with auranofin-resistance signature upregulation were further investigated in pan-cancer cell lines and human cancer datasets. Then, we carried out cell viability assays (MTT method) in a panel comprising 20 cancer cell lines (7 hematological and 13 solid cancer types) treated *In vitro* with auranofin at different concentrations for 72 h. DISCUSSION AND RESULTS: In silico analysis showed that upregulation of the signature occurred mainly in tumors carrying genetic alteration in the NRF2 pathway genes (i.e. KEAP1 and NFE2L2), whereas no genetic alteration was significantly enriched in tumors and cell lines with downregulation of auranofin-resistance signature in pan-cancer analysis. Based on signature expression, non-small cell lung cancers, liver hepatocarcinoma and esophageal carcinoma – especially tumor subsets harboring mutations in the NRF2 pathway – were predicted as resistant while leukemias, lymphomas, and multiple myeloma were inferred as sensitive to auranofin. Further, the antiproliferative (IC50) indexes obtained from *In vitro* screen in cancer cell lines showed that blood cancer cells are more sensitive to auranofin when compared to those from solid malignancies. CONCLUSION: These results indicate that hematopoietic cancers should be prioritized in auranofin repurposing strategies due to low expression of auranofin resistance genes.

Keywords: Blood cancer, Redox vulnerabilities, Thioredoxin reductase-1
Supported by: Fundação de Amparo à Pesquisa e Inovação de Santa Catarina (FAPESC), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

S.31 - Evaluation of the activity of coordination compounds against hydroxyl radical stress

Hyan Melo Hottum [1], Daniele Cabral Pires[1], Larissa Maura De Melo Mattos[1], Lucas Brandalise Menezes[2], Christriane Fernandes Horn[2], Adolfo Horn Junior[2], Marcos Dias Pereira[1]
[1]Departamento de Bioquímica, Universidade Federal do Rio de Janeiro (, Brasil), [2]Departamento de Química, Universidade Federal de Santa Catarina (, Brasil)

INTRODUCTION: Oxidative stress is the consequence of reactive oxygen species (ROS) unbalance within cell. Amongst ROS, the hydroxyl (HR) anion is a non-selective, highly reactive radical, capable of oxidize all biomolecules. Since the antioxidant system cannot cope with this agent, the use of coordination compounds with antioxidant activity has been a promising alternative to eliminate the hydroxyl radical (HR). OBJECTIVES: This work aims to evaluate the protective role of coordination compounds containing Cu(II), Fe(III) or Mn(II) in *Saccharomyces cerevisiae* exposed to HR generating conditions. MATERIALS AND METHODS: Antioxidant activity was analyzed using S. cerevisiae, a well-known model for oxidative stress studies. S. cerevisiae growing on fermentative metabolism were used to assess the antioxidant potential of synthetic compounds under oxidative stress generated by HR. Cells were treated for 1 h with the compounds by direct addition to the culture cell. After treatments, cells were collected by centrifugation, washed with sterile H 2 O and then stressed with the generating HR conditions (0.25mM H 2 O 2 plus 0.1mM Fe(NH 4) 2 (SO 4) 2) for 1 h in water. Survival was determined by plating and the lipid peroxidation was determined by TBARS. DISCUSSION AND RESULTS: It was observed that all complexes increased yeast cell survival after exposure to HR stress.The treatments with synthetic antioxidants increased the survival rates of cells exposed to HR stress from 7.5% to 30-40%. Lipid peroxidation caused by HR stress was also attenuated in cells treated with synthetic antioxidants. CONCLUSION: We can conclude that all complexes tested presented a relevant antioxidant activity against the HR stress, the compounds FeHP and Fe 2 HP showed greater protection and greater reduction in lipid peroxidation levels.
Keywords: Antioxidant, Coordination compounds, Hydroxyl radical

S.32 - Toxicology Of Insulin-Producing Cells Front Of Exposure To Defensives And Effects Of Bioactive Compounds

Desirée Roque Villard [1], Ingrid Batista Borges Rodrigues[1], Kleber Luiz Araujo e Souza[1]
[1]UFRJ - Campus Duque de Caxias Prof. Geraldo Cidade, Núcleo Multidisciplinar de Pesquisas em Biologia (NUMPEX-BIO) - UFRJ (, Brasil)

INTRODUCTION: Malathion is an organophosphate pesticide capable of inducing oxidative stress in beta cells and compromising antioxidant capacity, triggering tissue and cellular damage. On the other hand, non-enzymatic compounds, such as glycyrrhizin and rutin, have antioxidant activities and may act in the prevention or protection against possible oxidative damage caused by organophosphates. OBJECTIVES: In this study, we evaluated the cytotoxicity of the malathion, as well as test possible cytoprotective effects of bioactive compounds on RINm5F insulin-producing cells. MATERIALS AND METHODS: RINm5F cells were cultured in RPMI medium supplemented with 10% fetal bovine serum, 1% antibiotics and incubated at 37ºC. Cells were exposed to different concentrations of malathion (0.001 to 100µM) at three different incubation times (3h, 24h and 48h). Otherwise, cells were pre-incubated for 24h in the absence and presence of 10 µM glycyrrhizin, past the period the cells were exposed to different concentrations of malathion (0.01 to 100 µM) for another 24h. The MTT reduction method was used for cell viability curves. DISCUSSION AND RESULTS: A significant decrease in cell viability was observed when exposed to the pesticide in the incubation period of 24h and 48h. On the other hand, no significant decrease in cell viability was observed at concentrations of up to 0.1µM of the pesticide in the 3h incubation period. The exposure of cells with different concentrations of malathion concomitantly to glycyrrhizin did not demonstrate any statistically significant cytoprotective effect. CONCLUSION: The organophosphate pesticide has a more evident effect when exposed to cells with higher concentration and also for a longer time, demonstrating that it has cytotoxic effects for cells under these conditions. Otherwise, glycyrrhizin did not show a cytoprotective effect on cells against the cytotoxic effects caused by exposure to different concentrations of malathion.
Keywords: organophosphate pesticide, oxidative stress, antioxidant / **Supported by:** FAPERJ

S.33 - *In vitro* Toxicology of Insulin-Producing Cell Lines Grown in Ultraoxidative Environments

Ingrid Batista Borges [1], Kleber Luiz Araújo Souza[1]
[1]NUMPEX-BIO, Federal University of Rio de Janeiro (Rio de Janeiro, Brazil)

INTRODUCTION. Oxidative stress is involved in the progression diabetes. The oxidative stress can be a common link in beta-pancreatic cell dysfunction and use of chemical compounds that mimic the situations encountered in diabetes, has helped to clarify the various deleterious or protective molecular mechanisms that operate in the beta cell. On the other hand, bioactive compounds that may act to prevent or mitigate the consequences of this process are of great interest. OBJECTIVE: To assess the toxicity of oxidizing agents and nitric oxide donors, and to test the possible cytoprotective effects of natural compounds on insulin-producing cells RINm5F. MATERIALS AND METHODS: RINm5F insulin producing cells were cultured in an appropriate medium and exposed to cytotoxic compounds (hydrogen peroxide, menadione, sodium nitroprusside) and to antioxidants of interest (glycyrrhizin and rutin). The MTT was used for cell viability tests. The state of the redox (nitroxy) cells, production of intracellular mitochondrial superoxide and the detection of phosphatidylserine in the cytoplasmic membrane were determined by the fluorogenic probes by fluorescence microscopy. RESULTS AND DISCUSSION: A significant decrease in cell viability has been observed, as well as an increase in the production of reactive species of intracellular oxygen in the face of oxidative challenge. However, co-incubation of RINm5F cells with glycyrrhizin had three different types of effects: a partially protective against the use of hydrogen peroxide; another deleterious exposure to exposure to menadione and no effect on the use of the sodium nitroprusside donor (SNP). CONCLUSION: It is possible that the cell type and the sub-localization of the production of reactive oxygen species can determine the final effect of glycyrrhizin and cell fate. Although rutin had no protection against oxidative damage induced by hydrogen peroxide, menadione and SNP, no cytotoxic effect on RINm5F cells was observed under the conditions tested.

Keywords: antioxidants, diabetes, reactive species / **Supported by:** FAPERJ

T - Natural Products

T.01 - Extract Eugenia uniflora Reverses Long- and Short-term Memory Deficits in a Parkinson's disease model

Anne Suély Pinto Savall [1], Eduarda Monteiro Fidelis[1], Jhully Dorneles de Melo[1], Cristiane Denardin[1], Caroline Brandão Quines[1], Tuane Bazanella Sampaio[2], Simone Pinton[1]
[1]PPG Bioquímica, Universidade Federal do Pampa - Campus Uruguaiana (Rio Grande do Sul , Brazil), [2]Departamento de Farmácia, Universidade Estadual do Centro-Oeste do Paraná (Paraná, Brazil)

INTRODUCTION: Parkinson's disease (PD) is a multisystemic neurodegenerative disorder that includes motor and non-motor symptoms, its main characteristic is the depletion of dopaminergic neurons, mainly in the substantia nigra pars compacta and consequent reduction of dopamine levels in the striatum. However, impairment of other brain structures and neurotransmission pathways has been evidenced. OBJECTIVES: Thus, the proposal to investigate the neurorestorative potential of the Eugenia uniflora (purple variety of pitanga) against memory impairments in a PD model induced by MPTP in rats. MATERIALS AND METHODS: Adult male and female wistar rats (90 days old) were used, MPTP (0.1 mg/nostril) or saline solution (day 0) were administered via intranasal. After recovery, the animals were divided into: Control; MPTP; Pitanga 2000; MPTP+Pitanga 300 or 2000. From day 1, animals received a volume of 3 ml/animal/day of distilled water or hydroalcoholic extract of E. uniflora (300 or 2000 mg/kg) for 14 days, via oral. Object recognition behavior test (ORT) was performed at days 12 and 13 in order to assess explicit short-(STM) and long-term memory (LTM). DISCUSSION AND RESULTS: Evaluation revealed that animals receiving MPTP had both short- and long-term memory decline. Time of exploration of novel object was lower in the evaluation of STM in MPTP males and females rats ($p < 0.0001$ and $p < 0.0002$, respectively), and in LTM as well ($p < 0.0001$ and $p < 0.0004$, respectively), compared to control. Both doses of E. uniflora reversed this effect at STM in male and female rats. Similarly, both doses of 300 mg/kg and 2000 mg/kg of E. uniflora restored the memory of rats exposed to MPTP. CONCLUSION: No differences were observed in the effects of MPTP or E. Uniflora in relation to the sex of the animals. Thus, it is implied that other important structures, such as the hippocampus, may be involved in these processes, and neurochemical evaluations are essential to clarify possible mechanisms.
Keywords: MPTP, olfactory vectorization, bioprospection / **Supported by:** CAPES, CNPq and FAPERGS

T.02 - Purification of a Trypsin Inhibitor from Libidibia Ferrea Seeds

Luís Antônio De Souza Junior [1], Marielle Paniago Brambilla[1], Luzia Aparecida Pando[2], Silvana Cristina Pando[1]
[1]Coordenação de Curso , Universidade Federal do Mato Grosso do Sul (Mato Grosso do Sul, Brasil), [2]Departamento de Ciências e Tecnologias , Universidade Estadual do Sudoeste da Bahia (Bahia , Brasil)

INTRODUCTION: L. ferrea (Mart. EX. Tul.) L.P. QUEIROZ, belongs to the Fabaceae family, is endemic of Brazil with prevalence in the northeast and southeast regions and its seeds contain proteolytic inhibitors. These molecules can be classified by the type of protease they inhibit or by their mechanism of action. Most studies aim to understand biochemical and physiological features that may be useful for understanding various subcellular mechanisms. OBJECTIVES: This study aimed the purification and partial characterization of a trypsin inhibitor present in L. ferrea seeds and its possible effect on the blood clotting cascade. MATERIALS AND METHODS: The seeds were ground and subjected to saline extraction (0.15M NaCl – 10%, w/v) and precipitation with 80% acetone (v/v). The acetone precipitate was resuspended and applied to a DEAE Sepharose Fast Flow column. Fractions with inhibitory activity were eluted in a 0.15 M saline gradient (0-1M NaCl), pooled, dialyzed, lyophilized and applied to a tryspin Sepharose column. The fractions with inhibitory activity was named LfTI. Antitrypsin activity was monitored using DL-BAPNA as substrate and quantification of total proteins was performed according to the method of Bradford. DISCUSSION AND RESULTS: Preliminary results suggest that LfTI acts on clotting factors by prolonging activated partial thromboplastin time (aPTT). The inhibitor also showed reduction of 90% in the activity of trypsin but did not inhibited chymotrypsin. Furthermore, the inhibitor maintained 85% of its activity up to 60 °C and remained stable over a wide pH range (2-10). SDS-PAGE (12.5%) revealed an inhibitor with apparent molecular mass of 18 kDa with single polypeptide chain after treatment with DTT. CONCLUSION: This study allowed to obtain a plant trypsin inhibitor, its biochemical properties and preliminary evidences of its effect on the intrinsic pathway of the blood clotting cascade, therefore, studies are needed for its use in biotechnological and terapeutic applications.
Keywords: Serine protease, Coagulation, Trypsin

T.03 - Toxicity Evaluation of Bauhinia monandra Fraction Containing Phytochemicals and Leaf Lectin (BmoLL) on Embryos of Biomphalaria glabrata

Thierry Wesley de Albuquerque Aguiar[1], **José Josenildo Batista[1]**, Silvio Assis de Oliveira Ferreira[2], Magda Rhayanny Assunção Ferreira[3], Luiz Alberto Lira Soares[3], Ana Maria Mendonça De Albuquerque Melo[4], Mônica Camelo Pessoa de Azevedo Albuquerque[5], Hallysson Douglas Andrade de Araújo[1], André de Lima Aires[5], Luana Cassandra Breitenbach Barroso Coelho [1]

[1]Departamento de Bioquímica, Universidade Federal de Pernambuco (, Brazil), [2]Centro Acadêmico de Vitória, Universidade Federal de Pernambuco (, Brazil), [3]Departamento de Ciências Farmacêuticas, Universidade Federal de Pernambuco (, Brazil), [4]Departamento de Biofísica e Radiobiologia, Universidade Federal de Pernambuco (Brazil), [5]Departamento de Medicina Tropical, Universidade Federal de Pernambuco (, Brazil)

INTRODUCTION: Biomphalaria glabrata mollusks are the main vector of schistosomiasis in Brazil and plants of the Bauhinia genus are widely found in endemic continents for the disease; leaves of Bauhinia monandra contain a BmoLL lectin with biocidal action. OBJECTIVES: To analyze the fraction (F) from leaves of B. monandra and the embryotoxic effect on B. glabrata. MATERIALS AND METHODS: F was obtained with powder from B. monandra leaves in 10 mM citrate-phosphate buffer, pH 6.5, containing 0.15 M NaCl which was passed through gauze and centrifuged. Ammonium sulfate (60%) was added to precipitate proteins; the material was kept under gentle agitation, centrifuged and lyophilized. Secondary metabolites were investigated by thin-layer chromatography (TLC); quantitative protein, hemagglutinating activity (HA) and specific HA (SHA) were evaluated. B. glabrata embryos at blastula, gastrula, trochophore, veliger and hippo stages (300 of each) were exposed to F for 24 h at different concentrations (12.5-600 µg/mL). Subsequently, the embryos were washed and transferred to plates with filtered and dechlorinated water, analyzed with a magnifying glass for their viable (normal) and unviable forms (malformations and death). DISCUSSION AND RESULTS: TLC detected cinnamic derivatives and flavonoids; protein concentration (2.5 mg/ml) and SHA (809.6) confirmed the lectin presence. In the concentration of 100 µg/mL, F provided 65% and 88% of unviable blastula and gastrula, respectively, while at 200 µg/ml 100% of unviable embryos were observed for both stages. In trochophore 400 and 600 µg/mL of F made 95.7% and 100% of non-viable embryos. At the same F concentrations, the veliger and hippo stages showed unviability of 92.7% and 75.3%, respectively, and 100% of malformations and/or deaths at 600 µg/mL. CONCLUSION: F showed teratogenic/toxic effects in all evolutionary stages of B. glabrata, proving to be an efficient molluscicide.

Keywords: Lectin, Embryotoxicity, Schistosomiasis

T.04 - Evaluation of Kaempferol Against Rattlesnake sPLA2 Edema and Myotoxicity

Airam Roggero [1], Laila Lucyane Ferreira de Moraes[1], Gustavo Antônio Fernandes [1], Caroline Ramos da Cruz Costa[2,1], Mariana Novo Belchor[2,1], Igor Nicodemo De Oliveira[1], Marcos Antônio de Oliveira [1], Marcos Hikari Toyama[1]

[1]Biologia Geral, A Univ. Estadual Paulista "Júlio de Mesquita Filho" (SP, Brasil), [2]Biologia Geral, Univ. Federal do ABC (SP, Brasil)

INTRODUCTION: Kaempferol (KPF) a known antioxidant and anti-inflammatory agent was not able to counteract the Edema and myotoxicity induced by secretory PLA2 from *Crotalus durissus terrificus (Cdt.)* . OBJECTIVES: Our results present that PLA2 from *Cdt.* venom induces increased production of PAF, and the control of Edema induced by this toxin involves the presence of Plasma-platelet activating factor acetylhydrolase (PAF-AH). Studies show that KPF was able to inhibit the enzymatic activity of PAF AH and this would have increased the Edema induced by sPLA2 in animals previously treated with KPF. MATERIALS AND METHODS: Our studies demonstrate the myotoxic activity of sPLA2 from *Cdt.* is besides its action directly on skeletal muscles also stems from the neurotoxic action of rattlesnake sPLA2, the control of myotoxic activity involves the activity of acetylcholinesterase, which is inhibited by the action of KPF and resulted in a massive increase in myotoxicity induced by rattlesnake sPLA2 in animals previously treated with KPF. DISCUSSION AND RESULTS: KPF has antioxidant and biological beneficial properties but is a compound that also plays the role of potential inhibitors of those proteins with alpha-beta hydrolase motif that is found in common with the presence of Ser-His-Asp found in PAF-AH and Acetylcholinesterase but is not found in the active site of sPLA2 or cPLA2. CONCLUSION: Therefore, KPF, despite being an anti-inflammatory and antioxidant agent with proven pharmacological activities, was not able to inhibit the inflammatory and myotoxic activity of *Cdt.* sPLA2. venom, the compound also interacts with Acetylcholinesterase (AChE) and has a potential inhibitor PAF-AH enzymes.

Keywords: Kaempferol, secretory phospholipase A2, Crotalus durissus terrificus / **Supported by:** FAPESP

T.05 - Effect of Crude and Ethereal Senna Occidentalis Extract on Catalase Enzymatic Activity in Cattle Liver
Larissa Tenfen Lehmann[1], Taís Da Silva Rosa[1]
[1]IFMT, Instituto Federal de Educação, Ciência e Tecnologia de Mato Grosso (Mato Grosso, Brasil)

INTRODUCTION: Senna Occidentalis, commonly known as fedegoso is an annual legume, found in the most diverse Brazilian pasture areas. This plant could provide human health benefits, but also, could be toxic to grazing animals. The toxicity is related to a bioactive compound that, exposed to the cell, can cause an intense oxidation and reduction of electrons in cellular metabolism, resulting in the formation of free radicals, chemical reactions that interfere in the organism physiological maintenance. In this sense, cells mobilize defense systems that involve enzymes action, or antioxidant compounds to protect the cell from oxidative damage. One of these compounds is the catalase, a cytoplasmic hemeprotein that, through the catalysis of peroxidation, modify hydrogen peroxide into non-toxic molecules such as water and oxygen. OBJECTIVES: Thus, the study aimed to evaluate the effects of crude and ethereal fedegoso extractVEs on the cytosolic catalase activity in beef cattle liver, focused in the ability and mechanisms to eliminate toxic compounds. MATERIALS AND METHODS: Assays performed to determine the catalase activity were performed according to Zoppi et al. (2003). Crude extracts in distilled water, and ethereal in ethyl ether from leaves, seeds, pods and flowers of fedegoso were used and cattle liver cytosolic fraction were tested by spectroscopy at an absorbance of 240 nm, the absorbance obtained were standardized with a control activity, without extracts. DISCUSSION AND RESULTS: All extracts of fedegoso plants inactivated the enzyme, especially those with ethers, which inhibited 100% of the activity. Leaves showed the greatest capacity for this function, fact that could be explained by the greatest concentration of bioactive compounds presented in these leaves, such as saponin, tannin, flavonoids and anthraquinone. CONCLUSION: The extracts used could inhibit catalase activity, however more studies are needed to understand the correlation between the extraction forms and the enzyme potential of action.
Keywords: Catalase, Senna Occidentalis, Inhibition / **Supported by:** IFMT

T.06 - Inhibitory Activity Of Yeast Encapsulated Orange Essential Oil (YEOO) On Digestive Enzymes Of Aedes aegypti
Juliana Welbert Pereira Winter [1], Bianca Monteiro Henriques Santos[1], Fernando Ariel Genta[1]
[1]Instituto Oswaldo Cruz, Fundação Oswaldo Cruz (Rio de Janeiro, Brasil)

INTRODUCTION: Yeast encapsulated orange essential oil (YEOO) is a potent larvicide against Aedes aegypti. YEOO's mechanism of action is unknown, but it is possible that it interferes on the action of digestive enzymes. OBJECTIVES: Verify the inhibitory capability of YEOO on A. aegypti larvae digestive enzymes. MATERIALS AND METHODS: A. aegypti larvae were exposed to YEOO for 30 or 120 minutes, at 20 mg/L or 30 mg/L. Controls were incubated with yeast cells only or were fasting. Heads, guts and the rest of the bodies were used to each assay, using fluorogenic or spectrophotometric specific substrates. DISCUSSION AND RESULTS: Larvae showed a strong gut β-glucosidase activity, with minor activities in the head and in the rest of the body (substrate Methylumbelliferyl-β-glucoside). Two substrates for lipase were applied. Minor activities against Methylumbelliferyl-butirate (MUC4) were observed in the head and in the rest of the body, and strong activities in the gut. Activities against Methylumbelliferil-heptanoate (MUC7), showed the same general pattern that was observed with MUC4. Strong chitinase activities were observed in the head and in the rest of the body, with significant gut activities. Very high Trypsin activities were detected in the gut, with minor activities in the rest of the body and in the head. Strong β-1,3-glucanase activities were observed in all samples studied. Treatment with YEOO did not result in any significant changes in all the activities above. CONCLUSION: The results suggest that digestive enzymatic activities are not changed after the treatment with YEOO. In this respect, the digestion of the yeast cell wall, a necessary step in the proposed mechanism of action of the larvicide, seems to be normally active during the intoxication of larvae.
Keywords: Aedes aegypti, Enzymes, Orange essential oil

T.07 - *In vitro* Cytotoxicity and Antiproliferative Effects of Snake Venom L-amino acid oxidase (BmooLAAO) in Cancer Prostate Cells

Lorena Morais [1], Lorena Polloni[1], Tássia Costa[1], Thales Alves de Melo Fernandes[1], Renata Santos Rodrigues[1], Veridiana de Melo Rodrigues Avila[2]
[1]Instituto de Ciências Biomédicas, Universidade Federal de Uberlândia (Minas Gerais, Brasil), [2]Instituto de Genética e Bioquímica, Universidade Federal de Uberlândia (Minas Gerais, Brasil)

INTRODUCTION: Cancer cells are characterized by enhanced reactive oxygen species (ROS) generation and accumulation that is crucial during tumorigenesis. In consequence, cancer cells upregulate their antioxidant capacity to maintain ROS levels below the toxicity threshold. In this scenario, oxidative stress induced by exogenous ROS-generating agent presents an interesting therapeutic strategy once cancer cells have their antioxidant system overload, while non-cancerous cells can still maintain their antioxidant capacities. Snake venom L-amino acid oxidases (svLAAOs) are well known to generate hydrogen peroxide by catalyzing the oxidative deamination of amino acids. OBJECTIVES: In this study, we aimed to understand the oxidative effects induced by a snake venom L-amino acid oxidase from Bothrops moojeni (BmooLAAO) in non-tumorigenic (PNT2) and tumorigenic (PC3) prostate cells lines. MATERIALS AND METHODS: To further investigate the oxidative stress we performed MTT and proliferation assays. The proliferation assay was made with two approaches: (i) cell treatment with BmooLAAO (ranging from 3.125 to 0.04 µg/mL) and (ii) co-treatment with BmooLAAO and catalase for 24, 48 and 72 h. DISCUSSION AND RESULTS: We observed that BmooLAAO showed a selective cytotoxicity towards PC3 cells when compared to PNT2 cells for MTT assay. BmooLAAO significant inhibited PC3 cells proliferation time-dependent when compared to PNT2 cells. Assays performed with catalase abolished BmooLAAO effects in PC3 and PNT2 cells. CONCLUSION: These results suggest that ROS play a crucial role in tumor progression since BmooLAAO caused more damage for PC3 cells.
Keywords: ROS, oxidative stress, LAAO / **Supported by:** FAPEMIG, Capes, CNPq

T.08 - Chrysin Restores the Memory Deficit Induced by the Hypothyroidism in Mice: Assessment of Different Types of Memory

Vandreza Cardoso Bortolotto [1,2], Stífani Araujo[1], Mustafa Dahleh[1], Cristini Viana[1], Franciane Pinheiro[1], Franciéle Balok[1], Luana Meichtry[1], Cristina Nogueira[2], Marina Prigol[1]
[1]Department of Nutrition , Federal University of Pampa (RS, Brasil), [2]Department of Biochemistry and Molecular Biology, Federal University of Santa Maria (RS, Brasil)

INTRODUCTION: Hypothyroidism is a disease associated with neuropsychiatric disorders and closely related to Alzheimer's disease, similarly, causing memory deficit, thereby, the study of new substances with the potential to improve memory deficits becomes important. OBJECTIVES: We investigated if flavonoid chrysin improves declarative, aversive and working memory deficits induced by hypothyroidism in female mice. MATERIALS AND METHODS: Hypothyroidism was induced by continuous exposure to 0.1% methimazole (MTZ) in drinking water for 31 days. Exposure to MTZ was associated with low plasma levels of thyroid hormones T3 and T4 compared with the control group. Thereafter, euthyroid and MTZ-induced hypothyroid mice were intragastrically administered vehicle or chrysin (20 mg/kg), once a day for 28 consecutive days. After treatments, were performed the open field test (OFT) and three different learning tasks: habituation in an open field box for object recognition, inhibitory avoidance and Y-maze (declarative, aversive and working memories, respectively). DISCUSSION AND RESULTS: In the OFT, there was no significant difference, in this way, we can validate all the memory tests used in this work. Hypothyroid mice showed memory deficit both short- (STM) and long-term memory (LTM) of the recognition task, the LTM in inhibitory avoidance and in Y-maze, the chrysin treatment reversed the memory deficit in all types of memory. These findings demonstrate a comprehensive effect of chrysin on different types of memory, being able to exert a beneficial effect on declarative, aversive and working memory deficits caused by hypothyroidism in female mice. CONCLUSION: Our findings indicate that chrysin was able to reverse the behavioral changes associated with memory deficit induced by hypothyroidism. Proper experiments are in course to understand how chrysin act to improve the deficit of these memories.
Keywords: Flavonoid, Memory loss, Memory improvement
Supported by: FAPERGS, CNPq and CAPES

T.09 - Effect of D-limonene and Hydroxypropyl-β-cyclodextrin Inclusion Complex on Doxorubicin-Induced Cardiotoxicity in mice

Aimee Obolari Durco [1], Diego Souza[2], Samuel Beserra[2], Michael Conceição[2], Paula Araujo[3,4], Alexandre Costa[4], Jader Cruz[4], Danilo Campos[2], Adriano Araujo[1], Bruno Lima[1], Andre Barreto[6], Aldeidia Oliveira[7], Marcio Santos[1]
[1]Pós-Graduação em Ciências da Saúde, Universidade Federal de Sergipe (Sergipe, Brasil), [2]Departamento de Biofísica, Universidade Federal de São Paulo (São Paulo, Brasil), [3]Department of Physiology and Membrane Biology, University of California Davis (California, Estados Unidos), [4]Departamento de Bioquímica e Imunologia, Universidade Federal de Minas Gerais (Minas Gerais, Brasil), [5]Departamento de Farmácia; [6]Departamento de Educação em Saúde, [7]Departamento de Biofísica e Fisiologia, [8]Departamento de Fisiologia, Universidade Federal de Sergipe (Sergipe, Brasil)

INTRODUCTION: Anthracyclines-induced cardiotoxicity is a common problem in cancer treatment, causing both mechanical and electrocardiographic cardiac changes. The monoterpene D-limonene (DL) has been successfully tested as a cardioprotective agent in animal models; however, it is volatile and has low water solubility, which may limit its clinical use. In this context, complex DL with hydroxypropyl-β-cyclodextrin (HPβCD) could improve its properties. OBJECTIVES: This work aimed to investigate the effect of DL and its complex (DL-HPβCD) on anthracycline-induced cardiotoxicity in mice. MATERIALS AND METHODS: Animals were divided into groups and treated or with DL (10 mg/kg), or DL-HPβCD (10, 30, and 100 mg/kg), or HPβCD (100 mg/kg), or saline solution (0.9%, 10 ml/kg); cardiotoxicity was induced with doxorubicin (DOXO) (20 mg/kg). For assessment of cardiac injury, CK-MB was measured in the blood serum of all groups. However, for other evaluations, such as electrocardiography, patch-clamp technique, and fluorescence experiments (DHE, Mito SOX, and MitoTracker), were used just DL-HPβCD 10 mg/kg. DISCUSSION AND RESULTS: CK-MB was significantly higher in DOXO than in the control group, and that was reverted just by DL-HPβCD in all doses. Electrocardiographic parameters such as QRS complex, QTc, and BPM were increased in DOXO and reverted by DL-HPβCD (10 mg/kg). Arrhythmia in action potential (AP), AP duration, calcium transient amplitude, reactive species in the cytoplasm and mitochondria, as well mitochondrial mass, were increased in the DOXO group and reduced by DL-HPβCD. CONCLUSION: So far, the results have validated the cardiac injury study design and show a promising potential cardioprotective effect of DL-HPβCD. Considering that cardiotoxicity is well related to oxidative stress, further investigations will be carried out to assess lipoperoxidation, carbonylation, and sulfhydrylation of proteins; protein expression of antioxidant pathways, and pro and anti-apoptotic signaling.
Keywords: d-limonene, cardiotoxicity, cyclodextrin / **Supported by:** CNPq (305345/2019-2) and CAPES

T.10 - In Silico And *In vitro* Evaluation Of 3-O-Methylquercetin inhibitory Potential Against Secretory Phospholipase A2 From Crotalus durissus terrificus Venom

Mariana Novo Belchor [1], Caroline Ramos da Cruz Costa[1,2], Airam Roggero[1], Laila Lucyane Ferreira de Moraes[1], Agatha S. Manzi[1], Bruno Paes de Carli[1], Isabelly Annunciato[1], Marcos Antonio de Oliveira[1], Marcos Hikari Toyama[1,2]
[1]Biosciences Institute, São Paulo State University (SP, Brazil), [2]Biotechnosciences, Federal University of ABC (SP, Brazil)

INTRODUCTION: 3-O-methylquercetin (3MQ) is a flavonoid found in several plants revealing anti-inflammatory properties. Secretory phospholipase A2 (sPLA2) is an essential enzyme in the inflammatory process, and it is widely found in diverse animals, such as mammals and snake venoms. 3MQ has already demonstrated a weak enzyme inhibition against sPLA2 from Bothrops jararacussu (Bj), when compared with Quercetin, Rhamnetin and Rhamnazin, hence, the initial hypothesis was that these compounds would similarly inhibit sPLA2 from Crotalus durissus terrificus (Cdt). OBJECTIVES: This work aimed to evaluate the quercetin derivatives inhibitory potential against sPLA2 from Cdt. MATERIALS AND METHODS: 4-Nitro-3-(octanoyloxy)benzoic acid was used as a substrate to analyze enzymatic activity and the compounds inhibitory potential. To reveal enzyme structural modifications, High Performance Liquid Chromatography (HPLC), using a reverse phase column xb-c18 phenomenex, with a linear gradient of Buffer A (trifluoracetic acid (0,1%)) and Buffer B (66% Acetonitrile in 0.1% TFA) was made. In silico tools, such as AutoDockTools-1.5.6, were used to generate compounds interactions with PLA2. DISCUSSION AND RESULTS: Enzymatic assay shows that only 3MQ (0,5 mg/mL) was capable to inhibit sPLA2 (1mg/mL), a higher concentration than that used against sPLA2 from Bj venom1. Although there are similarities between both sPLA2, Cdt sPLA2 assumes a tetrameric form, and Bj sPLA2 tends to exhibit a dimeric form, which would explain the need of more compound concentration. HPLC results reveal 3MQ diminished the protein affinity with the column, since it was observed a decrease in the retention time. In silico assays show great interactions between 3MQ and the enzyme catalytic site: ASP49, TYR52, TRP31 and calcium CONCLUSION: 3MQ was the unique compound that exhibits a dose-dependent activity against Cdt sPLA2. Future studies concerning compounds structure-function and the role of methylations on quercetins are essential to elucidate interaction mechanism of these compounds with Cdt sPLA2.
Keywords: Inflammation, Natural compounds, Snake Venom / **Supported by:** UFABC

T.11 - Effect of Ylang-ylang (Cananga Odorata) essential oil on leukocyte recruitment in an experimental arthritis model

Paloma Kênia de Moraes Berenguel Lossavaro [1], Iluska Senna Bonfá [1], Joyce dos Santos Lencina [1], Monica Cristina Toffoli Kadri[1], Saulo Euclides Silva Filho[1]
[1] pharmacology, Pharmaceutical Sciences, Food and Nutrition College, Federal University of Mato Grosso do Sul, Campo Grande, MS, Brazil (Mato Grosso do Sul, Brasil)

INTRODUCTION: The use of anti-inflammatory drugs becomes limited in therapy due to the adverse effects. The search for natural products with anti-inflammatory activity and lower incidence of these effects becomes relevant. Several biological activities have been described for Ylang-ylang essential oil (YEO). However, studies on the effect of YEO on the inflammatory response are still limited. OBJECTIVES: To evaluate the effect of YEO on leukocyte recruitment in mice submitted to experimental zymosan-induced arthritis. MATERIALS AND METHODS: Swiss male mice were treated with YEO (50, 100 and 200 mg/Kg, v.o.); dexamethasone (1mg/kg, v.o., reference drug) or vehicle (saline solution and 1% Tween, v.o.) (n=5 animals/group). One hour after pre-treatment, 200 µg of zymosan diluted in 10 µL of sterile saline were injected into the joint cavity of the right knee of the mice, in the left knee of the mice only saline was injected. Seven days after Zy induction, leukocyte influx into the joint cavity was evaluated, which was exposed by surgical incision and washed with phosphate-buffered saline (pH 7.2), containing (PBS/EDTA). After, joint exudate was collected. From this cell suspension, aliquots were used to determine total leukocytes. For the total leukocyte count, 10 µl of exudate were removed (50 µl of wash plus 90 µl of Turk's liquid), and the count was performed in a Neubauer chamber, by light microscopy, and the results were expressed as the total number of leukocytes per joint cavity. DISCUSSION AND RESULTS: After seven days of i.a. de Zy, an increase in the influx of total cells (44.75 ± 6.32 cel/mm ^{3}) into the joint cavity of mice was noted. Pre-treatment with dexamethasone (reference drug) inhibited cell migration by 70.95% and animals' pre-treatment with OEY of 100 and 200MG/Kg showed a reduction of total cells by 59.21% and 63 .68%, respectively. CONCLUSION: YEO has anti-inflammatory activity, reducing leukocyte migration in the mouse knee cavity.

Keywords: inflammation, essential oil, experimental arthrits. / **Supported by:** CAPES e CNPq

T.12 - *In vitro* Antiangiogenic Effects of BmooLAAO: A Snake Venom L-amino acid oxidase from Bothrops moojeni

Lorena Polloni [1], Tássia Rafaella Costa[1], Lorena Pinheiro Morais[2], Lucas Ian Veloso Correia[1], Samuel Cota Teixeira[2], Thales Alves de Melo Fernandes[2], Bruna Cristina Borges[2], Marcelo José Barbosa Silva[2], Eloisa Amália Vieira Ferro[2], Daiana Silva Lopes[3], Veridiana de Melo Rodrigues Ávila[1]
[1]Instituto de Biotecnologia, Universidade Federal de Uberlândia (MG, Brazil), [2]Instituto de Ciências Biomédicas, Universidade Federal de Uberlândia (MG, Brasil), [3]Departamento de Bioquímica e Biofísica, Univ. Federal da Bahia (Bahia, Brasil)

INTRODUCTION: Reactive oxygen species (ROS) are considered a key role in cancer hallmarks like tumor angiogenesis. Angiogenesis is the major mechanism of tumor vascularization, which is crucial for solid tumors to growth and metastasize. Thus, tumor angiogenesis has been considered a promising target for therapeutic interventions. In this context, exploiting ROS-pathways regulation may create possibilities to develop more effective antiangiogenic drugs, since high ROS levels are cytotoxic. Snake venom can present enzymes like L-amino acid oxidases (LAAOs), which are able to catalyze a stereospecific oxidative deamination of L-amino acids producing α-keto acids, ammonia and hydrogen peroxide. Several studies have demonstrated the antimicrobial and antitumor effects induced by LAAOs from snake venoms, however little is known about its antiangiogenic potential. OBJECTIVES: In this scenario, the present study investigated the oxidative stress effects in tumor angiogenesis induced by a snake venom L-amino acid oxidase from Bothrops moojeni (BmooLAAO). MATERIALS AND METHODS: To evaluate the antiangiogenic activity of BmooLAAO in human umbilical vein endothelial cells (HUVECs), we performed MTT, adhesion in different substrates, migration, invasion and co-culture assays in the presence of BmooLAAO (0.09, 0.19 and 0.39 µg/mL) or in presence of BmooLAAO and catalase simultaneously. DISCUSSION AND RESULTS: BmooLAAO treatment of HUVECs inhibited critical steps of tumor angiogenesis such as proliferation, adhesion (on collagen IV, fibronectin and matrigel), migration, invasion and tubulogenesis. Catalase abolished BmooLAAO antiangiogenic effects. In the co-culture assay, BmooLAAO decreased tube formation. CONCLUSION: These results add more complexity to the role of oxidative stress during tumor progression since BmooLAAO demonstrated antiangiogenic effects in absence of catalase treatment.

Keywords: angiogenesis, oxidative stress, LAAO / **Supported by:** FAPEMIG, CNPq and CAPES

T.13 - Purification and Partial Characterization of Trypsin Inhibitor Isolated from Inga Capitata Desv. Seeds and Investigation of Anticoagulant Activity.

Vinicius Pereira da Silva Xavier [1], Luis Antonio de souza Junior[1], Luzia Aparecida Pando[2], Luiz Gustavo Bernardes[1], Silvana Cristina Pando[1]
[1]Coordenação de Curso, Universidade Federal do Mato Grosso do Sul (Mato Grosso do Sul, Brasil), [2]Depto de Ciências e Tecnologias, Universidade Estadual do Sudoeste da Bahia (Bahia, Brasil)

INTRODUCTION: Plant protease inhibitors are proteins that are widely distributed in nature and inhibit the activity of different proteolytic enzymes such as serine, cysteine, aspartic and metalloproteases. OBJECTIVES: The objective of this work was to isolate and characterize the inhibitor present in I. capitata seeds and investigate the anticoagulant activity. MATERIALS AND METHODS: Saline extraction 0.15M 10% (v/v), followed by precipitation with 80% (v/v) acetone, purification by ion exchange chromatography and gel electrophoresis (SDS-PAGE) were used to obtain the desired inhibitor. After these steps, thermal and pH stability assay were performed, as well as tests for anticoagulant activity, consisting of prothrombin time (PT) and activated partial thromboplastin time (aPTT). DISCUSSION AND RESULTS: The inhibitor (4.3 µg) reduced 99% of bovine trypsin activity and presented a single polypeptide chain with an apparent molecular mass of 20 kDa in the absence and presence of DTT. It was thermostable up to 70ºC and resistant to pH variations (2-10) and prolonged aPTT by approximately three times compared to the control, but had no effect on PT. CONCLUSION: The preliminary results of this study point to a new plant inhibitor with a potential effect on serine protease of the blood clotting cascade.
Keywords: Inhibitors , Serine protease, Inga capitata

T.14 - Coumarins antiviral candidates for the hydrophobic pocket at the nucleoprotein/phosphoprotein interaction site of the human respiratory syncytial virus

Jéssica Maróstica de Sá [1], Eduardo Maffud Cilli[2], Marcelo Andres Fossey[1], Fabio Ceneviva Lacerda Almeida[3], Fatima Pereira de Souza[1], Ícaro Putinhon Caruso[1]
[1]Física, Universidade Estadual Paulista "Júlio de Mesquita Filho" (São Paulo, Brasil), [2]Química, Instituto de Química (São Paulo, Brasil), [3]Centro de Estrutura Biologica e bioimagem, Centro Nacional de Ressonância magnética Nuclear de moléculas (Rio de Janeiro, Brasil)

INTRODUCTION: The human Respiratory Syncytial Virus (hRSV) is the causative agent of acute respiratory disease in children and the elderly, such as bronchiolitis and pneumonia. Currently, there is no vaccine against the virus. Within the proteins encoded by the virus, N-nucleoprotein excels in the protection of viral RNA by providing nucleocapsid (NC) formation and its role in viral replication and transcription. Recognition of NC by viral polymerase is mediated by P-phosphoprotein due to the interaction of C-terminal residues (especially the latter, Phe241) with N-terminal N protein (N-NTD). OBJECTIVES: The present study aims to characterize the interaction of N-NTD with coumarins (coumarin (1,2 Benzopyrone), esculetin (6,7- Dihydroxycoumarin), esculin(6,7- Dihydroxycoumarin- 6- glucoside) and 4-methyl esculetin (5,7- Dihydroxycoumarin- 4- methylcoumarin)) MATERIALS AND METHODS: by NMR saturation transfer difference (STD) and 2D 1H-15N HSQC-NMR, and to use fluorescence anisotropy analysis and STD to verify the C-terminal competition of the P protein (peptide-Ppep11) with the coumarins for the hydrophobic hotspot of N-NTD. DISCUSSION AND RESULTS: STD data revealed that all coumarins interact with N-NTD. Fluorescence anisotropy and STD analyzes showed that Ppep competes with coumarin (1,2 Benzopyrone) for the hydrophobic hotspot of N-NTD. The HSQC analyzes indicated that chemical displacements of some residues present in the hydrophobic pocket of N-NTD occurred in the presence of coumarin. The coumarin epitope map suggests that its A benzene ring is most buried at the N-NTD binding site. CONCLUSION: The results suggest that coumarin binds specifically to the nucleoprotein interaction site, while esculetin, esculin and 4-methyl esculetin bind near or together with Ppep11 in the nucleoprotein pocket.
Keywords: Coumarins, hRSV, Molecular biophysics / **Supported by:** FAPESP 2020/13582-1

T.15 - Evaluation of Antitumor Effects of Bougainvillea glabra Choisy Extract in Caenorhabditis elegans

Maria Eduarda Oliveira de Souza [1], Flávia Suelen de Oliveira Pereira[1], Aline Castro Caurio[2], Elton Luis Gasparotto Denardin[2], Daiana Silva de Ávila[1]
[1]Programa de pós-graduação em bioquímica - GBToxCe, Universidade Federal do Pampa (Rio Grande do Sul, Brasil), [2]Programa de pós-graduação em bioquímica - LEFQPN, Universidade Federal do Pampa (Rio Grande do Sul, Brasil)

INTRODUCTION: The use of plant-derived compounds as anticancer drugs is already well established, however new compounds are still being investigated in order to reduce the aggressive cancer adverse effects. *In vitro* studies have demonstrated the antitumor effects of *Bougainvillea glabra Choisy* extract (BGCE). Therefore, for initial research *In vivo* , it is necessary the use of alternative models, and *Caenorhabditis elegans* has been standing out. OBJECTIVES: In this work we aim to evaluate the antiproliferative effect and the mechanism of action of the BGC extract in the *let-60* gain-of-function mutant in *C. elegans*. MATERIALS AND METHODS: The worm strains used were: MT4244 (unc-24(e138); let-60(n1046) IV), CL2166 (dvIs19 [(pAF15)gst-4p::GFP ::NLS] III), TJ356 zIs356 [daf-16p::daf-16a/b::GFP + rol-6(su1006)] and MD701 bcIs39 [lim-7p::ced-1::GFP + lin-15(+)]. At L1 stage larvae were exposed to concentrations of 10, 50 and 75 µg/mL of chlorogenic acid equivalents (CAE/mL), being solubilized in deionized distilled water, vehicle to which the control group was exposed. The larvae were placed in the nematode growth medium (NGM) with *Escherichia coli OP50*, and the parameters evaluated after 48h, at the L4 stage. DISCUSSION AND RESULTS: The concentration of 50 µg/mL showed a decrease in both the formation of the phenotype and the area of the multivulva, consequently increasing the lifespan of the worm treated in this same group. Its action may be due to the increase in the translocation of the DAF-16 transcription factor to the cell nucleus, thus modulating the expression of the enzyme glutathione-S-transferase at a concentration of 50 µg/mL. The extract also showed to act in the apoptotic cascade, with a significant increase of apoptotic corpses of the germline cells (75 µg/mL). CONCLUSION: This study demonstrates for the first time in an *In vivo* model the promising antitumor action of BGCE, being essential data for future research with the extract.

Keywords: alternative model, Bougainvillea glabra, let-60 / **Supported by:** CAPES e CNPQ

T.16 - Isolation and Partial Characterization of Albizia inundata Seed Lectins (MART) Barneby & J. W. Grimes

Erika Janaina Rodrigues Barbosa DeSa [1], Wagner Pereira Felix[1]
[1]Laboratório de Bioquímica (CCA), Universidade Federal do Vale do São Francisco (Pernambuco, Brasil)

INTRODUCTION: Primary metabolites such as lectins are widely found in nature. In plants they are isolated and purified in the most diverse parts of the plants including the seeds. These metabolites have wide applicability, such as indetification agents of carbohydrates, tumor cells, antibacterial agents, bactericides, antifungals among other applications. *Albizi inundata* is popularly known as "canafístula" or "biguazeiro" endemic to the Caatinga and has potential in its biological activities. OBJECTIVES: Isolation, purification and characterize partial lectins in *A. inundata* seeds. MATERIALS AND METHODS: Lectins extractions were performed with 100 mM phosphate buffer pH 6.6, which were purified by ion exchange chromatography on a DEAE−Sephadex A−50 column, followed by gel filtration on a Sephadex G−75 column and their homogeneities were evaluated by SDS−PAGE. DISCUSSION AND RESULTS: Lectins were isolated and called AlbiH−B and AlbiH−M.They presented a protein band with apparent molecular mass of 55 kDa for AlbiH−B and 37 kDa for AlbiH−M. Both lectins demonstrated hemagglutinating activity in glutarized rabbit blood. AlbiH−B lost its thermal stability after incubation at 80 °C for 1 h and AlbiH−M after 60 °C. They are resistant over a wide pH range from 2 to 10. AlbiH−B lectin did not inhibit the bacteria tested. CONCLUSION: The information obtained is of great importance for the know of typical species of the Caatinga biome, through which it was possible to isolate, for the first time, two lectins from the seeds of *A. inundata*.

Keywords: Albizia inundata, Lectins, Partial characterization
Supported by: UNIVASF

T.17 - Development of bioadhesive oral films incorporated with lectins with antifungal properties

José Manuel Noguera Bazán[1], Vitor Lopes Chagas[1], Karla Lilian Rodrigues Batista[1], Gustavo Henrique Rodrigues Vale de Macedo[1], Adrielle Zagmignan[1], Claudener Souza Teixeira[2], Edilausson Moreno Carvalho[3], **Luís Cláudio Nascimento da Silva [1]**

[1]Biologia Microbiana, Universidade CEUMA (Maranhão, Brazil), [2]Centro de Ciências Agrárias e da Biodiversidade, Universidade Federal do Cariri (Ceará, Brazil), [3]Odontologia, Universidade CEUMA (Maranhão, Brazil)

INTRODUCTION: The emergence of *Candida* strains with resistance towards antifungal drugs have driven the search for new therapeutic alternatives. Some lectins have been reported as anti-Candida agent, such as ConBr (from *Canavalia brasiliensis*) and MaL (from *Machaerium acutifolium*). Furthermore, the immunomodulatory and antinoceptive properties described for ConBr and MaL make these proteins promising alternatives in the management of candidiasis. Thus, the development of vehicles for drug delivery in the oral cavity, such as oromucosal films, is important. OBJECTIVES: This study aimed to develop and characterize sodium alginate-derived bioadhesive films containing lectins with antifungal activities. MATERIALS AND METHODS: In preventive tests, lectins (128 µg/kg) were inoculated two hours before *C. albicans* CA40 infection (1×10^4 yeast cells/larva). In treatment assays, the infection was established two hours before lectin administration. Following, bioadhesive films were developed using sodium alginate and each lectin (50 µg/mL of filmogenic solution). The prepared films were evaluated for physical-chemical and mechanical characteristics (uniformity of weight and content, film thickness, roughness, color, pH, swelling index). DISCUSSION AND RESULTS: Larvae infected with CA40 had a survival rate of 1.5 days and a survival rate of 40%. Lectins showed prophylactic effects at the concentrations tested, with survival rates between 80% and 90%. However, in the treatment assays only the MaL lectin significantly increased larval survival (70% survival rate). The alginate films containing the lectins presented parameters compatible with their application in the oral cavity. In general, the addition of lectins did not induce significant changes in the physicochemical properties and conformation of the alginate film. CONCLUSION: The data obtained in this work demonstrate, for the first time, the anti-infective effect of the ConBr and MaL lectins in an *In vivo* model of candidiasis. The lectins-based bioadhesive films presented physical-chemical and mechanical characteristics suitable for application in the oral cavity.

Keywords: Oral candidiasis, bioactive products, polymers

Supported by: FAPEMA, CNPq

T.18 - Green Tea and Purple Tea Differ Largely in their Inhibitory Potential Against Fat Digestion

Tamires Barlati Vieira da Silva[1], Adelar Bracht[1], **Rosane Marina Peralta [1]**

[1]Bioquímica, Universidade Estadual de Maringá (PR, Brasil)

INTRODUCTION: One of the most common treatments for obesity involves the prescription of inhibitors of pancreatic lipases, which reduce hydrolysis of triglycerides with a consequent reduction in fatty acids availability for absorption by intestinal cells. Purple tea is a rare variety of tea grown in Kenya. It is made from a new mestizo variety of the common tea leaf, *Camellia sinensis* (green tea). Due to the high content in anthocyanins of its leaves, the beverage obtained from this variety has a purplish color, what originated the name purple tea. Although green tea is considered a pancreatic lipase inhibitor, no study has been carried out to evaluate the potential lipase inhibitory action by purple tea. OBJECTIVES: To compare the lipase inhibitory lipase potentials of green tea and purple tea as well as their inhibitory potentials against fat digestion. MATERIALS AND METHODS: Aqueous extracts mimicking the usual consumption of teas were prepared. Bioactive contents were determined by chromatography and mass spectrometry. Enzyme activity measurements were complemented by *In vivo* assessment of triglyceride absorption in mice. DISCUSSION AND RESULTS: Both extracts are rich in catechin derivatives. Only the purple tea contains significant amounts of anthocyanins, especially O-glycosylated derivatives of cyanidin and peonidin. With reference to the *In vitro* inhibition of the pancreatic lipase, an IC50 value (concentration of purple tea extract causing 50% inhibition of the enzyme) of 100 µg/mL and >500 µg/mL were found for purple tea and green tea, respectively. This inhibition reflected on triglyceride digestion *In vivo* , an activity that was strongly inhibited solely by the purple tea extract. CONCLUSION: The data obtained suggest that the purple tea extract, thanks to its inhibitory activities on digestive enzymes, may be an adjunct or even to serve as a basis for a future drug useful in the control of obesity.

Keywords: Camellia sinensis, lipase inhibition, obesity

Supported by: CNPq, CAPES, Fundação Araucária

T.19 - Evaluation of Polyphenolic Compounds and Kunitz-Type Inhibitor on the Enzymatic and Pharmacological Activity of Serine Protease from Crotalus durissus terrificus.

Caroline Ramos da Cruz Costa[1], Mariana Novo Belchor[1], Airam Roggero[1], Laila Lucyane Ferreira de Moraes[1], Camila Ramalho Bonturi[2], Maria Luiza Vilela Oliva[2], Marcos Antonio de Oliveira[1], Marcos Hikari Toyama[1]
[1]Biociências, Universidade Estadual Paulista (SP, Brasil), [2]Bioquímica, Escola Paulista de Medicina (SP, Brasil)

INTRODUCTION: Serine protease from snake venom (SVSP) interferes with the regulation and control of important biological reactions in homeostasis and can be classified as an activator of the fibrinolytic system, and platelet aggregation. Our group has recently isolated a new serine protease from Crotalus durissus terrificus total venom (Cdtsp-2). This protein exhibits edematogenic capacity and myotoxic activity. Two glycosylated flavonoids (quercetin-3-O-arabinoside (Qa) and quercetin-3-O-ramnoside (Qn)) isolated from Laguncularia racemosa thrombin inhibition capacity. The Kunitz-like inhibitor protein EcTI with a molecular mass of 20 kDa was isolated from Enterolobium contortisiliquum, and showed high trypsin inhibition. OBJECTIVES: To verify the possible inhibition of the pharmacological activities of Cdtsp-2 by the flavonoids (Qa) and (Qn), and the Kutinz-type inhibitor EcTi. MATERIALS AND METHODS: For the isolation of Cdtsp-2 from total C. d. terrificus venom we used HPLC in 3 chromatographic steps following each one by enzymatic assays with BAPNA. The first step was with a Superdex 75 column with a mobile phase of ammonium bicarbonate pH 8, the second was on an ion exchange column in DEAE and the third with a reverse phase column to confirm the degree of molecular homology. Using the Swiss rat paw edema model we observed an edematogenic effect and myotoxicity through increased creatine kinase (CK) levels caused by the new serine protease. DISCUSSION AND RESULTS: The glycosylated flavonoids (Qa and Qn) responded to Cdtsp-2 inhibition, with Qn being more effective in such inhibition, however, *In vitro* and *In vivo* experiments show that EcTI inhibits significantly better the enzymatic and pharmacological activities of Cdtsp-2 than Qn. CONCLUSION: EcTi shows to be a molecule of great potential for future studies and may help to counter the effects of poisoning that are not fully countered by serum therapy.

Keywords: Serine Protease Cdtsp-2, Kunitz-type inhibitor, Crotalus durissus
Supported by: Programa de Pós graduação Biotecnociências - UFABC

T.20 - Embryotoxic and Molluscicidal Effect of the Extract and Seed Fraction of Parkia pendula on the snail Biomphalaria glabrata (Say 1818)

José Josenildo Batista [1], Hallysson Douglas Andrade de Araújo[1], Thierry Wesley de Albuquerque Aguiar[1], Dewson Rocha Pereira[2], Maíra de Vasconcelos Lima[2], Ana Maria Mendonça de Albuquerque Melo[2], Mônica Camelo Pessoa de Azevedo Albuquerque[3], André de Lima Aires[3], Luana Cassandra Breitenbach Barroso Coelho[1]
[1]Depto de Bioquímica e Fisiologia, Univ. Federal de Pernambuco (PE, Brasil), [2]Depto de Biofísica e Radiobiologia, Univ. Federal de Pernambuco (PE, Brasil), [3]Laboratório de Imunopatologia Keizo Asami, LIKA, Univ. Federal de Pernambuco (PE, Brasil)

INTRODUCTION: Introduction: The only molluscicide available for the control of schistosomiasis causing snails, niclosamide, has shown low selectivity, which leads to the search for new alternatives. OBJECTIVES: Objective: To evaluate the toxicity of the extract (E) and fraction (F) of Parkia pendula (seeds) in developmental stages of Biomphalaria glabrata. MATERIALS AND METHODS: Materials and methods: The E obtained with 0.15 M NaCl and the F with 0-60% ammonium sulfate were quantified by the presence of total sugars (S), proteins (P) and hemagglutinating activity (HA). Embryos (n=100) in the blastula, gastrula, trochophora, veliger, hippo stage and adult snail (n=10) stages were exposed (24 h) to E and F at different concentrations. Then, washed (filtered/dechlorinated water) and the embryos analyzed and classified as viable (normal) and non-viable (malformed-dead) and the snails analyzed for reproductive parameters (fertility/fecundity) and survival, as well as cytotoxicity and genotoxicity (comet) in hemocytic cells. DISCUSSION AND RESULTS: Results: E showed 0.86 mg/mL S, 5.1 mg/mL P, HA of 128 and specific HA (SHA) of 116.36, while F, 0.91 mg/mL P, HA 128 and SHA 140.65. E and F showed alterations such as hydropic embryos, shell malformation and developmental delay with an inviability rate ranging from 11% to 100%. Adult snails (24 h/exposure) had mortality of 10.0%, 26.0%, 33.0%, 36.0%, 50.0% (E) and 0%, 3.0%, 10.0%, 13.0%, 20.0% (F) at concentrations of 1.0, 2.0, 3.0, 4.0 and 5.0 mg/mL and after seven days, a decrease in fecundity (35% to 96%) and increased mortality (20% to 100%). In cytotoxicity, morphological changes such as micronucleus, binuclear and apoptotic cells, as well as genotoxic action on DNA by the comet assay (levels 1, 2 and 3) were found.⌈ CONCLUSION: Conclusion: E and F are promising molluscicides in the control of B. glabrata, with changes in embryonic stages, decrease in eggs and defense cells, causing changes in the physiology of these animals.

Keywords: Parkia pendula, Preparations, Schistosomiasis / **Supported by:** Fundação de Amparo a Ciência e Tecnologia do Estado de Pernambuco (FACEPE); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

T.21 - The effectiveness and safety of a natural product inducing cell death of malignant melanoma cells

Camila Ramalho Bonturi [1], Juliana Rodrigues Oliveira[1], Rita de Cassia Sinigaglia[2], Tiago Rodrigues[3], Maria Luiza Vilela Oliva[1]

[1]BIOCHEMISTRY, Universidade Federal de São Paulo (São Paulo, Brazil), [2]Electron Microscopy Center, , Universidade Federal de São Paulo (São Paulo, Brazil), [3]Center for Natural and Human Sciences, Universidade Federal do ABC (SP, Brazil)

INTRODUCTION: Malignant melanoma is a highly aggressive tumor that typically arises from melanocytic nevus or by the transformation of melanocyte cells. Patients with an early diagnosis are more prone to be cured, however, the cells have the potential to invade adjacent organs within a short period. Current therapy includes multi-strategy, which will depend on melanoma stage, body's location, and patient's health. Nonetheless, these treatments face numerous challenges. In this view, we suggest a plant-derived natural therapy that could reduce side effects and show a promising compound with anti-cancer properties, *Enterolobium contortisiliquum* Trypsin Inhibitor (EcTI). OBJECTIVES: To this purpose, the study was developed to provide new insight into melanoma treatment, since plant proteins are being increasingly used in diverse cancer therapy with success. MATERIALS AND METHODS: The effects of EcTI were evaluated on proliferation, migration, invasion and the mechanisms that culminate in apoptosis were accompanied by caspase-3/7 activation, through a western blotting analysis and electron microscopy. DISCUSSION AND RESULTS: EcTI showed no effect on healthy cells, although inhibited viability, proliferation, migration, and invasion of melanoma cancer cells (SK-MEL-28 and CHL-1). The inhibitor was shown to induce caspase-3/7 activation and proteins related to survival, apoptosis, and autophagy, confirmed by electronic microscopy CONCLUSION: Taken together, these results contribute to new insights into the cancer field, demonstrating EcTI as a promising inhibitor of melanoma cell growth.

Keywords: melanoma, natural products, protease inhibitors / **Supported by:** 2019/22243-9

T.22 - Chemical characterization, ADMET properties, leishmanicidal and anti-inflammatory potential of essential oil from Plinia cauliflora (Mart.) Kausel

Vanderlan Nogueira Holanda[1,2], Thaíse G. S. Brito[2], Vera Lucia De Menezes Lima[2], Regina Celia Bressan Queiroz De Figueiredo[1]

[1]Microbiologia, Instituto Aggeu Magalhães (PE, Brasil), [2]Bioquímica, Univ. Federal De Pernambuco (PE, Brasil)

INTRODUCTION: Cutaneous leishmaniasis (CL), caused by protozoans belong to the genus *Leishmania*, is one of the most devastating infectious diseases, leading to disability and social stigma due to the severe skin lesions. The treatment of CL relies on few chemotherapeutic agents, most of which having high toxicity. Furthermore, the appearance of drug resistance has been reported. In the search of new chemotherapeutic alternatives for CL treatment, the essential oils have been promising due to their diverse biological activities. OBJECTIVES: In this study we aimed to investigate the leishmanicidal and anti-inflammatory potential of the essential oil isolated from the leaves of *Plinia cauliflora* (PCEO). MATERIALS AND METHODS: The chemical composition of PCEO was assayed by GC/MS. The effects of PCEO on the growth and survival of promastigote and amastigote of *Leishmania amazonensis* and *Leishmania braziliensis* were assayed by cell counting in a light microscope. The cytotoxic potential of PCEO on mammalian cells was assayed using the Cell Titer-Glo Luminescent Cell Viability Kit. The *in-silico* prediction of pharmacokinetics properties of major compounds was also performed. The effects of PCEO on the promastigote morphology were analysed by scanning electron microscopy. The anti-inflammatory activity was assayed in mouse carrageenan-induced paw edema model. DISCUSSION AND RESULTS: β-Caryophyllene (24.4%), 10-epi-γ-Eudesmol (8%), 2-Naphthalenemethanol (8%) and trans-calamenene (8%) were the major constituents of the PCEO. The PCEO has moderate cytotoxicity for mouse peritoneal exudate (mPEC) and Vero cells. The PCEO was able to significantly decrease the mPEC infection by *L. amazonensis* and *L. braziliensis*. PCEO induced drastic ultrastructural changes in both species of Leishmania. The *in-silico* ADMET analysis showed that PCEO has a good potential for using in oral or/and topical formulations. PCEO also has an *In vivo* anti-inflammatory effect, with 95% of reduction of mouse paw edema. CONCLUSION: Our results pointed PCEO as promising therapeutic agent against CL.

Keywords: CHEMOTHERAPY, ESSENTIAL OILS, LEISHMANIA / **Supported by:** CNPQ, CAPES, FACEPE AND FIOCRUZ

T.23 - A rapid and simple method to purify phosphatidylethanolamine plasmalogens from bovine or porcine brains

Rodrigo Lucas de Faria [1], Sayuri Miyamoto[1]
[1]Departamento de Bioquímica,, Instituto de Química da Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: Plasmalogens are present in all human tissues and represent approximately 20% of the total phospholipids. The brain is especially enriched in phosphoethanolamine plasmalogens (pPE). Plasmalogens may be endogenous antioxidants and several *In vitro* studies show the antioxidant action under different types of oxidative stress. However, the reaction of plasmalogens with reactive oxygen species (ROS) generates reactive products such as aldehydes. Therefore, plasmalogens antioxidant activity is controversial and more studies, especially *In vivo* , are needed to better understand the action of plasmalogens. Despite the great interest, commercial plasmalogens are costly and the purification methods are not productive and require robust equipment such as HPLC. OBJECTIVES: Here, we report a quick and easy purification of pPE from the brain (bovine and porcine) using simple equipment and techniques. MATERIALS AND METHODS: Lipids were extracted by chloroform and ethyl acetate method using the least amount of solvent possible. Then, the lipids were treated with Phospholipase A1 from *Aspergillus oryzae* to hydrolyze the diacyl PE while the PE plasmalogens remain intact. Therefore, the purification of these species by silica column is facilitated. Purified products were characterized by lipidomic analysis (UPLC-ESI-QTOF-MS/MS). Alternatively, TLC analysis was performed to identify pPE with 2,4-Dinitrophenylhydrazine (DNPH) stain and quantified by charring after incubation with 0.1% HCl. DISCUSSION AND RESULTS: Approximately 50 mg of pPE (80% purity) was obtained from 8 g of wet tissue (bovine or porcine brains). Purity was determined by UPLC-ESI-QTOF-MS/MS and same value was obtained by TLC. Plasmalogens were easily identified by TLC analysis (DNPH). Furthermore, approximately 80 mg of cerebrosides and 10 mg of sulfatides were also obtained in the same purification. CONCLUSION: The procedure requires only two days and good amounts of plasmalogen are obtained using common laboratory techniques. Here we describe a simple and economical method to obtain high purity pPE and detailed characterization of pPE molecular species by mass spectrometry analysis.

Keywords: Lipidomics, Plamalogen, Mass spectrometry / **Supported by:** FAPESP, CNPq, Capes and Pró-Reitoria de Pesquisa da USP

T.24 - Production and yield of essential oil from growth and regrowth of basil cultivated in the central-south region of Bahia.

Bárbara Almeida Dutra [1], Meira M.R.[1], Fonseca R.C.[1], Maria Caroline Rizério Costa[1], Gilberto G.N.[1], Marcondes Dos Santos M.S.[1], Simone Andrade S.A.[1], Crislene Silva[1], Janaína Silva Freitas[1], Esaul Lucas Oliveira[1]
[1]Department of Exact and Natural Sciences, State University of Southwest Bahia (BA, Brasil)

INTRODUCTION: Introduction: Basil produces essential oils of economic interest. OBJECTIVES: Objective: To evaluate the growth and yield of essential oil of Ocimum basilicum L. sprout and regrowth under protected cultivation and full sun in the region of Itapetinga-BA. MATERIALS AND METHODS: Materials and methods: The plants were grown in 15cm high containers and distributed in a protected environment and full sun. Harvest interval was 30 days for sprouting and 45 for regrowth. Irrigation was individual. They were weighed on an analytical balance to determine the fresh matter and taken to hydrodistillation for 2h. The essential oil was collected and weighed on analytical balance. Essential oil content was calculated based on dry matter and data expressed as average. DISCUSSION AND RESULTS: Results and discussion: The cultivation in the environment showed a difference between sprouting and regrowth in fresh matter, height and essential oil. The sprout registered fresh mass of 14g in protected environment and 13g in full sun, and the regrowth 17g in protected environment and 9g in full sun. The sprout showed height of 28 cm in protected environment and 27.5 cm in full sun, and the regrowth 29 cm in protected environment and 22 cm in full sun. The bud obtained an essential oil content of 0.6% in a protected environment and 0.4% in full sun and the regrowth 0.5% in a protected environment and 0.2% in full sun. Because it is a species with C3 metabolism, it showed better adaptation in protected environment. CONCLUSION: Conclusion: Regrowth in the environment is unfeasible due to high evapotranspiration and loss of a large number of experimental subjects. Cultivation for commercial purposes in protected environment from sprouting and regrowth is suggested for the production of biomass and essential oils of basil under protection from photosynthetically active irradiation at 50%, given the reduction of evapotranspiration and protection against herbivory.

Keywords: cultivation, medicinal plants, essential oil / **Supported by:** FAPESP, CNPq and CAPES

U - Gene Regulation and Cell Signaling

U.01 - Exossomes isolated from macrophages culture médium after contact with Aspergillus fumigatus conidia impact lung cells mitochondrial function

Lucas Oliveira Sousa [1], Juliana Silveira Ruas[2], Emerson de Souza Santos[1], Roger Frigério Castilho[2], Sérgio Akira Uyemura[1]
[1]Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto - USP (São Paulo, Brasil), [2]Patologia Clínica, Faculdade de Ciências Médicas - UNICAMP (São Paulo, Brasil)

INTRODUCTION: Exosomes are one type of extracellular vesicles produced and released by cells with the size range from 30 to 150 nm. Exosomes contain several regulatory molecules, such as miRNAs. Invasive Pulmonary Aspergillosis is one of the most severe infectious diseases in immunocompromised patients. The inhalation of fungal spores is the gateway of the disease. Alveolar macrophages are one of the first immune cells to be in touch with the fungi, but the biological impact of the exosomes secreted by macrophages in lung cells after contact with A. fumigatus is not determined yet. OBJECTIVES: Verify if the exosomes isolated from macrophages after A. fumigatus exposure impact mitochondrial functions in lung cells. In addition, to screen for altered miRNAs present inside the exosomes that possibly regulates mitochondrial functions in lung cells. MATERIALS AND METHODS: Macrophages cells were exposed for 2 hours to A. fumigatus conidia (MOI 1:3). Exosomes were isolated using the KIT ExoQuick® TC ULTRA. The characterization of exosomes was carried out through Nano Tracking Assays (NTA),Transmission Electronic Microscopy (TEM) and Western blotting (WB). Mitochondrial function was measured in lung cells using SeaHorseXF94, after exposing them to exosomes isolated from macrophages after contact with A. fumigatus conidia. The levels of miRNAs were screened in exosomes through real time RT-qPCR. DISCUSSION AND RESULTS: NTA revealed average size of exosomes around 113nm. TEM showed that their shape and size were as expected, while WB was positive to Alix, Actin-Beta and CD81. The Oxygen Consumption Rate assay showed reduction of parameters involving oxidative phosphorylationandrespiratory spare. On the other hand, the Extracellular Acidification Rate showed increasing ofglycolysis in lung cells. Real time RT-qPCR revealed altered levels of miR-34a, miR-146a and miR-181c. CONCLUSION: The specific impact of these miRs on mitochondrial function will be investigated using the mimetic and inhibitors miRs.

Keywords: Exossomes , Aspergillus fumigatus, MiRNAs
Supported by: FAPESP; CAPES, CNPq

U.02 - Molecular Pathways in Pancreatic Cancer Stem Cells

Gabriel Lucas da Fonseca [1], Camila Gomes das Neves[1], Daniela Sanchez Bassères[1]
[1]Departamento de Bioquímica, Instituto de Química (São Paulo, Brazil)

INTRODUCTION: Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal disease characterized by early and extremely aggressive metastatic dissemination. One of the most important hallmarks of metastatic capability is the acquisition and maintenance of a stem-like phenotype by cancer stem cells (CSCs), a process that is still poorly understood in PDAC. OBJECTIVES: Therefore, our goal was to identify molecular pathways enriched in PDAC CSCs. MATERIALS AND METHODS: We analyzed the global transcriptional profile of primary cultures derived from 5 PDAC patient-derived xenograft (PDX) tumors comparing tumorspheres (enriched for CSCs) with adherent cells (not enriched for CSCs). For that purpose, we analyzed the RNAseq raw data using the RSEM and DeSeq2 pipelines to identify differentially expressed genes (DEGs). In addition, we used publicly available PDAC datasets (PAAD-US and PACA-AU) to investigate the association of DEGs with patient survival and used gProfiler to identify biological pathways enriched in CSCs. DISCUSSION AND RESULTS: We found 1777 DEGs, including genes that have been shown to be promising therapeutic targets in other cancers, such as AURKB. Of these, 51 DEGs were significantly altered in tumorspheres of all 5 PDX tumors and 5 of them were significantly associated with PDAC survival (MALAT1, PDLIM7, RAD54B, KIF18A and POLD1). Interestingly, a comparison with genes belonging to the surfaceome identified 5 DEGs that encode surface proteins that could be used as biomarkers, including PIGR, a polymeric immunoglobulin receptor associated with hepatocellular carcinoma stemness. Finally, gProfiler analysis identified pathways enriched in CSCs, such as cell cycle, cytoplasmatic translation and DNA repair. CONCLUSION: We have identified multiple DEGs that may be important in pancreatic cancer stem cell acquisition, including enriched pathways, potential therapeutic targets and potential novel biomarkers, that could be explored to improve PDAC therapy and prognosis. Future studies will aim to functionally validate these targets.

Keywords: Cancer Stem Cells, Metastasis, Pancreatic Ductal Adenocarcinoma / **Supported by:** FAPESP, CNPq, CAPES

U.03 - In Silico analysis of protein phosphatases role in the COVID-19

Giulia Isabelle Marinho Garrito [1], Luciana Elena de Souza Machado[1]
[1]Genética e Biologia Evolutiva, Universidade de São Paulo (São Paulo, Brasil)

Introduction: Severe Acute Respiratory Syndrome virus (SARS-CoV-2) is the cause of COVID-19, a respiratory syndrome that caused a pandemic. Much of the published work on the subject focuses on proteomic and transcriptomic analyses of sick and infected individuals, and demonstrates changes in protein expression and protein-protein interaction involved in various signaling pathways. Protein phosphatases are important in many cellular functions, such as cell cycle control, energy metabolism, and gene expression. Objective: We sought to understand the role of protein phosphatases and the signaling pathways that may be involved during infection. In addition, we analyzed the interaction of protein phosphatases directly and indirectly (via intermediate proteins) with viral proteins; their degree of expression, and the degree of intermediate proteins phosphorylation during infection. Methods: Protein phosphatases were selected from databases of articles with proteomic analyses. The protein interaction network and signaling pathway was performed using Cytoscape and ClueGo software. Graphs of the results obtained from protein phosphatase expression and statistical analysis were performed in GraphPad Prism 9. Results: Protein interactions between protein phosphatases, SARS-CoV-2 proteins, and intermediate proteins were identified. We revealed two phosphatases with more evident interactions, PP2CA and PTEN, and found several functions of the proteins involved in SARS-CoV-2 infection, especially in protein synthesis, degradation, growth, and apoptosis, such as: modulation of the host defense response symbiosis, ribosome assembly, mRNA export from the nucleus, anaphase promoter complex, and positive regulation of protein ubiquitination. Finally, we identified decreased phosphorylation levels of RBM8A and CDC20, intermediate proteins that interact with PP2CA and PTEN. Conclusion: The degree of expression of protein phosphatases does not change in model cells and in samples analyzed in SARS-CoV-2 infection. The activity of protein phosphatases increases through dephosphorylation of intermediate proteins, involved in synthesis/degradation. These data show protein phosphatases as a potential target for drug development for disease control.
Keywords: bioinformatics, Covid-19, protein phosphatases / **Supported by:** CNPQ e FAPESP

U.04 - Effect of Prenatal Alcohol Exposure on the Expression of Genes Related to Cardiovascular Dysfunction in Adult C57Bl/6 Mice

Allan Luis Barboza Atum [1], Bruna Calixto de Jesus[1], Leonardo Paroche de Matos[1], Caio Perez Gomes[2], João Bosco Pesquero[2], Stella Regina Zamuner[1], José Antônio Silva Júnior[1]
[1]Detpo Bioquimica, Univ. Nove de Julho (São Paulo, Brasil), [2]Depto Bioquimica, Univ. Federal de São Paulo (São Paulo, Brasil)

Introduction: The potential impact of prenatal alcohol exposure (PAE) varies considerably between individuals, affecting several organs. Its impact on the cardiovascular system can result in structural changes and alterations in genes related to the functioning of the heart. However, silent molecular changes in the myocardium can lead to harmful changes in adulthood. **Objective:** We investigated PAE's effects on gene transcription of genes related to CH signaling in mouse myocardium and morphological changes. **Materials and Methods:** Isogenic C57Bl/6 mice were subjected to a PAE protocol at 10% alcohol (v/v) diluted in drinking water throughout the gestation and 10 days of weaning (PN10). Morphometric measurements occur at PN2 and PN60. At PN60, the hearts were collected and the mRNA expressions of 47 genes participating in nine myocardial signal transduction pathways related to cardiovascular dysfunction were analyzed using qPCR in customized TaqMan plates. Trichrome and HE staining were used to detect myocardial morphologic alterations. **Results:** Animals from the PAE group presented low birth weight than the Control group, but the differences were abolished in adult mice. In contrast, the mice's size was similar in PN2; however, PAE mice were oversized at PN60 compared with the Control group. Cardiac and ventricular indexes were increased in PAE mice. We found that PAE modulated the mRNA expression of 43 genes related to cardiac dysfunctions at PN60, especially the reprogrammed, increased expressions of atrial natriuretic peptide and β-myosin heavy chain mRNA in adult PAE ventricle, genes essential for maladaptive tissue remodeling. The morphometric evaluation revealed that PAE hearts presented mild cardiac hypertrophy compared to the Control group. Increased interstitial collagen deposition in the myocardium was also observed in PAE animals compared to the Control group. **Conclusion:** Our data suggest that PAE modifies the transcription of myocardial genes related to cardiac dysfunction and morphometric changes in adult mice.
Keywords: prenatal alcohol exposure, myocardium, gene expression. / **Supported by:** CAPES, CNPq

U.05 - Gene Expression Of PTEN Was Increased In Reticulocytes Of Patients With Beta-Thalassemia Intermedia

João Pedro Maia de Oliveira da Silva [1], Caroline Valério Moraes[1], Karen Simone Romanello[1], Karina Kirscner Lopes Teixeira[1], Marcos André Cavalcanti Bezerra[2], Aderson da Silva Araújo[2], Igor de Farias Domingos[2], Diego Antonio Pereira Martins[2], Iran Malavazi[1], Anderson Ferreira da Cunha[1]
[1]Departamento de Genética e Evolução, Universidade Federal de São Carlos (São Paulo, Brasil), [2]Centro de Ciências Biológicas, Universidade Federal de Pernambuco (Pernambuco, Brasil)

INTRODUCTION: Beta-thalassemias (BT) are a group of hereditary diseases that affects the synthesis of beta globin chains. Although the genotype is widely known, the disease is classified according to the frequency of transfusions and other clinical manifestations into three distinct categories: minor (BTMi), intermedia (BTI) and major (BTM) which is the most severe form, with patients requiring regular transfusions (every 20 to 30 days). OBJECTIVES: Identification of the correlation among *PTEN* expression with pathways triggered by oxidative stress in BT. MATERIALS AND METHODS: Using previous studies carried out by our group, we selected *PTEN* as a possible target because of its widely recognized antioxidant role and its interactions with several differentially expressed genes found between BTI and BTM reticulocytes. qPCR and western blotting analysis were used to evaluate the gene expression and content of this protein. DISCUSSION AND RESULTS: Gene expression of *PTEN* was found up-regulated (7 fold) in BTI reticulocytes compared to healthy controls, however, the analysis of protein content showed no significant difference among the groups. Since our group showed that reactive oxygen species (ROS) were increased in BTI patients and other studies showed that in high contents of oxidative stress PTEN can be targeted to the nucleus, we suggest that this difference among qPCR and western blot analysis is due to the loss of the content of PTEN localized in the nucleus, which is lost during erythroid differentiation. CONCLUSION: *PTEN* was associated with several diseases in which it plays an important role against oxidative damage. However, the association with BT is still not clear. The understanding of this correlation could provide new insights into the pathophysiology of the disease.
Keywords: Beta-thalassemia, gene expression, oxidative stress / **Supported by:** FAPESP e CAPES

U.06 - Characterization of the Role to the Protein CWC24 in Pre-mRNA Splicing in *Saccharomyces cerevisiae*

Moises Ricardo de Almeida Barros [1], Carla Columbano de Oliveira[1]
[1]Bioquímica, Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, Brazil; (SP, Brasil)

INTRODUCTION: All RNAs transcribed in eukaryotic cells undergo maturation stages. One of the maturation steps that pre-mRNAs go through is the splicing process, through which the non-coding sequences (introns) are removed and the coding sequences (exons) joined. For this process to occur, two transesterification reactions are necessary, which are mediated by a multimeric complex called spliceosome, composed of five snRNPs (small nuclear ribonucleoproteins), U1, U2, U4, U5 and U6, and several proteins. The spliceosome assembly occurs during the splicing process, requiring several conformational changes of the subcomplexes, so that the two reactions occur during splicing. These conformational and composition changes of the complex are mediated by the action of helicases and other ATPases, and consequently, involve ATP consumption. In addition to the snRNPs and ATPase complexes, the pre-mRNA splicing requires the NTC complex (NineTeen Complex), which participates in the two transesterification reactions, with its composition changing throughout the process. OBJECTIVES: Among the proteins that make up the NTC complex, we highlight Cwc24, which transiently binds to the spliceosome and has the function of interacting with the 5' end of the intron to prevent a premature transesterification reaction, which could lead to a non-functional mRNA. The objective of this work is the characterization of the mechanisms that lead to the association and disassociation of Cwc24 from the yeast spliceosome MATERIALS AND METHODS: by constructing strains expressing TAP-tagged splicing factors in the presence or absence of Cwc24. DISCUSSION AND RESULTS: The protein interactions are analyzed by western blot, co-immunoprecipitation, and functional analysis are performed by northern blot. CONCLUSION: By observing the effect of the Cwc24 absence on the interactome of spliceosome subcomplexes, it is possible to infer its function in splicing.
Keywords: Splicing, Pre-mRNA, Spliceosome / **Supported by:** FAPESP

U.07 - Caveolae-Dependent Endocytosis of QSOX1b Drives Cell Migration

Pierina Alexandra Martinez Huamani [1], Lia Nakao[1], Silvio Zanata[1]
[1]Patologia Básica, Universidade Federal do Paraná (Paraná, Brasil)

INTRODUCTION: Quiescin/sulfhydryl oxidase 1 (QSOX1) is a predominantly secreted flavoprotein (isoform b) that contributes to extracellular matrix assembly and also modulates cellular proliferation, migration and adhesion. We showed that QSOX1 is upregulated in the neointimal tissue of balloon-injured arteries in rats, and that extracellular QSOX1b induces proliferation and migration of smooth muscle cells (SMC) through intracellular superoxide and hydrogen peroxide, respectively. We also observed that recombinant QSOX1b can be endocytosed by rat SMC and mouse fibroblasts (L929 cells). However, the molecular mechanisms involved in these processes remain unknown. OBJECTIVES: To characterize the endocytosis of recombinant QSOX1b and its function in fibroblasts of the L929 lineage. MATERIALS AND METHODS: Internalization of recombinant QSOX1b was evaluated by indirect immunofluorescence and western blot. The migration induced by endocytosed recombinant QSOX1b was evaluated by the scratch technique. Endocytosis inhibitors were used to characterize this process. DISCUSSION AND RESULTS: The images acquired by confocal microscopy showed fast internalization of recombinant QSOX1b and colocalization with EEA1 and Rab5, from 5 to 30 minutes, indicating the presence of the recombinant in early endosomes. Scratch assays showed that incubation with 500 nM of endocytosed recombinant QSOX1b for 30 minutes was sufficient to induce cell migration 24 hours after stimulation. Pre-incubation with inhibitors of endocytosis indicated that 1 mM of methyl-β-cyclodextrin (inhibitor of caveolae-mediated endocytosis) dramatically decreased the promigratory effect of QSOX1. By immunofluorescence, it was also observed that endocytosed QSOX1b colocalizes with caveolin1 and pretreatment with 1mM methyl-β-cyclodextrin inhibited QSOX1b internalization. CONCLUSION: Recombinant QSOX1b is endocytosed by caveolae and this process is important for cell migration.

Keywords: cell migration, endocytosis, QSOX1 / **Supported by:** CAPES

U.08 - The Neurospora crassa PCL-1 is a cyclin with multifunctional activities and together with PHO85-1 (PGOV) protein kinase directs the complex to glycogen metabolism

Jonatas Erick Maimoni Campanella [1], Thiago de Souza Candido[1], Antoniel Augusto Severo Gomes[2], Paula A. Barbugli[3], Maria Célia Bertolini[1]
[1]Depto de Bioquímica e Química Orgânica, Instituto de Química, Universidade Estadual Paulista (SP, Brazil), [2]Depto de Física e Biofísica, Instituto de Biociências de Botucatu, Universidade Estadual Paulista (SP, Brazil), [3]Departamento de Materiais Dentários e Prótese, Faculdade de Odontologia de Araraquara, Universidade Estadual Paulista, (SP, Brazil)

INTRODUCTION: Cyclins are a family of structurally related and highly conserved proteins that regulate cell cycle, transcription, and other cellular events. They can associate with proteins called cyclin-dependent kinases (CDKs), such as the protein PHO85-1 (PGOV in *Neurospora crassa*), regulating their activities and determining the substrate specificity of the kinase complex. OBJECTIVES: To characterize the *Neurospora crassa* PCL-1 cyclin and to investigate its involvement in glycogen metabolism and stress. MATERIALS AND METHODS: We combined molecular approaches such as biochemical characterization, cell localization, molecular modeling, and genetics to get information regarding this cyclin in a filamentous fungus. DISCUSSION AND RESULTS: PCL-1 possesses a cyclin-like region at C-terminus, which corresponds to a classical cyclin domain by AlphaFold. The Δ*pcl-1* strain accumulates higher amounts of glycogen than the wild-type (WT) strain and the glycogen synthase (GSN) enzyme is less phosphorylated and, therefore, more active in the mutant strain. Recombinant PCL-1 and PHO85-1 phosphorylates *In vitro* GSN at a single residue (Ser636) and the structural model of PHO85-1/PCL-1 complex was predicted using AlphaFold Multimer based on the structures of the yeast Pho85/Pcl orthologous proteins. The predicted structure of the complex presented high confidence only with PCL-1 suggesting that some PHO85-1 regions may assume stable conformations when in complex with PCL-1. The cellular localization of PCL-1-GFP was investigated, and we observed a complex localization pattern both at cytoplasm and nucleus. The Δ*pcl-1* strain is involved in the response to calcium stress, since the mutant strain exhibits higher growth than the WT strain under 300 mM calcium. We demonstrated that PCL-1 regulates the expression of calcium related genes and may be involved in the phosphorylation of the CRZ-1 transcription factor, a protein involved in calcium response, by influencing its subcellular localization. CONCLUSION: PCL-1 is the first CDK cyclin characterized in *N. crassa* and exhibits multifunctional activities.

Keywords: Kinase/Cyclin, Calcium , Glycogen metabolismo / **Supported by:** FAPESP, CAPES e CNPq

U.09 - The STK11 (LKB1) gene edition in NSCLC-A549 by CRISPR-Cas9 and the response to metformin and cisplatin.

Matheus Brandemarte Severino [1], Ana Paula Morelli[1], Isadora Carolina Betim Pavan[1], Mariana Camargo Silva Mancini[1], Mariana Marcela Góis[1], Luiz Guilherme Salvino Silva[1], Nathália Quintero Ruiz[1], Érika Pereira Zambalde[1], Daniel Francisco Guimarães dos Santos Júnior[1], Fernando Moreira Simabuco[1]
[1]LabMAS, Universidade Estadual de Campinas (São Paulo, Brasil)

INTRODUCTION. Cancer is a complex pathology, that originates from the accumulation of mutations in cells. Regarding tumors, lung tumors have the highest mortality rates. The lung cancer A549 cell line is widely used, this model has an important mutation in the *STK11* gene that encodes the LKB1 protein, an important tumor suppressor that regulates the mTOR pathway. These cells often have a low response to chemotherapy, requiring synergistic treatments for a better prognosis such as metformin. OBJECTIVE. By generating cells with the repair of *STK11* mutation in A549 cells by CRISPR-Cas9, to evaluate the response to cisplatin and the combination of cisplatin with metformin. METHODS. The CRISPR-Cas9 system was used, besides Western Blotting, immunofluorescence, analysis of O2 consumption, H_2O_2 generation, MTT viability, and functional assays of clonogenic and migration capacity. DISCUSSION AND RESULTS. The comparisons were made between A549 Wt (LKB1 K.O) vs A549 LKB1+. Clones generated by CRISPR-Cas9 presented LKB1 expression, but the rescue of expression probably occurred by NHEJ (nonhomologous end joining), detected by a higher molecular weight isoform. The cells with an expression of this isoform showed an increase in AMPK phosphorylation ($p < 0.01$) that resulted in alterations in mTORC1 ($p < 0.05$), autophagy ($p < 0.01$), and metabolism ($p < 0.03$) pathways. In addition, we found modulation in the redox system of cells that resulted in apoptotic signaling in the genetically edited cells. In this sense, we found that the clones responded better to cisplatin (IC50 10 vs 5,5μM), which was associated with an accumulation of H_2O_2. The use of metformin improved the response of A549 Wt, at the same level as the edited clones. CONCLUSION. In lung tumor cells with alterations in LKB1 expression, modulations in theautophagic and oxidative processes were detected, and the use of metformin seemed to contribute to the response to cisplatin in the LKB1 knockout cell lines.

Keywords: LKB1, CRISPR-Cas9, autophagy / **Supported by:** FAPESP 2020/13660-2

U.10 - Identification of Long Non-Coding RNAs Involved in the Cellular Development of Human Trophoblasts

Thalles Souza-Lopes [1,2], Ana Carolina Tahira[1], David Abraham Morales-Vicente[1,3], Mayana Zatz[4], Murilo Sena Amaral[1], Sergio Verjovski-Almeida[1,3]
[1]Laboratório de Parasitologia, Instituto Butantan (São Paulo, Brazil), [2]Instituto de Matemática e Estatística, Universidade de São Paulo (São Paulo, Brazil), [3]Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo (São Paulo, Brazil), [4]Centro de Pesquisas sobre o Genoma Humano e Células Tronco, Inst. de Biociências, Univ. de São Paulo (São Paulo, Brazil)

INTRODUCTION: The molecular mechanisms by which viral agents bypass the placenta, responsible for the maintenance of fetal development and homeostasis in addition to acting as a key immunological mediator are yet to be unraveled. A recent study evaluated trophoblasts (TBs) derived from human induced pluripotent stem cells (hiPSCs), which were reprogrammed from erythroblasts isolated from dizygotic twins discordant for the congenital Zika syndrome (CZS), revealing a differential expression of genes between TBs from CZS-affected and non-affected twins. Nonetheless, the involvement of long non-coding RNAs (lncRNAs) in the development of TBs and the placental susceptibility to Zika virus (ZIKV) were not investigated. OBJECTIVES: To identify and validate, using RNA-Seq data and differential expression analysis, the lncRNAs involved with ZIKV infection and with TBs differentiation from hiPSCs. MATERIALS AND METHODS: RNA-Seq quality was evaluated by FastQC and Fastp algorithms. Reads were mapped to the human reference genome (GRCh38) using the GENCODE annotation (v36) and the alignment tool STAR. Differential expression analyses used edgeR and voom R packages. Functional validation assays are yet to be performed. DISCUSSION AND RESULTS: We found 169 lncRNAs differentially expressed (DE) between TBs and hiPSCs, 150 lncRNAs DE between non-infected and ZIKV-infected TBs, 19 lncRNAs DE between non-infected TBs from CZS-affected and non-affected twins, and 10 lncRNAs DE between ZIKV-infected TBs from CZS affected and non-affected twins. CONCLUSION: Several pieces of evidence suggest the involvement of lncRNAs in the cellular development and differentiation of stem cells into many different specialized cell populations; still, the role of these agents in the regulation of TBs development are poorly understood. Thereby, our data will provide substantial information to describe a set of lncRNAs involved in the development of human TBs, as well as involved in the placenta susceptibility to ZIKV infection.

Keywords: Cellular development, Human trophoblast, Long non-coding RNA / **Supported by:** (FAPESP)

U.11 - AKT-Mediated Regulation of FOXK2 Transcription Factor: Molecular Mechanisms and Potential Role in Breast Cancer Drug Resistance

Luciana da Torre Carneiro [1,2], Bruna dos Santos Mendonça[2], Wallace Martins de Araújo[3], Raquel Ciuvalschi Maia[2], Eric Wing-Fai Lam[4], Andrew David Sharrocks[5], Gabriela Nestal de Moraes[1,2]

[1]Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro (RJ, Brazil), [2]Programa de Hemato-Oncologia Molecular, Instituto Nacional de Câncer (RJ, Brazil), [3]Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro (RJ, Brazil), [4]Department of Surgery and Cancer, Imperial College London (UK), [5]Faculty of Biology, Medicine and Health, University of Manchester (UK)

INTRODUCTION: FOXK2 is a transcription factor which modulates drug sensitivity in breast cancer cells. In contrast, drug-resistant cell lines exhibit constitutively high FOXK2 protein levels, suggesting that post-translational modifications might impair its functions. An in silico analysis revealed AKT oncogenic kinase as the main putative regulator of FOXK2 by phosphorylation. OBJECTIVES: To evaluate the effect of AKT modulation in the regulation of FOXK2 protein levels and the impact of the AKT-FOXK2 axis on drug resistance in breast cancer. MATERIALS AND METHODS: The MTT and clonogenic assays were performed to assess drug-induced cytotoxicity in MCF-7 (chemosensitive) and MDA-MB-231 (chemoresistant) cell lines. AKT1 and GSK3 knockdown was done by RNA interference. LY294002 and MK-2206 were used for pharmacological inhibition of AKT. Overexpression of FOXK2 and constitutively active AKT was performed by transient transfections. The NE-PER kit was used for cytoplasmic and nuclear extractions. Protein levels in whole and fractioned lysates were examined by Western Blotting following electrophoresis in SDS-PAGE and Phos-tag gels. DISCUSSION AND RESULTS: Inhibition of AKT, but not GSK3β, resulted in a reduction in exogenous and endogenous FOXK2 protein levels, particularly in the lower mobility bands. Differently from MCF-7, FOXK2 and phosphorylated AKT are predominantly found in the nucleus of MDA-MB-231 cells. Experiments involving lambda-phosphatase treatment and Phos-tag gels suggest that FOXK2 mobility is altered in AKT-inhibited cell models. Notably, an in silico analysis identified higher levels of phosphorylated FOXK2 at different sites in breast tumor samples. Finally, inhibition and overexpression of AKT impact drug response, which is associated with modulation of FOXK2 protein bands. CONCLUSION: In summary, our results suggest that AKT can regulate FOXK2 protein levels in drug-resistant breast cancer cells. Future investigations will determine whether AKT directly phosphorylates FOXK2, as well as identify the crucial sites for AKT-FOXK2 interaction and biological functions. **Keywords:** Breast cancer drug resistance, FOXK2 transcription factor, AKT oncogenic kinase
Supported by: L'óreal-UNESCO-ABC Para Mulheres na Ciência, FAPERJ, INCA-Ministério da Saúde, CNPq and Programa de Oncobiologia.

U.12 - Epigenetic Regulation Of The Tetrahydrobiopterin Pathway In Lipopolysaccharide-Induced Neuroinflammation

Jennyffer Souza[1], **Thomas Creczynski Pasa** [1], Samantha Izildinha Veloso[2], Alexandre Solano[1], Rodrigo Augusto da Silva[2], Alexandra Latini[1]

[1]Bioquímica, Univ. Federal de Santa Catarina (SC, Brasil), [2]Patologia Ambiental e Experimental, Universidade Paulista (Brasil)

INTRODUCTION: Robust evidence suggests an involvement of the metabolism of tetrahydrobiopterin (BH4), an essential cofactor for biogenic amines and nitric oxide biosynthesis, and epigenetic modulation of gene expression in the physiopathology of neurodegenerative diseases. OBJECTIVES: Since BH4 synthesis is regulated by inflammation, and key component of neurodegeneration, we evaluated whether BH4 metabolism is regulated by epigenetic mechanisms during neuroinflammation, leading to impaired neurotransmission. MATERIALS AND METHODS: Male C57BL/J6 mice received saline solution or lipopolysaccharide (LPS; 0.33 mg/kg i.p.) and were euthanized after 4 and 24 h. Behavioral analysis, BH4 metabolism, neurotransmitter levels, epigenetic profiles, oxidative stress, and inflammasome activation markers were analyzed in the plasma and the hippocampus after 4 or 24 h of LPS administration. DISCUSSION AND RESULTS: LPS-treated mice showed impaired locomotor activity after the inflammatory challenge (4h/24h), denoting LPS-induced sickness behavior. Increased neopterin levels, evidencing exacerbated BH4 synthesis, were observed in the plasma (4h/24h) and in the hippocampus (4h). A dysregulation in dopamine and serotonin metabolisms, denoting perturbed neurotransmission, were observed in the hippocampus (24h). Epigenetic enzymes DNA methyltransferase enzymes (DNMTs) were increased, while ten eleven translocations 1 and 3 (TET; DNA demethylases) were reduced in the hippocampus (24h). Enhanced global DNA hypermethylation resulted in permissive hippocampal expression of pro-inflammatory cytokines, including interleukin 1-β (24h). The inflammatory scenario also promoted upregulation of genes involved in the activation of the inflammasome and BH4 synthesis, perpetuating inflammation. Genes related to cellular antioxidant defenses, i.e., expression of erythroid-related nuclear factor 2 were also increased (24h), suggesting that the LPS triggered scenario stimulated protective mechanisms. CONCLUSION: The inflammatory stimulus provoked hippocampal DNA hypermethylation favoring persistent neuroinflammation, which led to upregulation of the BH4 metabolism. Although the latter was not under direct epigenetic control, the inflammation-induced pathological regulation of the BH4 pathway provoked impaired neurotransmission characteristic of neurodegenerative diseases.
Keywords: DNA hypermethylation, neopterin, neurotransmission / **Supported by:** Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

U.13 - Redox Regulation of Mitochondrial Protein Phosphatases Manganese-Dependent and Investigation of Their Network Interactions

Naira Bellissimo Oliveira de Almeida [1], Luciana Elena de Souza Fraga Machado[1]
[1]Departamento de Genética e Biologia Evolutiva, Instituto de Biociências Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: PP2Cm and PPTC7 (serine/threonine protein phosphatase 2C mitochondrial and canonical PP2C, respectively) are mitochondrial matrix metal-dependent serine/threonine phosphatases that regulates cellular functions through dephosphorylation. PP2Cm has been described as a regulator of the homeostasis of branched-chain aminoacids and the mitochondria permeability transition pore. PPTC7 plays an important role in metabolism through the dephosphorylation of citrate synthase, an enzyme of tricarboxylic acid cycle. OBJECTIVES: We sought to understand the PP2Cm and PPTC7 regulation based on the identification and analysis of interaction with ligands. MATERIALS AND METHODS: in silico studies were performed to identify ligands. The softwares used were BioGRID, STRING and other references. To analyze the mitochondrial localization of the ligands identified, we used the softwares MitoProt and DeepMito, as well as the mitochondrial gene inventory MitoCarta. In addition, we performed manual analysis on the presence of a mitochondrial peptide signal. Cytoscape and ClueGo were used to make network interaction and identify the signaling pathways in which PP2Cm and PPTC7 might be involved. DISCUSSION AND RESULTS: The databases indicate 33 and 90 proteins as ligands of PP2Cm and PPTC7, respectively. However, based on our analysis only 18 proteins seem to be real ligands of PP2Cm and 37 of PPTC7. We identified previously described cell signaling pathways such as branched-chain aminoacids catabolic process for PP2Cm and tricarboxylic acid cycle for PPTC7. We also identified not yet described cell signaling pathways for PPTC7 such as regulation of mitochondrial autophagy. CONCLUSION: Less than 50% of the proteins identified by databases seems to be real ligands of PP2Cm and PPTC7. Although these proteins are from the same family, they are specific for their signaling pathway. Therefore, we expect to elucidate the regulation of PP2Cm and PPTC7 activity, as well as the signaling pathways in which they participate, seeking to understand important cellular processes. **Keywords:** serine/threonine protein phosphatase, PP2Cm, PPTC7 / **Supported by:** FAPESP

U.14 - Expression of Nuclear XIAP Reveals Molecular Signatures Associated with Most Proliferative and Migratory Features in Breast Cancer

Bruna dos Santos Mendonça[1], Renata Binato Gomes[2], Eliana Saul Furquim Werneck Abdelhay[2], Raquel Ciuvalschi Maia[1], Renato Sampaio Carvalho[3], **Gabriela Nestal de Moraes [1,4]**
[1]Laboratório de Hemato-Oncologia Celular e Molecular; Programa de Hemato-Oncolog, and [2]Laboratório de Células-Tronco, Unidade de Transplante de Medula Óssea, Instituto Nacional de Câncer (RJ, Brasil), [3]Departamento de Biotecnologia Farmacêutica, Faculdade de Farmácia and [4]Programa de Oncobiologia, Instituto de Bioquímica Médica Leopoldo de Meis (IBqM), Universidade Federal do Rio de Janeiro (RJ, Brasil)

INTRODUCTION: X-linked inhibitor of apoptosis protein (XIAP) is an inhibitor of apoptosis protein aberrantly expressed in cancer. XIAP performs its canonical antiapoptotic functions at the cytoplasm, but growing evidence implicate an unexplored role at the nucleus. Our group has demonstrated that nuclear XIAP is associated with unfavorable outcome in breast cancer patients as well as increased *In vitro* cell growth and chemoresistance. OBJECTIVES: Here, we aimed to explore transcriptomic signatures associated with XIAP nuclear oncogenic functions in breast câncer. MATERIALS AND METHODS: MCF-7 human breast cancer-derived cells overexpressing XIAP variants: pEBB (empty vector), XIAPwildtype, XIAPH467A (cytoplasmic location) (lack of ubiquitin ligase activity; cytoplasmic location), XIAPΔRING (RING deletion; nuclear location) and XIAPNLS (Nuclear Localization Signal insertion; nuclear location) were compared by RNA microarray considering a ≥ 2- fold-change as criteria to define the differentially expressed genes (DEGs). Pathway analysis and related processes were obtained through gene enrichment analysis using MetaCoreTM and gene ontology (GO) dataset. The Venn diagram was produced using InteractiVenn. The STRING® database was used to construct a protein network. Interest genes were validated by qRT-PCR. DISCUSSION AND RESULTS: XIAPNLS overexpression induced a global repression in gene expression in comparison to XIAPwild type and XIAPΔRING. The most representative DEGs were involved in the regulation of cellular processes such as localization, transport, cell proliferation, migration, motility and protein phosphorylation. Following validation, MMP13, IGFPB6 and TFPI2 turned out to be the most differentially expressed in XIAPNLS in relation to XIAPΔRING, genes closely linked to cell proliferation and migration. Interestingly, MMP13 expression was found increased, and IGFBP6 and TFPI2 reduced in invasive breast cancer versus normal tissues. Notably, TFPI2 was also associated with favorable clinical outcome. CONCLUSION: Functional assays with candidate genes and XIAP nuclear interactomes are currently being performed and will help understand nuclear XIAP role in breast cancer aggressiveness. **Keywords:** Breast cancer, Nuclear XIAP, Transcriptomic signatures / **Supported by:** Fundação do Câncer (Programa de Oncobiologia), FAPERJ, CNPq-Universal, L'óreal-UNESCO-ABC Para Mulheres na Ciência and Ministério da Saúde/INCA

U.15 - Caffeine Attenuates Lipopolysaccharide-induced Neuroinflammation by Regulating the Transcription of Nrf2/Txn1/Nlrp3 Axis in the Adult Mice Hippocampus

Camila Cristina Dos Santos Afonso [1], Paula Lemes dos Santos Sanna[1], Liebert Bernardes Carvalho[1], Alexandre F. Solano[3], Jennyfer Souza[3], Alexandra Latini[3], Rodrigo Augusto da Silva[2]
[1]Dentistry, University of Taubaté (SP, Brazil), [2]CEEpiRG - Program in Environmental and Experimental Pathology, Paulista University (SP, Brazil), [3]LABOX - Department of Biochemistry, Center for Biological Sciences, Federal University of Santa Catarina (SC, Brazil)

INTRODUCTION: Caffeine is the most widely used psychostimulant in Western countries, with antioxidant, anti-inflammatory and anti-apoptotic properties. Although caffeine exerts its action predominantly through non-selective antagonism of adenosine receptors in the brain, numerous studies have demonstrated its potential as an epigenetic modulating agent by increasing the gene expression of enzymes involved in DNA methylation, which can control the expression of inflammatory genes. OBJECTIVES: Here, we investigated the impact of caffeine pretreatment on the epigenetic control of Nrf2/Txn1/Nlrp3 axis in the hippocampus. MATERIALS AND METHODS: The LPS administration (LPS) (0.33mg/kg) was performed 15 minutes after the caffeine pretreatment (6mg/kg). Gene expression was evaluated by qPCR and methylation status by qPCR with genomic DNA previously treated with MspI and HpaII endonucleases 24 h after LPS administration. DISCUSSION AND RESULTS: Caffeine pretreatment was shown to have a significant inhibitory effect on *Il 1β* gene transcription after LPS administration. It was also observed that caffeine itself was able to modulate the expression of genes encoding DNA-modifying enzymes and had an inverse effect with LPS for the *Nrf2* gene. For the *Nrlp3,* and Txn1 genes, only the administration of LPS reduced the expression of both. Furthermore, it was observed in the Caffeine+LPS group that the changes related to the reduction of *Nrf2* expression and the increase of *Nrlp3* and *Txn1* expression promoted by LPS were significantly prevented and/or reversed by the pretreatment with caffeine. Even having seen significant changes in the methylation status of the gene characterized by the 5-meC (hypermethylation) and 5-hmeC (hypomethylation) marks, no significant differences were observed in the 5-meC/5-hmeC ratio and in the correlation between the methylation status. versus gene expression. CONCLUSION: Therefore, the data strongly indicate that the neuroprotective action of caffeine may be associated with its anti-inflammatory effect by transcriptionally controlling the expression of LPS-activated Nrf2/Txn1/Nlrp3 axis genes and that this control is not being mediated by DNA methylation.
Keywords: caffeine, Nrf2/Txn1/Nlrp3 axis, neuroepigenetics / **Supported by:** FAPESP

U.16 - Inositol and Phosphate Metabolism crosstalk with the DNA Damage Response in *Saccharomyces cerevisiae*

Marina Rodrigues Pires [1], Giovanna Marques Panessa[1], José Renato Rosa Cussiol[1]
[1]Bioquímica, Universidade Federal de São Paulo (São Paulo, Brasil)

INTRODUCTION: The DNA Damage Response (DDR) involves several effector proteins that act in many cellular processes promoting changes such as reestablishment of nuclear function, transcriptional and metabolic reprogramming among others. Failure to trigger an appropriate cellular response can promote genomic instability with loss of cellular viability. The inositol metabolism is responsible for the production of several molecules such as phospholipids and inositol polyphosphates that play key structural and signaling roles. Moreover, phosphate metabolism is responsible to keep the nutritional homeostasis, even in conditions of low nutritional levels. Previous studies proposed that proteins from the inositol and phosphate metabolism are important to trigger an effective DDR. Therefore, we sought to investigate the relevance of inositol and phosphate metabolism for genomic stability in budding yeast. OBJECTIVES: Therefore, we sought to investigate the relevance of inositol and phosphate metabolism for genomic stability in yeast. MATERIALS AND METHODS: Trough a combined genetic and biochesmistry/molecular biology approach we have studied the role of Opi1 and Pho81, two proteins that regulate inositol and phosphate metabolism respectively, for the DDR. DISCUSSION AND RESULTS: Notably, Pho81 accumulates in the cell after genotoxic stress induced by Methyl methanesulfonate (MMS), suggesting an increase in its expression during the DDR. Besides that, knockout of genes related to the PHO pathway show sensitivity to genotoxins such as MMS and Hydroxyurea (HU). In addition, deletion of Opi1 confers sensitivity to MMS and expression of Opi1 from a plasmid rescues the MMS sensitivity of *opi1Δ*. Finally, we have created a phosphomutant of Opi1 (Opi1 S396A) that cannot be phosphorylated by the DDR kinase Mec1 (ATR in humans). We will test in the future if cells expressing Opi1 S936A show defects in inositol metabolism under conditions of genotoxic stress. CONCLUSION: Together, these results strengthen the notion that inositol and phosphate metabolism contributes to trigger an effective DDR through a mechanism yet to be explored.
Keywords: DNA Damage Response, Inositol, Phosphate / **Supported by:** CNPq e FAPESP

U.17 - Analysis of the Ligands and the Interaction Network of the Mitochondrial Protein Phosphatase PGAM5

Sofia de Oliveira Farias [1], Luciana Elena de Souza Fraga Machado[1,1]
[1]Departamento de Genética e Biologia Evolutiva, Instituto de Biociências, Universidade de São Paulo (São Paulo, Brazil)

INTRODUCTION: The protein phosphoglycerate mutase 5 (PGAM5) is a mitochondrial histidine-dependent serine threonine phosphatase that has been described in cellular apoptosis processes and in mitophagy and mitochondrial fission. However, the sub-mitochondrial localization of PGAM5 remains unknown as there is evidence of interaction between PGAM5 and cytosolic proteins. OBJECTIVES: We intended to analyze the possible ligands and cell signaling pathways that the mitochondrial protein phosphatase PGAM5 is involved. MATERIALS AND METHODS: A bioinformatical analysis was carried out to identify PGAM5 ligands and the cellular signaling. The softwares used were Biogrid, String and other references to analyze the PGAM5 ligands. Other softwares such as Mitoprot and DeepMito, as well as inventories (Mitocarta) and manual analyses were used to study the mitochondrial peptide signal. Lastly, the softwares Cytoscape, MCode and ClueGo were used to make the network interaction and identify the signaling pathway. DISCUSSION AND RESULTS: Through database, 201 proteins were identified as potential PGAM5 ligands, however, only 62 proteins appear to be real PGAM5 ligands. From this analysis, 6 proteins were identified solely as mitochondrial, 33 had functions related to apoptosis, mitophagy and/or mitochondrial fission and 23 appears to be mitochondrial proteins with, at least, one of these functions. Most of the 62 proteins are involved in necroptotic processes and regulation of cytochrome c release. CONCLUSION: Only thirty percent of the initial ligand seems to be real PGAM5 subtracts and we identified pathways that had not yet been described for PGAM5, such as necroptotic processes. Considering the importance of protein dephosphorylation in cellular regulatory processes, the analyses of PGAM5 ligands and signaling pathways is of great significance as this protein appears to be involved in processes such as cellular apoptosis and it is still greatly misunderstood.
Keywords: protein serine/threonine phosphatase, mitochondria, PGAM5
Supported by: FAPESP

U.18 - The NPF protein, a component of the Polycomb Repressive Complex 2 (PCR2) in *Neurospora crassa,* displays a role in adaptative stress response

Karina G Resges Orives[1], Jonatas E Maimoni Campanella[1], Maria Célia Bertolini[1], **Fernanda Zanolli Freitas** [1]
[1]Bioquímica e Química Orgânica - DBQO, Instituto de Química de Araraquara, UNESP (SP, Brasil)

INTRODUCTION: It has become increasingly clear that covalent modifications of chromatin, such as methylation of specific histone residues can have profound effects on genome functions. In plants, animals and fungi, PRC2 is a regulator that trimethylates H3Lys27 (H3K27me3), leading to transcriptionally repressed chromatin state. Although absent in yeasts, the core components of PCR2 are conserved in several fungi, including *Neurospora crassa* (SET-7, EED, SU(Z)12, and NPF). Using a proteomic approach to search for TF downregulating *gsn* gene transcription under heat-stress, we found the PCR2 NPF component, suggesting a role for this protein in heat stress response. OBJECTIVES: Considering that repression mediated by H3K27me3 is largely unknown in fungi, and that we previously correlated NPF to heat-stress, we took advantage of the relatively simple *N crassa* system to explore possible relationships between NPF-PCR2 and stress-adaptative responses. MATERIALS AND METHODS: Growth behavior of a Δ*npf* strain was analyzed after exposing to stressing conditions, such as heat (45C), acid (4.5) and alkaline pH values (7.2 and 7.8), osmotic (sorbitol and NaCl) and oxidative chemicals (menadione, H_2O_2, paraquat), using the wild-type strain as control. DISCUSSION AND RESULTS: Δ*nfp* strain displayed several phenotypical defects at standard growth conditions, as an impaired conidiation, defective carotenoid production, shorten-hyphae extension, and retarded hyphae extension rate. Mutant cells cultivated at alkaline pH displayed drastic colony-diameter reduction, and impaired growth at high levels of osmotic and oxidative chemicals, suggesting that fungi detoxification pathway is somehow modulated by NPF. For heat stress, we found for Δ*nfp* a defective radial growth and reduction of glycogen content. CONCLUSION: Our results suggest that NPF is important to cells properly modulate gene expression and respond to environmental challenges in fungi, and participating in stress-adaptive response. If PCR2-chromatin-remodeling is also involved, needs to be further investigated, since the slow-growth Δ*nfp* can be due to a role of this protein in complexes other than PCR2.
Keywords: chromatin remodeling, gene expression regulation, stress response / **Supported by:** CAPES, FAPESP

U.19 - Identification of Mitochondrial DUSPs Ligands through Bioinformatics Tools

Luca Paulino Otvos [1], Luciana Elena Souza Fraga Machado[1]
[1]Genética e Biologia Evolutiva, Instituto de Biosciências da Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: The DUSPs are protein tyrosine phosphatase capable of dephosphorylating their targets to serine, threonine and tyrosine residues. Four DUSPs have been identified in mitochondria: PTPMT1 (DUSP23), DUSP18, DUSP21 and DUSP26. Few or no mitochondrial functions has been unraveled for these DUSPs. OBJECTIVES: This work aims to investigate their ligands within the mitochondria. MATERIALS AND METHODS: For this, we carried out a search for partners of mitochondrial DUSPs in silico, using the BioGRID and STRING platforms. Determined whether these possible partners would be mitochondrial proteins, using the programs Mitoprot and DeepMito and the mitochondrial proteomics database MitoCarta3.0. As DUSP18 is released into the cytosol during apoptosis, cytosolic proteins related to this cell death process were considered as possible partners of this protein phosphatase. DISCUSSION AND RESULTS: 83, 33, 17 and 9 proteins were identified as real partners of the PTPMT1, DUSP21, DUSP26 and DUSP18 protein phosphatases, respectively. From the final list of ligands, the interaction networks and groups of functions performed by them were obtained. 14 groups of functions were obtained for PTPMT1, one of them related to cardiolipin metabolism, and four of them to cellular respiration and ATP synthesis, roles already associated with this protein phosphatase. For DUSP21, three functional groups were found: oxidative phosphorylation, transmembrane protein transporter activity and heart growth. Two functional groups were identified for DUSP26: regulation of mitochondrial gene expression and mitochondrial protein processing. DUSP18 was mainly related to apoptotic proteins. CONCLUSION: An amount less than or equal to half of the binding proteins appear to be real partners of mitochondrial DUSPs. For all, new cellular functions were identified, which are specific for their signaling pathway. These results will make it possible to expand the panorama of knowledge about mitochondrial DUSPs, in addition to guiding future works that seek to deepen the characterization of the interactions and functions of these phosphatases.

Keywords: DUSP, Phosphatase, Mitochondria / **Supported by:** FAPESP

U.20 - Knockdown of the Oncogenic lincRNA PVT1 Decreases Prostate Cancer Cell Line Proliferation

Gabriel Nakanishi Fortes [1], Sergio Verjovski Almeida[1], Maria Gabriela Berzoti Coelho[1]
[1]Parasitologia, Instituto Butantan (São Paulo (SP), Brasil)

INTRODUCTION: Prostate cancer (PCa) is the second cancer most diagnosed and the lead cause of death cancer-related in men. The development of PCa is highly dependent on androgen receptor (AR), a transcription factor that modulates the effect of androgenic hormones and gene transcription in prostatic cells, promoting their activation or inhibition. Long non-coding RNAs (lncRNAs) are a class of nucleic acids with 200 or more nucleotides that are not translated into proteins and play important roles in cellular processes. Some lncRNAs are associated with cancer development since their regulation seems to impact cells' functions like proliferation, apoptosis, and induction of metastasis. Our research group has previously reported that lncRNA *PVT1* is associated with AR in the PCa cell line LNCaP, suggesting *PVT1* is involved in a repressive transcriptional program androgen-dependent, leading to the inhibition of tumor suppressor genes expression. OBJECTIVES: This study aims to investigate the cellular mechanisms that are affected by *PVT1* on prostatic cancer *in vitro*. MATERIALS AND METHODS: LNCaP cell line was transfected with lentivirus for *PVT1 knockdown*, using the *CRISPR-Cas13d* system components. The level of gene expression was detected by quantitative real-time PCR (RT-qPCR). Control cells (CTRL) or *knockdown* cells (sg #13.4 or sg #17.10) were seeded in 6-well plates and the trypan blue exclusion test was performed daily, for five days, to count the number of stained and unstained cells. Results were normalized to cell counts at day zero. DISCUSSION AND RESULTS: *Knockdown* of *PVT1* was confirmed by RT-qPCR and it decreased prostate cancer cell proliferation when compared to the control. CONCLUSION: Results suggest that lncRNA *PVT1* may work as a novel biomarker for PCa and could also be a candidate as a therapeutic target for the treatment of PCa.

Keywords: Long non-coding RNAs, Prostate cancer, PVT1 / **Supported by:** FAPESP

U.21 - The transcriptional repressor Opi1 links the DNA Damage Response with inositol metabolism in budding yeast

Giovanna Panessa[1], Rafaella Jekabson[1], Rodrigo Rodrigues Felix[1], Marina Rodrigues Pires[1], Fernanda Marques da Cunha[1], **José Renato Rosa Cussiol [1]**
[1]Bioquímica, Universidade Federal de São Paulo/Escola Paulista de Medicina (SP, Brasil)

INTRODUCTION: Inositol is a precursor for signaling molecules such as phosphoinositides and inositol polyphosphates that regulate a myriad of cellular functions both in normal and stress conditions. Therefore, it is an imperative for the cells to regulate inositol synthesis in order to promote cell viability. In budding yeast, transcription of genes involved in inositol metabolism are regulated by the transcriptional repressor Opi1. Cells lacking Opi1 overproduces inositol through the constitutive activation of the inositol 3-phophate synthase *INO1*. Overproduction of inositol affects expression of several other genes from different metabolic processes, which is highlighted by the fact that *opi1Δ* cells show a plethora of phenotypes. Notably, yeast cells lacking Opi1 are hypersensitive to DNA damage agents. Because Opi1 deletion leads to inositol overproduction, we decided to investigate if inositol accumulation is causing sensitivity to genotoxins such as methyl methanesulfonate (MMS). OBJECTIVES: We aim to investigate how the inositol metabolism crosstalk with the DNA damage response. MATERIALS AND METHODS: We have generated knockout and epitope tagged strains, employed cell viability, fluorescence microscopy, RT-qPCR, western blot and flow cytometry analyses. DISCUSSION AND RESULTS: Here, we report that cells under genotoxic stress repress *INO1* expression and that lack of Opi1 exponentially increases *INO1* expression under MMS treatment. Moreover, *opi1Δ* cells show defects to resume replication after MMS treatment. Importantly, we show that deletion of the inositol pyrophosphate kinase Kcs1 ^{IP6K} rescues the MMS sensitivity of *opi1Δ;* suggesting that cells must control intracellular inositol pyrophosphate levels in condition of stress in order to trigger an effective DDR. Strengthening this notion, cells lacking the inositol pyrophosphate phosphatase Siw14 also causes MMS sensitivity. CONCLUSION: Here, we show that inositol levels must be tight regulated during the DDR and overproduction of inositol leads to DNA damage sensitivity and cell cycle defects probably by accumulation of inositol pyrophosphates generated by Kcs1 ^{IP6K} .
Keywords: Inositol pyrophosphates , DNA Damage Response, Replication Stress / **Supported by:** FAPESP; CNPq; CAPES

U.22 - The Role of Inositol Metabolism and its Phosphorylated Derivatives in the Response to Genomic Damage in *Saccharomyces cerevisiae*

Giovanna Marques Panessa [1], Rafaella Jekabson[1], José Renato Rosa Cussiol[1]
[1]Departamento de Bioquímica, Universidade Federal de São Paulo (São Paulo, Brasil)

INTRODUCTION: Cells are constantly exposed to agents capable of altering the DNA leading to genomic instability. To cope with that, cells have mechanisms to tackle damage to the DNA, collectively termed the DNA damage response (DDR). Interestingly, it has been shown that proteins from the inositol pathway are putative targets of the DDR kinases Mec1 ^{ATR} /Tel1 ^{ATM} suggesting that these proteins might contribute to the DDR. Inositol is a precursor of a broad range of signaling molecules, like inositol polyphosphates (IPs) and pyrophosphates (PP-IPs). It has been shown that these highly energetic molecules modulate cellular processes either by allosteric regulation or non-enzymatic pyrophosphorylation of protein targets. OBJECTIVES: We aim to study the role of IPs and PP-IPs for the modulation of the DDR and maintenance of genomic stability. MATERIALS AND METHODS: We have generated knockout cell lines for genes related to the inositol metabolism. In order to access the contribution of inositol metabolism for the DDR we employed cell viability assays as well as western blot and flow cytometry analyses. DISCUSSION AND RESULTS: Here, we show that cells lacking the inositol pyrophosphate kinase Kcs1 ^{IP6K} are sensitive to replication stress induced by methyl methanesulfonate (MMS) and hydroxyurea (HU). Interestingly, *kcs1Δ* cells challenged with genotoxins show delayed Rad53 activation, increased accumulation of gamma-H2A and replication defects such as an intra-S-phase delay and failure to resume replication after MMS treatment. In addition, deletion of Vip1, the other inositol pyrophosphate from yeast does not lead to genotoxin sensitivity and replication defects. Finally, deletion of Kcs1 ameliorates MMS sensitivity of cells lacking Mec1 ^{ATR} which is an indication that PP-IPs might contribute to DNA damage signaling. CONCLUSION: These results suggest that PP-IPs generated by Kcs1, but not Vip1, regulate the DDR. However, further investigation is required to understand how inositol metabolites contributes for DDR modulation.
Keywords: DNA damage signaling, Budding yeast, Genotoxic stress / **Supported by:** Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP

U.23 - Evaluation of Angiogenic Capacity of Human Adenocarcinoma Cell Line Knockout for NF-κB1 Protein

Luiz Felipe S. Teixeira[1], Maria Helena Bellini[1]
[1]Departament of Biotechnology, Nuclear and Energy Research Institute (São Paulo, Brazil)

INTRODUCTION: Renal cell carcinoma (RCC) is the most common adult renal epithelial cancer. The most frequent subtype of RCC is clear cell (ccRCC). Most of ccRCC patients have a mutation in the Von Hippel-Lindau (VHL) tumor suppressor gene. The VHL gene encodes a protein, the VHL, which can up-regulate a series of intracellular proteins, including the hypoxia inducible factor (HIF). The transcription factor NF-κB is increased in the ccRCC. OBJECTIVES: To evaluate the impact of the NF-κB1 gene knockout on the VEGF and IL6 expression in the human RCC cells under normoxia and hypoxia. MATERIALS AND METHODS: The CRISPR/Cas-9 technique was used to obtain 786-0 cells knockout for the NF-κB1 protein. Western Blot assay was used to selected the clones. A hypoxia-inducing humid chamber was used and its effectiveness was validated its effectiveness was certified by the analysis of HIF-2α expression levels. The quantification of VEGF and IL-6 levels was measured using Real Time-PCR and MILLIPLEX assay. DISCUSSION AND RESULTS: The VEGF gene expression in the clones was significantly lower than that presented by the control both in normoxia (786-0-sg1 99.68±0.09%, 786-0-sg2 78.55±0.85%, 786-0-sg3 91.70±0.87%) and in hypoxia (786-0-sg1 98.30±1.49%, 786-0-sg2 75.21±4.14%, 786-0-sg3 98.44±0.18%). The expression of IL-6 gene was also significant lower in normoxia (786-0-sg1 49.03±0.80%, 786-0-sg2 76.59±12.43%, 786-0-sg3 66.98±10.89%) and in hypoxia (786-0-sg1 95.85±0.36%, 786-0-sg2 96.45±0.49%, 786-0-sg3 91.08±1.42%). The MILLIPLEX results show that there was a significant reduction of both VEGF and IL-6 in the culture medium of cells knocked out in normoxia and hypoxia compared to control group. CONCLUSION: Suppression of p50 expression in the clones resulted in the reduction of VEGF and IL6 in both conditions. The reduction in the IL-6 relative expression hypoxia/normoxia demonstrates a change in cellular responsiveness to decreased levels of oxygen.
Keywords: clear cell renal carcinoma (RCC), nf-κb1, hypoxia
Supported by: CNPq, FAPESP

U.24 - Impact of Neuroinflammation on Epigenetic Transcriptional Control of SHH Pathway Members in the Central Nervous System

Costa Mariana Ribeiro[1], Santos Amanda Yasmin Ilario dos[1], Miranda Tais Browne[1], Silva Ericka Patricia[2], Coque Alex A.[2], **Rogerio Luiz Aires Lima [2]**, Bernardi Maria Martha[2], Birbrair Alexander[4], Latini Alexandra[3], Silva Rodrigo Augusto[1,2]
[1]Dentistry, School of Dentistry, University of Taubaté, 12020-340, Taubaté, São Paulo, Brazil (SP, Brazil), [2]Environmental and Experimental Pathology, CEEpiRG, Program in Environmental and Experimental Pathology, Paulista University, São Paulo (SP, Brazil), [3]Center of Biological Sciences, LABOX, Department of Biochemistry, Center for Biological Sciences (SC, Brazil), [4]Department of Pathology, Department of Pathology, Federal University of Minas Gerais (MG, Brazil)

INTRODUCTION: Sonic Hedgehog signaling plays a fundamental role in the development of the central nervous system, and alterations in this signaling can lead to neurological disorders that are often irreversible. Although well studied, little is known about its regulation of Shh signaling in the adult brain and its influence on diseases that affect humans. OBJECTIVES: In this study, we investigated the contribution of DNA methylation in the transcriptional control of members of the Hedgehog signaling pathway and the impact of neuroinflammation in the gene expression of DNA-modifying enzymes. MATERIALS AND METHODS: Through inducing a murine model of neuroinflammation by single-dose i.p. of LPS (0.33mg/kg) we determined the profile of gene expression and methylation of Hedgehog signaling pathway members by qPCR. DISCUSSION AND RESULTS: We were able to show that in the adult brain, the methylation of CpG-promoting regions of the members of the Hedgehog pathway was fundamental determining the differential transcription pattern observed between distinct brain regions. Another result was that neuroinflammation differentially modulates the gene expression of DNA-modifying enzymes, revealing the basal transcriptional profile of DNMTs and TETs-modifying enzymes in the central nervous system and demonstrating the effect of neuroinflammation on the transcriptional control of members of the Hedgehog pathway in the central nervous system in the adult brain. CONCLUSION: Altogether, our results support the hypothesis that epigenetic mechanisms, such as DNA methylation, might be involved in determining the endogenous expression pattern of Hedgehog pathway members, in the adult brain and that neuroinflammation modulates both gene expression of DNA-modifying enzymes and the Hedgehog pathway members, but only the reduction in Sufu gene expression was accompanied by increased DNA methylation levels of the promoter region.
Keywords: DNA methylation, Neuroepigenetics, Sonic Hedgehog / **Supported by:** Capes

U.25 - Investigation of XIAP on Survival and Death Events in Leishmania amazonensis-Infected Macrophages
Thayane Motta Fagundes [1], Alberto Nogueira Neto[1], Luiz Dione Barbosa De-Melo[1]
[1]departamento De Biotecnologia, Instituto Federal De Educação Ciência E Tecnologia, Campus Rio De Janeiro (RJ, Brazil)

INTRODUCTION: Pathogenic trypanosomatids appropriate signaling pathways in the vertebrate host cell for their own benefit, neutralizing cell death that would normally occur in the host in a pathological stress condition such as infection. The success of the intracellular replication step of these parasites in the vertebrate host depends on multiple mechanisms of inhibition of death and pro-survival for the time required for the differentiation/proliferation of the pathogen in a safe niche. Previous data of our group demonstrate that XIAP (X-linked inhibitor of apoptosis) is downregulated at the level of mRNA and protein during infection of macrophages by Leishmania amazonensis, but resistance to apoptosis is still observed in these macrophages. OBJECTIVES: To better understand how XIAP-mediated signaling pathways occur during infection, our project aims to produce wild-type and different mutant versions of XIAP in Raw 264.7 model (murine macrophages) and analyze. MATERIALS AND METHODS: We intend to produce wild-type XIAP and mutants with deletion of the different domains of XIAP (BIR1, BIR2, BIR3, UBA or RING) by PCR and express them in Raw 264.7 macrophages by transfection. Will be used mutant with deletion of BIR1 domain (deltaBIR1) to investigate the phenotypes in the absence of NF-kB1 signaling. Mutant with deletion of the BIR1/2 domains (deltaBIR1/2) to investigate the loss of selective binding to Casp3 and Casp7 caspases, and loss of binding to Smac/DIABLO. Mutant with deletion of BIR3 domain (deltaBIR3) with loss of selective binding to Casp9 and its consequent activation. A mutant with only BIR1/2/3 domains but with C-terminal deletion of UBA and RING domains (deltaUBA/RING) to investigate ubiquitination mediated by E3 ligase activity of RING domain. Besides, we intend to overexpress mutants obtained by site-directed mutagenesis in residues of essential aminoacids for the interaction with XIAP effector proteins. DISCUSSION AND RESULTS: CONCLUSION: Furthermore, unravel the XIAP-mediated signaling pathways important for successful of L. amazonensis infection.
Keywords: apoptosis, Leishmania amazonensis, XIAP
Supported by: FAPERJ e IFRJ

U.26 - Histone deacetylase 1 (HDAC1) plays a role in antiviral gene expression reprogramming
Marina Bonzoumet[1], Luan Rocha[2], Yasmin Mustafá[2], Luciana Arruda[2], Caroline de Carvalho[3], Teresa Calegari[3], Ulisses Lopes[3]
[1]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil), [2]Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro (RJ, Brasil), [3]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro (RJ, Brasil)

INTRODUCTION: Histone acetyltransferases (HATs) and histone deacetylases (HDACs) regulate the chromatin accessibility to transcription factors (TFs), therefore, controlling the gene expression. OBJECTIVES: The present study investigates the role of pharmacological inhibition of HDAC activity, particularly HDAC1, in the interface of epigenetics events, notably histone acetylation levels for gene expression programming in human endothelial cells and the consequences for viral killing activity. MATERIALS AND METHODS: Human brain microvascular endothelial cells (HBMECs) were treated with 500nM of MS-275 (HDAC1 inhibitor) during 24 h. The histone 3 acetylation levels was verified by western blot as well as and the levels of mRNA of antiviral genes (OAS1 and IRF6) by quantitative RT-PCR. To evaluate the impact of HDAC1 activity inhibition in viral infection, HBMEC cells were treated with MS-275 during 24 h and then infected with VSV (Vesicular Stomatitis Virus). The viral load was verified by plaque assay after 18h of infection. DISCUSSION AND RESULTS: Preliminary results revealed that the treatment of HBMEC with MS-275 increased H3K9/14 acetylation levels and favored the transcription activity observed by the increased mRNA levels of the antiviral genes OAS1 and IRF6. Moreover, the pre-treatment of HBMEC with MS-275 inhibited the VSV replication. CONCLUSION: The pharmacological inhibition of HDAC1 activity showed an important impact on antiviral gene expression and, consequently, the control of viral infection. We aspire to unveil a comprehensive molecular mechanism of the role of HDAC1 for gene expression reprogramming in endothelial cells.
Keywords: HDAC1, epigenetic, HBMEC / **Supported by:** FAPERJ, CNPq and CAPES

U.27 - MicroRNAs analysis and their correlation with intracellular pathways associated with the development of cutaneous ulcers in sickle cell anemia: in silico study

Rafael Souza de Almeida[1], Victor de Barros Serrano Neves[1], Vitor Antônio Foturna[1,2], Simone Garcia Macambira[1,2]
[1]Bioquímica e Biofísica , Universidade Federal da Bahia (Bahia, Brasil), [2]Bioquímica e Biofísica, Fundação Oswaldo Cruz (Bahia, Brasil)

INTRODUCTION: Sickle cell anemia is a chronic, hereditary, autosomal recessive hematological disorder. In Brazil, sickle cell anemia is a social and public health problem. Sickle cell ulcers are among the most serious complications of sickle cell anemia (SCA). Until recently, treatment strategies for sickle cell ulcers were limited to wound bed preparation, removal of necrotic tissue, and infection control. The investigation of biomarkers to assess the degree of ulcer healing appears as an interesting therapy for controlling the condition OBJECTIVES: To study the miRNA expression profile and its correlation with intracellular pathways associated with the healing of skin ulcers MATERIALS AND METHODS: Public data of transcriptomes, GEO access code of GSE121996 were analyzed. The samples were examined using bioinformatics tools through the Galaxy platform. Subsequently, a filtering was performed based on the ratio of the “fold-change” expression level and the adjusted p-value < 0.05, in order to identify the differentially expressed microRNAs comparing the moments: 0h vs 24hr, 0h vs 5 days and 24hr vs 5 days DISCUSSION AND RESULTS: We identified 56, 82 and 46 differentially expressed miRNAs at 0h vs 24hr, 0h vs 5days and 24h vs 5 days, respectively. Among the identified microRNAs, Mir129, Mir155, Mir204 and Mir211, Mir34c, Mir298 and Mir224 stand out, which regulated genes involved in the stages of inflammation and cell proliferation in wound healing CONCLUSION: Based on the results, it was possible to evidence a set of microRNAs differentially expressed throughout the healing process. Of these, Mir34c, Mir298 and Mir224 stand out, which in the literature are involved with cancers, but which in this work are associated with pathways of signaling associated with wound healing, aiding in the inflammatory response, migration of keratinocytes and fibroblast differentiation. The future perspective is to establish a correlation between these microRNAs and their target genes during wound healing of sickle cell ulcers in a biological system

Keywords: Sickle cell ulcers, transcriptome, Bioinformatics / **Supported by:** FAPESB

V - Phase Separation in Biological Systems

V.01 - Nucleic Acid-Driven Condensation of Coronavirus Nucleocapsid Protein: SARS-CoV2 versus hCoV-HKU1

Clara Malizia Leal Ferreira da Motta[1], Mariana Juliani do Amaral[2], Ana Elisa Ciambarella[1], Jéssica de Azevedo[3,4], Fábio Almeida[3], Marcius da Silva Almeida[3], Anderson de Sá Pinheiro[1]
[1]Depto de Bioquímica, Instituto de Química, [2]Depto de Biotecnologia, Faculdade de Farmácia, [3]IBqM, Instituto de Bioquímica Médica Leopoldo de Meis, [4]CENABIO, Centro Nacional de Biologia Estrutural e Bioimagem, Univ. Federal do Rio de Janeiro (RJ, Brazil)

INTRODUCTION: Coronavirus nucleocapsid protein (N) is a multifunctional RNA-binding protein that play major roles in nucleocapsid assembly and discontinuous transcription through the specific interaction with transcriptional regulatory sequences (TRSs). OBJECTIVES: Here, we analyzed the ability of SARS-CoV2 and HKU1-CoV N-terminal domain of N (N-NTD), with or without the serine-rich C-terminal region (SR), to undergo liquid-liquid phase separation (LLPS) *in vitro*. MATERIALS AND METHODS: SARS-CoV2-N-NTD and N-NTD-SR as well as hCoV-HKU1-N-NTD and N-NTD-SR were cloned into pET28a and expressed in Escherichia coli BL21 DE3 as His6-fusion proteins. All proteins were purified by a combination of nickel-affinity and size exclusion chromatography, and further analyzed by phase contrast microscopy and turbidity measurements. DISCUSSION AND RESULTS: SARS-CoV2-N-NTD-SR, but not N-NTD, formed condensates in the presence of nucleic acid, suggesting that the SR region is required for nucleic acid-driven phase separation. In contrast, both hCoV-HKU1-N-NTD and N-NTD-SR formed droplets at same conditions, indicating that hCoV-HKU1-N-NTD(SR) has a higher tendency for phase separation. Interestingly, hCoV-HKU1 droplets were disposed in bunches, suggesting a gel-like property. dsTRS triggered condensation of SARS-CoV-2 and hCoV-HKU1-N-NTD-SR at 1:1 and 2:1 (protein:DNA) molar ratios. However, excess DNA completely dissolved the condensates. In the presence of single-stranded TRS (ssTRS(+) or ssTRS(-)), N-NTD-SR condensates were smaller. Addition of 1,6-hexanediol decreased the number of condensates by about 30%, while salt excess completely dissolved the N-NTD-SR condensates, suggesting that electrostatic contacts play a major role in phase separation. At acidic pH (5.5), the number and size of condensates increased, suggesting that lower pH induces LLPS. CONCLUSION: LLPS exists in cellular systems and are related to various mechanisms in cells. The presence of the SR region seems to play an important role in LLPS, thus is probably one of the regulators of this phenomenon. Results obtained provide a possible mechanism by which the N protein of coronavirus can employ its function of regulating viral transcription and replication. - **Keywords:** Liquid-liquid phase separation, Nucleocapsid protein, Coronavirus

V.02 - Investigation of the Phase Transitions of The Circadian Clock Regulated RNA-Binding Protein AtGRP7

Bruna Almeida Brito [1], Anderson de Sá Pinheiro[1]
[1]Departamento do Bioquímica, Universidade Federal Do Rio De Janeiro (rio de Janeiro, Brasil)

INTRODUCTION: AtGRP7 (Arabidopsis thaliana glycine-rich protein 7) is a highly studied member of the plant glycine-rich protein family, which is known to regulate key physiological processes, including circadian rhythm, flowering time and abiotic stress response. AtGRP7 is an RNA-binding protein formed by an N-terminal RNA recognition motif (RRM) and a C-terminal disordered region enriched in glycines. Due to its amino acid sequence bias and disordered nature, we hypothesized that AtGPR7 undergoes liquid-liquid phase separation, a physical phenomenon responsible for the formation of membraneless organelles. OBJECTIVES: Here, we investigated the ability of AtGRP7 to form biomolecular condensates *in vitro*. MATERIALS AND METHODS: AtGRP7 (residues 1-176) was cloned into RPB1B and expressed as a His6-tag fusion protein. His6-AtGRP7 was solubly expressed in Escherichia coli BL21 DE3 at 18 oC and 1 mM IPTG. AtGRP7 was purified by nickel-affinity and size exclusion chromatography, and further analyzed by phase contrast microscopy. DISCUSSION AND RESULTS: Bioinformatic predictors, such as PONDR, CIDER, PScore, PLAAC, and catGRANULE, suggested that AtGRP7 contains a C-terminal prion-like disordered region that may function as a liquid-liquid phase separation driver. Using optical microscopy, we showed that full-length AtGRP7 formed spherical, micron-sized droplets *In vitro* at concentrations as low as ~10 μM. However, its isolated RRM domain was unable to undergo phase separation, suggesting that that the C-terminal glycine-rich region drives AtGRP7 condensation. We are currently investigating the effects of physicochemical parameters, such as pH, temperature and ionic strength, on the phase transitions of AtGRP7, in order to map its phase diagram. In addition, we are investigating the effect of RNA binding on the number, size and material properties of AtGRP7 condensates. CONCLUSION: The ability of AtGRP7 to undergo liquid-liquid phase separation may be related to its function as a stress adaptor protein in Arabidopsis thaliana.
Keywords: AtGRP7, Arabidopsis, phase separation / **Supported by:** CAPES

V.03 - The Plant RNA-Binding Glycine-Rich Protein AtGRP2 Phase Separates into Gel-Like Condensates

Giovanna S Melo [1], Clara L F Malizia-Motta[1], Gilberto Sachetto-Martins [1], Anderson S Pinheiro[1]
[1]Departamento de Bioquímica, Universidade Federal do Rio de Janeiro (, Brasil), [2]Departamento de Genética, Universidade Federal do Rio de Janeiro (, Brasil)

INTRODUCTION: Membraneless organelles, cellular functional units that are not limited by a lipid bilayer, are supramolecular complexes of intrinsically disordered multivalent proteins and RNA assembled via liquid-liquid phase separation (LLPS). AtGRP2 (Arabidopsis thaliana glycine-rich protein 2) is an RNA-binding protein that plays key roles in the plant response to abiotic stress as well as regulation of flowering time. AtGRP2 consists of an N-terminal cold shock domain (CSD) followed by a disordered C-terminal region containing two CCHC-type zinc fingers and glycine-rich segments. OBJECTIVES: Here, we investigated the ability of AtGRP2 to form biomolecular condensates *in vitro*. MATERIALS AND METHODS: AtGRP2 (residues 1-203) was cloned into pETM30-MBP and expressed in Escherichia coli BL21 DE3 as a His6MBP-tag fusion protein. His6MBP-AtGRP2 was purified by nickel-affinity and anionic exchange chromatography. LLPS assays were initiated by cleaving the His6MBP tag with TEV protease and analyzed by phase contrast microscopy. DISCUSSION AND RESULTS: Bioinformatic tools, such as PONDR, CIDER, PScore, PLAAC, and catGRANULE supported the hypothesis that AtGRP2 LLPS is driven by its C-terminal glycine-rich region. Full-length AtGRP2, but not its isolated CSD, formed spherical, micron-sized droplets *in vitro*. AtGRP2 condensates were deposited at the bottom of the coverslip and did not undergo fusion, suggesting a gel-like structure. The increase in temperature (37 oC for 1h) led to an increase in number (but not size) and appearance of condensates arranged in bunches, supporting the gel-like property. SYPRO orange binding confirmed the proteinaceous nature of AtGRP2 condensates. Moreover, incubation with 10% 1,6-heaxanediol abrogated AtGRP2 LLPS, while 1 M NaCl had no effect on the number and size of condensates, suggesting that hydrophobic interactions play a major role in AtGRP2 phase separation. CONCLUSION: The ability of AtGRP2 to phase separate into gel-like condensates may be related to its role in cold stress response.

Keywords: AtGRP2, Arabidopsis, phase separation

V.04 - Phase Separation Research Applied to The Study of The Biological Function of Prion Protein

Maria Heloisa de Oliveira Freire [1], Mariana Juliani do Amaral[1], Rafael Linden[2], Yraima Cordeiro[1]
[1]Faculdade de Farmácia, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil), [2]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brasil)

INTRODUCTION: Although the biological function of the prion protein (PrP) is still unknown, putative functions are based on PrP-interacting molecules present on the cell surface, such as the co-chaperone hop/STI1, the laminin receptor precursor (LRP) and the neural cell adhesion molecule NCAM. It is currently considered that PrP is a cell surface scaffolding protein for a variety of signaling modules, which can be allosterically regulated and trigger differing functional consequences. Recombinant murine PrP (rPrP) undergoes *In vitro* liquid-liquid phase separation (LLPS) and the multivalency of interactions involving PrP suggests that LLPS may occur in the plasma membrane, a process associated with signal transduction. OBJECTIVES: Our goal is to test the interaction of PrP with multiple protein ligands simultaneously and whether the multivalency of these interactions results in LLPS. MATERIALS AND METHODS: Herein, we used full-length recombinant PrP (rPrP23-231), recombinant STI1 (His6-STI1) and synthetic peptides from STI1, NCAM and LRP, previously described to interact with PrP. We used isothermal titration calorimetry (ITC) to investigate the interaction of PrP with these ligands; circular dichroism (CD) to investigate allosteric effects on PrP as a result of interaction with ligands in different orders; microscopy, to investigate whether the association of rPrP with these ligands modulates its LLPS. DISCUSSION AND RESULTS: The interaction of rPrP with STI1 and LRP peptides was confirmed by ITC. Furthermore, our CD studies showed relevant changes in the secondary structure content of rPrP as a result of the interaction with ligands in different interacting orders. Finally, our microscopy data showed that the association of rPrP with His6-STI1 in solution produced LLPS. CONCLUSION: Subsequently, we will investigate whether rPrP undergoes LLPS in the presence of these ligands in a membrane mimetic system, which would support its role as a scaffold protein in the membrane and could provide essential elements to broaden the understanding of the biological function of PrP.

Keywords: Liquid–liquid phase separation, Prion, Scaffold protein
Supported by: FAPERJ, CNPq and CAPES

V.05 - Optimization of differential ultracentrifugation for the purification of exosomes from human biological fluids

Romuel Barros Costa Silva [1,5], Jenniffer Ione de Souza Silva[1,5], Eliana de Medeiros Oliveira[2,5], Letícia Barbosa Kawano-Dourado[4], Alexandra Susana Latini[1,5]

[1]Centro de Ciências Biológicas , Laboratório de Bioenergética e estresse Oxidativo (SC, Brasil), [2]Pró-reitoria de pesquisa, Laboratório Central de Microscopia Eletrônica (SC, Brasil), [3]Centro de ciências da saúde , Grupo de estudo de interação entre micro e macromoléculas (SC, Brasil), [4]Departamento de Pneumologia, Instituto de Pesquisa HCor (SP, Brasil), [5]Departamento de Bioquímica, Universidade federal de Santa Catarina (Santa Catarina, Brasil)

INTRODUCTION: Exosomes, membrane-bound extracellular vesicles (30-150 nm), have emerged as promising non-invasive biomarkers for the detection, prognosis and therapeutics of several diseases due to their ability to regulate cellular phenotypes. OBJECTIVES: To establish a method for the isolation of exosomes from human plasma and urine. MATERIALS AND METHODS: Samples from healthy volunteers were diluted (1:10; V/V) in phosphate buffered saline solution, filtered (hydrophilic 0.22 µm filter), and submitted to different centrifugation (from 3,000-12,000 x g) and ultracentrifuge conditions (from 45,000-200,000 x g) (Optima XE-100 ultracentrifuge; Beckman Coulter Life Sciences, USA). The exosomes' size was assessed by determining the mean hydrodynamic diameter (Z-average) (Zetasizer nano ZS equipment; Malvern Instruments, UK) by using the dynamic light scattering (DLS) technique. Transmission electron microscopy (TEM) was used to visualize the morphology and confirm the size of exosomes (JEM-1011 electron microscope; Jeol LTD, Japan). DISCUSSION AND RESULTS: The most purified vesicle preparations from plasma resulted from two centrifugations at 3,000 x g for 20 min and 12,000 x g for 30 min, followed by two ultracentrifugation steps of 45,000 x g for 2 h and 90,000 x g for 90 min. The preparations generated vesicle suspensions with a Z-average of 148 nm, which is comparable to exosome size. The same protocol was applied to urine samples; however, the ultracentrifugation steps were performed at 45,000 x g for 1 h and 100,000 x g for 90 min. DLS analysis showed vesicles with a Z-average of 34 nm. The efficacy of the two protocols was confirmed by TEM analysis that showed vesicles delimited by a lipid bilayer sizing between 30-130 nm. CONCLUSION: We established a protocol for the isolation of plasma and urine exosomes that guarantees high level of purification at a low cost, promoting the study of the role of exosomes in physiology and pathology.

Keywords: exosome isolation, electron microscopy, particle size analyzer / S**upported by:** CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

X - Biomimetic Systems and Membrane Biophysics

X.01 - Effect of Hydrotropic Anions on Zwitterionic Surfactant Micelles

Matheus Pires Cortez [1], Laura Mortara[1], Hernan Chaimovich[1], Iolanda Midea Cuccovia[1]
[1]Instituto de Bioquímica, Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: Zwitterionic Surfactants (ZS) are used in protein solubilization, vaccine preparation, and membrane models. Salts of the Hofmeister series promote specific effects on micelle formed by ZS. Here we analyzed salt effects on two ZS´s, namely dodecyl-phosphocholine (DPC) and dodecyl-dimethylammonium propanesulfonate (DPS). We compared critical micelle concentrations (cmc) and micellization enthalpies in the presence of sodium perchlorate (NaClO4), benzoate (NaBZO), benzene sulfonate (NaBZS), and trifluoromethanesulfonate (NaTf). OBJECTIVES: Understand the effect of anions on the thermodynamics of micellization of two ZW´s with opposed dipoles. MATERIALS AND METHODS: DPC (Avanti Polar Lipids). DPS, NaClO4, NaBZS and NaBZO (Sigma Chem. Co). NaTf was prepared neutralizing HTf with NaOH. Micellization Enthalpy and cmc were determined by Isothermal Titration Calorimetry (ITC) at 25°C and 100 mM for all salts. DISCUSSION AND RESULTS: In pure water, the cmc of DPS was higher (3.6 mM) than that of DPC (1.4 mM). DPS cmc decreased upon salt addition. Salts did not affect the cmc of DPC. With DPS, salt addition decreased micellization enthalpy, from 3 KJ/mol in water to -7.2 KJ/mol in NaClO4. With DPC, the micellization enthalpy decreased from 5.2 KJ/mol in water to 1.1 KJ/mol in NaClO4. Although to a higher degree for DPS, the enthalpy decrease followed the trend Bzo>BZS>Tfl> ClO4. CONCLUSION: In DPS, the positive charge is more internally localized in the micelle when compared to DPC, with a positive charge at the micelle interface. This different charge position in the surfactant chain causes a more significant effect of the anions on the physicochemical changes felt by the surfactant aggregates formed. This effect is sharper in DPS, where the positive charge is closer to the micelle hydrophobic core. **Keywords:** Dodecyl phosphocholine, Hofmeister series, Zwitterionic detergents

X.02 - Deuterated Polyunsaturated Fatty Acids Inhibit Photoirradiation-induced Lipid Peroxidation in Lipid Bilayers

Márcia Silvana Freire Franco [1], Alexander M Firsov[2], D. V Chistyakof[2], Sergey Goriainov[3], Marina G Sergeeva[2], Elena Avramovna Kotova[2], Maksim A Fomich[2], Andrei V Bekish[4], Olga L Sharko[4], Vadim V. Shmanai[4], Rosângela Itri[5], Maurício da Silva Baptista[1], Yuriy Nikolaevich Antonenko[2], Mikhail S Shchepinov[6]
[1]Biochemistry Department, University of São Paulo (São Paulo, Brazil), [2]Belozersky Institute of Physical-Chemical Biology, Lomonosov Moscow State University (Russia), [3]SREC, Peoples' Friendship University of Russia (, Russia), [4]Institute of Physical Organic Chemistry, National Academy of Science (Minsk, Belarus), [5]Applied Physics Department, University of São Paulo (São Paulo, São Paulo), [6]Inc., RETROTOPE (California, United States)

INTRODUCTION: Lipid peroxidation (LPO) of Polyunsaturated Fatty Acids (PUFA) is related to several diseases, making PUFAs an important target in Drugs Discovery. Deuterium Reinforced Polyunsaturated Fatty Acids (D-PUFA) are PUFA analogues with deuterium in the bis-allylic positions, which are known to reduce the rate of LPO oxidation. However, the protection efficiency of D-PUFA had never been investigated for photosensitized LPO OBJECTIVES: In this work, we induced type I and type II photooxidative damage and observed the protection of D-PUFAs. MATERIALS AND METHODS: We induced LPO photodynamically, using illumination in the presence of photosensitizer trisulfonated aluminum phthalocyanine (AlPcS3) in unilamellar vesicles (GUV) and Lipossomes. The membrane damage in GUVs was assessed by optical phase contrast intensity observations. Sulforhodamine B leakage, was detected by fluorescence correlation spectroscopy (FCS). Absorbance at 234nm was correspondet to dienes conjugates formation in liposomes. Ultra Performance Liquid Chromatograph - Tandem Mass Spectrometer (UPLC-MS/MS) detected the oxidized linoleic acid (Lin) derived metabolites. DISCUSSION AND RESULTS: No damage was observed in GUVs made mixture of 20% mol of D-PUFA (D2-Lin-PC or D10-docosahexanenoic-PC) in 80% mol H-Lin-PC.The presence of 20% mol of α-tocopherol in 80%mol H-Lin-PC leads dye leakage from liposomes, revealing a pro-oxidant action. The addtion of tocopherol in D2-PUFAs vesicles increased the resistance of PUFAs to oxidative damage. CONCLUSION: The replacement of the bis-allylic hydrogen atoms with deuterium atoms, arrests PUFA autoxidation due to the isotope effect. The synergistic action of alpha tocopherol relate to D2PUFA could be elucidated by Tocopherol-mediated Peroxidation (TMP) cycle model, in wich the termination of a radical chain occurs when a peroxidizing particle captures a second radical from the aqueous medium (ROO• + α-Toc• - nonradical products). However the presence of D-PUFAS in LUVs of α-tocopherol decrease the ROO• formation resulting in a protective effect to LPO. The protection against photodamage of D-PUFAs make then a potential preventive and/or therapeutic agent. **Keywords:** Photodynamic Therapy, Lipid peroxidation , Deuterium Reinforced PUFA / **Supported by:** RETROTOPE

X.03 - Phase diagrams of the lipid domains of BMP

Jhuann Piedro Nogueira [1]
[1]Department of Applied Physics, Institute of Physics (, Brazil)

INTRODUCTION: Micron-scale liquid domains are easily produced in vesicles composed of ternary mixtures of a high melting temperature lipid, a low melting temperature lipid, and cholesterol. The process of forming GUVs, also called Giant unilamellar vesicles, allows the use of such mixtures in varying concentrations. In this way, it is possible to mimic cell organelles and plasma membranes. The lipid bis(monoacylglycerol)-phosphate (BMP) is found in the composition of lysosomes and endosomes. However, its role in the organelle is poorly understood. Further, information about the phase diagram of systems containing BMP lacks in the literature. OBJECTIVES: To understand the importance of BMP in the composition of the lysosomes membrane. MATERIALS AND METHODS: To study the biophysical changes in the mimetic membranes, GUVs (Giant Unilamellar Vesicles) were grown by the electroforming method with some mixtures of the following lipids: DOPC, SM and Cholesterol to observe raft-like lipid domains in plasma membrane models. The formation and behavior of the lipid domains according to the concentration and temperature of formation is recorded using phase contrast and florescence microscopy. In the following, membranes mixtures containing BMP will be addressed. DISCUSSION AND RESULTS: Initially the phase diagram of ternary lipid mixtures composed of DOPC, SM and Chol was carried out with different molar ratio. It was possible to identify compositions displaying liquid-ordered (Lo)- liquid disordered (Ld) phase coexistence. New assays and new compositions including BMP are still necessary.

Keywords: lipid , phase , domains

X.04 - AN IMIDAZOLE-CONTAINING NOVEL CATIONIC SURFACTANT

Hernan Chaimovich [1], Laura Mortara[1], Carretero Gustavo B.[1], Sanapali Nagi Reddy[1], Samuel Kim[1], Karin Riske[2], Iolanda Midea Cuccovia[1]
[1]Bioquímica no Instituto de Química, Univ. de São Paulo (SP, Brasil), [2]Biofísica, Univ. Federal do Estado de São Paulo (SP, Brasil)

INTRODUCTION: Vesicles formed by cationic surfactants are used for gene and drug delivery and recently for vaccines. Imidazole-derived surface-active ionic liquid derivatives have many applications in nanotechnology and biotechnology. OBJECTIVES: Synthesis and characterized a novel cationic surfactant containing an imidazolium head group and two alkyl chains and comparison with a similar compound. MATERIALS AND METHODS: 1,3-Di-hexadecyl-imidazolium chloride (DHImC) was synthesized and characterized. Dihexadecyldimethyl-ammonium chloride (DHDAC) was prepared by ion exchange from commercial DHDAB. 4-trimethylammonium-2,2,6,6-tetramethylpiperidine-1-oxyl iodide (CAT) was encapsulated in SUVs of both surfactants in 5 mM NaCl (extruded with 100 nm pore membrane). EPR spectra for 5-MeSL and 16-MeSL (Methyl n-DOXYL-stearate) were incorporated in the surfactant bilayer. Differential Scanning Calorimetry determined transition temperatures (Tc). Optical Microscopy images were obtained for Giant Unilamellar Vesicles (GUVs). DISCUSSION AND RESULTS: Tc´s were: 30.1 0C (DHDAC) and 48.7 °C (DHImC). CAT-EPR spectra of SUVs, recorded after adding Ascorbic Acid (AA), showed a stable residual EPR signal attributed to aqueous core-entrapped CAT. 5- and 16- MeSL spin labels showed a more ordered region closer to the polar head (5-MeSL) and a less ordered region at the hydrophobic core of the bilayer (16-MeSL). The mobilities at different temperatures reflected a gel phase before the Tc. Optical Microscopy images also showed a clear difference in vesicle organization before and after the transition temperature. CONCLUSION: Conclusions. DHImC forms stable vesicles with an aqueous inner compartment. DHDAC and DHImC, characterized by EPR, showed differences in mobility along the bilayer and Tc´s. The remarkable differences in Tc´s for surfactants with the same alkyl chain size can be explained by the the π-π head group interactions on the imidazolium compounds. DHImC vesicles constitute a novel and potentially useful delivery system.

Keywords: Imidazole surfactant, Delivery Agents, Vesicle properties / **Supported by:** FAPESP, CNPq

X.05 - The Interaction of Levofloxacin with Ionic Membranes

Mariana Cunha de Souza [1], Evandro Luiz Duarte[1], Maria Teresa Moura Lamy[1], Gabriel Silva Vignoli Muniz[2]
[1]Departamento de Física Geral, Universidade de São Paulo (São Paulo, Brasil), [2]Divisão de Físico-Química, Universidade de Brasília (Goiás, Brasil)

INTRODUCTION: Levofloxacin's (LVX) molecule is naturally fluorescent and classified as a fluoroquinolone (FQ). DPPC and DPPG are, respectively, zwitterionic and anionic lipids that can model biomembranes. OBJECTIVES: In view of levofloxacin's relevance to clinical use, this work focused on understanding the drug's mechanism of action and how its interactions with membranes may affect itself and the biological structures involved. MATERIALS AND METHODS: Differential scanning calorimetry (DSC) and electron paramagnetic resonance (EPR), using 5-PCSL and 16-PCSL, were applied to investigate changes induced by LVX on 100 nm diameter vesicles. Considering LVX's intrinsic fluorescence, static and time-resolved fluorescence spectroscopies monitored alterations on LVX during the interaction with the lipid bilayer. DISCUSSION AND RESULTS: For DPPG, the addition of 10 mol% of LVX causes the existence of two thermal peaks, which were interpreted as the coexistence of regions with high antibiotic concentrations and regions of free lipids (bulks). From EPR studies, a crescent membrane packing is noticeable for DPPG vesicles. However, no significant alterations were observed using DSC, EPR, and fluorescence spectroscopy techniques for DPPC. LVX's emission on saturating conditions of DPPG presented a redshift of 29 nm. This effect is probably due to the lower local pH near DPPG vesicles caused by electrostatic attraction, and indicates that levofloxacin can be used to probe microenvironments of anionic subtracts; in accordance with previous experiments using other FQs. Partition constants for LVX in DPPG membranes, in both gel and fluid phases, were determined: $K_p = (1.9 \pm 0.2)\times10^2$ e $(2.9 \pm 0.2)\times10^2$, respectively. CONCLUSION: LVX strongly interacts with DPPG vesicles, and it is highly suggested that the binding between LVX and DPPG leads to the protonation of the antibiotic. Furthermore, the data obtained indicates that LVX presents low interaction with zwitterionic membranes. Such findings contribute to safer treatments, to the planning of new drugs and to the development of drug delivery systems.
Keywords: levofloxacin, membranes, antibiotic

X.06 - Betulinic Acid Interaction with Biomimetic Membranes Under Photooxidative Stress

itória Delfino Gonçalves [1]
[1]Departamento de Física Aplicada, Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION: Currently, autophagy modulation has emerged as a promising therapeutic approach not only for cancer, but also for other diseases. Betulinic Acid (BA) is a triterpenoid acid that can be extracted from various plant species. Important data from the literature show that BA acts as an antitumor agent, inducing apoptosis in a variety of human malignancies. It is also known that phenothiazine photosensitizers (FSs), when photoactivated, can modulate cellular and molecular responses. In this context, Methylene Blue (MB), which is a FS, has previously been shown to be able to promote autophagic cell death in the context of photodynamic therapy (PDT) by parallel damage in mitochondria and lysosome. In this work, we sought to explore the combined action of BA in photo-oxidized biomimetic membranes. Such a study provides light to think about a coupled treatment in tumor cells using PDT followed by treatment with BA. OBJECTIVES: Comprehend the action of BA coupled with MB in biomimetic membranes in the context of the photooxidative stress process. MATERIALS AND METHODS: To study the biophysical changes in mimetic membranes, GUVs (Giant Unilamellar Vesicles) containing BA were grown by the electroformation method with some mixtures of the following lipids: POPC, hydroperoxidized POPC (POPCOOH), DOPC, DPPC, SM and Cholesterol. Then, MB was added to the mixture with already grown GUVs. The morphological effects on the membranes were observed by optical microscopy in phase contrast and fluorescence during photo-activation of MB. Subsequently, analyzes of changes in the properties of the lipid bilayers were performed. DISCUSSION AND RESULTS: The methodology used in previous works to analyze the effect of betulinic acid on membranes was not reproducible, therefore, a methodological adaptation was carried out. Now, GUVs are being cultivated already containing BA. CONCLUSION: The results are still under discussion and should generate conclusions soon.
Keywords: Photo-oxidation, Betulinic Acid, Photodynamic Therapy / **Supported by:** Programa Unificado de Bolsas-Universidade de São Paulo

X.07 - DIBLOCK COPOLYMERS LEAK LIPOSOMES AND ARE ANTIMICROBIAL

Iolanda Midea Cuccovia [1], Greice Kelle Viegas Saraiva[1], Valdomiro Vagner de Souza[1], Luciana Coutinho de Oliveira[1], Alyne Procópio[1], Maisa Torres Martins[1], Isabel Nunes Aidar[1], Ronaldo Chan Fu Yi[1], Caroline Dutra Lacerda[1], Gustavo Penteado Battesini Carretero[1], Rafael Bezerra de Lira[2], Karin Amaral Riske[2], Roberto Kopke Salinas[1], Hernan Chaimovich[1], Fabio Herbst Florenzano[3]

[1]Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, Brazil (São Paulo, Brazil), [2]Departamento de Biofísica, Universidade Federal de São Paulo (São Paulo, Brazil), [3]Departmento Engenharia de Materiais, Escola de Engenharia de Lorena, Universidade de São Paulo (São Paulo, Brazil)

INTRODUCTION: Immobilized polymers are used to prepare antifouling surfaces, antimicrobial fabrics, and decontamination systems. Here we analyzed the interactions of poly (methyl methacrylate) (PMMA), poly[(dimethylamino ethyl) methacrylate] (PDMAEMA) and their diblock copolymers, prepared at different PMMAm/PDMAEMAn ratios (m/n): PMMA50, PDMAEMA265, PMMA94-b-PDMAEMA88, PMMA50-b-PDMAEMA269, and PMMA48-b-PDMAEMA324, with Large Unilamellar Vesicles (LUVs), Giant Vesicles, GUVs, and evaluated their antimicrobial potential. OBJECTIVES: To compare these polymers to proteins and verify its potential as antimicrobial compounds. MATERIALS AND METHODS: LUVs of phosphatidylcholine (PC) and phosphatidylglycerol (PG) were prepared by extrusion. The effects of polymer type, concentration, pH, and PG:PC ratios on vesicle-incorporated 5,6-carboxyfluorescein (CF) leakage, were analyzed by fluorescence spectroscopy. NMR, Dynamic Light Scattering, Zeta Potential, and microscopy were employed as characterization methods. Antimicrobial activity was measured with Escherichia coli (E.c.) and Bacillus subtilis (B.s.). DISCUSSION AND RESULTS: Positively charged polymers binding to anionic LUVs produced polymer/LUVs aggregates and CF leakage. PC:PG interactions with the cationic polymers were demonstrated by NMR and analysis of GUVs stability. Maximum vesicle leakage was obtained with PMMAm:PDMAEMAn copolymers. The interaction with PC:PG LUVs was greater at pHs where the cationic polymers were partially neutralized, while, at higher pH (pH=10), no interaction was observed. These results were confirmed by NMR, CF leakage, DLS, and zeta potential analyses. The cationic polymers inhibited the E.c. and B.s. growth while PMMA had no effect. CONCLUSION: The cationic polymers permeabilized LUVs with different PC:PG ratios varying with pH, polymer/lipid and PMMA/PDMAEMA ratios. PG favored polymer binding, inducing phase separation at the LUVs bilayer. The copolymers with higher PDMAEMA molar fractions were best for permeabilizing LUVs, while PMMA had no effect. These polymers can be used to prevent bacterial growth on surfaces and are good models to understand the interactions of other polymers with membranes. This system is straightforward compared with proteins, and has characteristics that resemble membrane/protein interactions.

Keywords: copolymers, liposomes, antimicrobial / **Supported by:** FAPESP; CNPq; INCT-FCx; NAP-FCx; CAPES

X.08 - Membrane Fission Induced by Polyunsaturated Lipid Photo-Oxidation

Maria Fernanda Marins [1], Maressa Donato[1], Rosangela Itri[1]

[1]Departamento de Física Aplicada, Universidade de São Paulo (São Paulo, Brasil)

INTRODUCTION. The contributions of proteins to the functions and integrity of cell membranes are widely-known regarding the membrane traffic system. However, the role of lipid composition in this process remains elusive. Depending on the lipid features such as shape, polar groups and alkyl chain composition, membrane curvature can change, interfering in the cell traffic system. Therefore, to understand the role of lipids in the generation and maintenance of membrane morphology, we performed photooxidation experiments. OBJECTIVES. This work aims to determine the effects of the photooxidation in membrane mimetic models composed by polyunsatutared lipids (PUFAs) with different numbers of unsaturations, and relate this to the fusion/fission mechanism. MATERIALS AND METHODS. To investigate the biophysical changes and fission in membrane mimetic models, giant unilamellar vesicles (GUVs) were grown by the electroformation technique. Different ternary lipid mixtures of PUFAS/DPPC(or SM)/Cholesterol at molar ratio 1:1:1, containing 10μM of the photosensitizer pheophorbide were irradiated at 395 nm. Moreover, to evaluate the evolution of membrane curvature during and after photooxidation on membranes, optical video microscopy in phase contrast and epifluorescence was applied. RESULTS AND DISCUSSION. So far, we have noticed that the irradiation of the pheophorbide present in phase separated liquid-ordered (Lo) - liquid disordered (Ld) GUVs maintained the phase coexistence, but it can promote bud formation, loss of optical contrast and, in some GUVs, the imminence of fissioning for the following compositions: DOPC:SM:Cholesterol, POPC:SM:Cholesterol and DOPC:DPPC:Cholesterol. The imminence of membrane fissioning is accompanied by Lo budding outwarding. CONCLUSIONS. Since not all experiments have been performed yet, it is reasonable to conclude that this work has potential to address crucial questions such as the role that lipid geometry has in the membrane fission process, the influence of phase separation and the presence of cholesterol in the fission mechanism, the consequence of oxidized lipids for the membrane spontaneous curvature. **Keywords:** Membrane fission; GUV; Photooxidation.
Acknowledgments: Unified Scholarship Program, University of São Paulo.

X.09 - INTERACTION OF POTENTIAL ANTIMICROBIAL PEPTIDES WITH MEMBRANE MODELS

Maressa Donato Ferreira de Souza [1], José Luiz de Souza Lopes[1], Rosangela Itri[1]
[1]Applied Physics, University of São Paulo (Brasil)

INTRODUCTION: In the recent years, the increasing number of antimicrobial resistance cases has urged the need for the development of new drugs. Antimicrobial peptides have great potential to act in the control of infections caused by resistant bacteria. The temporins, in particular, are a family of peptides isolated from the skin of the amphibian Rana temporaria, which attract attention due to the short size of the peptides, ranging from 10 to 14 amino acid residues OBJECTIVES: We aim at studying and understanding the lytic effect of a temporin peptide (Temporin 1Ca) with potential use as an antimicrobial peptide focusing on multiresistant bacteria, using giant unilamellar vesicles (GUVs) as a membrane model. MATERIALS AND METHODS: To understand the effect of the peptide 1Ca on membranes, GUVs of different lipid compositions (pure POPC lipid and mixed POPC/POPG in different proportions) were growth by electroforming technique. The growth by the electroforming technique consists on the use of two conductive glasses joined with a teflon to form an internal chamber that is filled with sucrose solution. Then, the glasses are connected to a current generator for 1h, and after that the GUVs are removed and diluted in the presence of the peptide for observation in optical microscopy (using phase contrast and fluorescence modes). By the time the GUVs are in contact with the peptide, the interaction is controlled and the solution observed by microscopy DISCUSSION AND RESULTS: It was possible to observe that in the presence of the 1Ca peptide, the vesicles that mimic bacterial membranes (composed of POPC:POPG) were the ones that showed the most contrast loss effects resulting from the formation of pores, thus mixing the internal solution (sucrose) with the external solution (glucose). In vesicles composed only of POPC, these effects were barely insignificant. CONCLUSION: The results indicate that the peptides studied have great potential against Gram positive bacteria bacteria.

Keywords: GUVs, antimicrobial peptides, antimicrobial peptides

X.10 - Luminescent Ruthenium(II) Complexes with Glucose Ligands as Molecular Probes of Lipids Membranes

Victoria Carolina Romero Colmenares [1], Maria Laura da Cruz Garcia[1], Rose Maria Carlos[1]
[1]Departemento de Química, Universidade Federal de São Carlos (São Paulo, Brasil)

Introduction: Alzheimer's disease (AD) and type 2 diabetes mellitus (DM2) are chronic diseases with high prevalence worldwide. The two diseases are directly related to glucose deficiency in the brain and damage to the cell membrane. Objectives: Prepare a glucose-containing Ru(II) luminescent complex to evaluate interaction with neuronal membrane models and evaluate protein cytotoxicity directly associated with these diseases: AD (Aβ) and DM2 (IAPP). Materials and Methods: The ligand (L) was synthesized and characterized by electron spectroscopy in the UV-Vis region (λ_{max} = 230 e 269 nm) e ^{1}H NMR confirming the H-6 (δ = 4.83) loss formation characteristic of precursor 5,6-Epoxy-5,6-dihydro-(1,10)-phenanthroline. The complex *cis*-$[Ru(L)_2(3,4\text{-}Apy)_2]\cdot(PF_6)_2$ was obtained from *cis*-$[Ru(dmso)_4(Cl)_2]$, 3,4-Apy = 3,4-Aminopyridine and L = (1,10)-phenantholine derivatized with glucose. The photophysical properties were obtained through fluorescence spectrophotometry, microscopic images and emission lifetime. Results and Discussion: The synthesis of the complex *cis*-$[Ru(L)_2(3,4\text{-}Apy)_2]\cdot(PF_6)_2$ has spectroscopic properties that allow application as a luminescent molecular probe in a physiological environment. For example, it exhibits intense absorption and emission of the visible region in a physiological environment and interacts with negative cell membrane models. Conclusion: The complex *cis*-$[Ru(L)_2(3,4\text{-}Apy)_2]\cdot(PF_6)_2$ is potential candidate for luminescent probe.

Key words: Ruthenium Complexes, Luminescence, Membrane Model.
Ackonowledgements: CNPq (140936/2021-1), CAPES, FAPESP.

X.11 – PROJECT: Unraveling Fusion Mechanism in Lysosomal Mimetic Membranes

Gabriela Forkel Bizerra DAvilla [1], Tayana Mazin Tsubone[2], Rosangela Itri[1]
[1]Applied Physics Department of Institute of Physics, University of São Paulo (São Paulo, Brasil), [2]Chemistry Department, University Federal of Uberlândia (Minas Gerais, Brasil)

INTRODUCTION: The lysosomal compartments are present in almost all eukaryotic cells and are responsible for digesting macromolecules by endocytosis or autophagy such as aged and/or damaged cellular components. Live-cell imaging has shown that fusion with lysosomes occurs by transient and full fusion events, and indeed, this view of lysosomes as fusogenic organelles is consistent with data showing that lysosomes can fuse with late endosomes, phagosomes, autophagosomes and the plasma membrane under appropriate circumstances. In spite of lysosomes fusion being a fundamental process, it is poorly known how lipid composition modulates the efficiency of fusion in lysosomes. A particular lysosomal membrane feature is that it contains a special lipid bis(monoaclyglycero)phosphate (BMP). It represents <1 mol % of the total phospholipid in most mammalian cells, its content increases to 15% in late endosomes and lysosomes and can comprise more than 70% of the internal membrane domains of these organelles, with an unusual structure and uncommon stereoconfiguration BMP is assumed to be important for the structural and functional integrity of this organelle, playing important roles in the lysosome membrane. However, just few reports related BMP on fusion properties of lysosomes. OBJECTIVES: Investigate fusion events by using lysosomal mimetic membranes containing BMP. Further, under photo-oxidation conditions or using a controlled composition of oxidized lipids, we hope to unravel the impact of membrane oxidation on fusion events on lysosomes. MATERIALS AND METHODS: Mimetic lysosome membranes will be composed of pure BMP and ternary mixtures of BMP:DPPC:Cholesterol or quaternary mixtures of BMP:POPC-OOH:DPPC:Cholesterol represented by giant and large unilamellar vesicles (GUVs and LUVs). We study fusion events by optical microscopy and FRET, using oppositely charged vesicles as a strategy for fusion. DISCUSSION AND RESULTS: Initial results of LUVs composed of lipids with charged membranes will be presented and discussed. CONCLUSION: This project is just starting.

Keywords: BMP, lysosome, Membrane fusion / **Supported by:** CNPq

X.12 - EXPERIMETALLY DETERMINED ASYMMETRIC PHASE DIAGRAM OF DPPC/DOPC

Thais Azevedo Enoki [1,2], Frederick A. Heberl[1]
[1]Department of Chemistry, University of Tennessee (Knoxville, TN, USA), [2]Department of Molecular Biology and Genetics, Cornell University (Ithaca, NY, USA)

The plasma membranes of eukaryotic cells are asymmetric with respect to their lipid composition. Model membrane studies consistently find that simplified outer leaflet mixtures tend to separate into coexisting liquid phases in symmetric bilayers, while inner leaflet mixtures do not. Among the major unanswered questions is how the phase behavior of the two leaflets is coupled in an asymmetric bilayer. To this end, mean field theory has been used to calculate asymmetric phase diagrams, where phase boundaries and tieline orientations depend on the relative strength of in-plane and out-of-plane lipid interaction energies. Here, we report what to our knowledge is the first experimentally determined asymmetric phase diagram for a binary lipid mixture, DPPC/DOPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine / 1,2-dioleoyl-sn-glycero-3-phosphocholine). Different from cholesterol-containing model membranes that separate into coexisting liquid phases, this simpler mixture exhibits gel + fluid coexistence over a range of composition and temperature. We choose this system because it enables a direct comparison with mean field theory. In our experiments, we used calcium-induced hemifusion to generate asymmetric GUVs which we then examined with confocal fluorescence microscopy. The extent of outer leaflet exchange, and hence the asymmetric vesicle composition, was determined through quantitative analysis of fluorescent probe intensities. The hemifusion method produces a wide range of exchange efficiencies in individual vesicles within the same sample preparation, which aids in the construction of phase diagrams. We found that in symmetric vesicles initially exhibiting gel+fluid domains, the phase separation is abolished beyond a threshold level of asymmetry.

Keywords: membranes, DPPC/DOPC, vesicles

X.13 - Ruthenium complex as pH and polarity sensitive sensor for biologic medium diagnosis

Rafaela Ribeiro Paixão [1], Rose Maria Carlos[1], Maria Laura da Cruz Garcia[1], Wallance Moreira Pazin[2], Rafael Cavalieri Marchi[1]

[1]Departamento de Química, Universidade Federal de São Carlos (São Paulo, Brasil), [2]Departamento de Física, Universidade Estadual de São Paulo (São Paulo, Brasil)

INTRODUCTION: Photoluminescent molecules have become essential tools in cell biology, diseases and diagnosis allowing real-time live-cells imaging and detect biologic changes as they occur. Most of fluorescent probes currently used are based on either small organic dyes although transition metal complexes have shown attractive photophysical biologic applications. OBJECTIVES: Herein we develop a luminescent Ru(II) complex for cellular and imaging applications that provides a readout of pH and polarity environment changes. MATERIALS AND METHODS: The *cis*-$[Ru(phen)_2(4ImAC)]^+$, (RuImAC), ImAC = 4-imidazole carboxylic acid complex was synthesized and characterized by NMR spectroscopy. UV–vis absorption and emission were done in buffer solution (pH 2-12) and using organic solvents with RuImAC [10^{-4} M]. Microscopic images were taken on a ZEISS-Axio Observer 7 inverted fluorescence microscope. DISCUSSION AND RESULTS: The RuImAC is stable in solid and fluid medium in the dark and under light irradiation and in aqueous solution shows a broad and strong visible absorption (ε450nm = 8600 mol^{-1} L cm^{-1}) and phosphorescent emission (λmax = 600 nm, τem = 402,68 ns) and large Stokes shift at approximately 5000 cm^{-1}, which eliminates self-quenching and does not interfere with most biomolecules' intrinsic fluorescence emission. Another feature of the emission behavior of RuImAC relevant to biological applications is its sensitivity to pH and polarity changes in the medium. There are significant changes of both maximum and intensity in the emission depending of pH. Emission is also sensitive to solvent polarity: polar and protic (λmax = 715 nm); nonpolar (λmax = 675 nm); polar and aprotic (λmax = 595nm). Luminescence imaging microscopy reveals the interaction of RuImAC with POPG and DOPG membranes, without perturbing its morphology in the dark and enables real time imaging of membrane rupture by light irradiation. CONCLUSION: RuImAC present suitable optic characteristics for luminescent imaging in physiological medium.

Keywords: Fluorescence, Membrane, Ruthenium / **Supported by:** FAPESP (Processo: 2021/09736-6)

X.14 – PROJECT: Correlation Between Oxidative Stress Damage and Aging of Biomembrane

Adriano Marchini Rodrigues Puerta da Silva [1], Koji Kinoshita[1], Rosangela Itri[1]

[1]Applied Physics Department, Institute of Physics (SP, Brasil)

INTRODUCTION: It is known that the photosensitizer-mediated oxidative stress can directly cause cell death, e.g. apoptosis and necrosis, by damaging the cell membrane However, the detailed mechanism behind changes to cell membrane mechanical properties under oxidative stress is poorly understood. Recently, our research group found that there is a significant correlation between singlet oxidative stress and aging for the red blood cell (RBC) membrane mechanical property change. We thus consider that the cell's aging could follow a very fundamental common process of oxidative stress for any type of cell membrane. This result gave us a big question, i.e. "How are the mechanical properties of biomembranes affected during the process of cell aging/death, especially brain cells?" This is because a brain cell typically lasts an entire lifetime (as a comparison: 2 – 4 months lifetime of human RBC). It is considered that the brain cells, which won't be replaced when they die, will be exposed to oxidative stress for the entire lifetime. In this project, we apply the oxidative stress to the target neuronal cell membrane of SH-SY5Y neuroblastoma and investigate the effect for the cell-cell adhesion force and the membrane elasticity by micropipette micromanipulation coupled to optical microscopy. OBJECTIVES: The overall goal is to characterize the cell aging/death of human RBC and SH-SY5Y neuroblastoma cells by investigating the mechanical properties changes of the respective cell membranes under the oxidative stress of photosensitizer porphyrin activation. MATERIALS AND METHODS: Measurements of cell-cell adhesion force and membrane elastic moduli will be carried out by micropipette micromanipulation technique coupled to optical microscopy. DISCUSSION AND RESULTS: This project is just starting.

Keywords: Cell aging, photo-oxidative stress, membrane elastic properties / **Supported by:** CNPq

X.15 - NPP1 and TNAP incorporated into liposomes cooperate synergistically on calcium phosphate-based minerals propagation.

Luiz Henrique da Silva Andrilli [1], Bruno Zocaratto Favarin[1], Heitor Gobbi Sebinelli[1], Mayte Bolean Correa[1], Ana Paula Ramos[1], Massimo Bottini[1,2,3], José Luis Millán[2], Pietro Ciancaglini[1,3]

[1]Department of Chemistry- FFCLRP, University of São Paulo (, Brazil), [2]La Jolla, Sanford Burnham Prebys (, USA), [3]Department of Experimental Medicine, University of Rome Tor Vergata (, Italy)

INTRODUCTION: Endochondral ossification is mediated by matrix vesicles (MVs)- a special class of extracellular vesicles responsible for the initial precipitation of calcium phosphates (CaP). These vesicles are enriched in tissue-nonspecific alkaline phosphatase (TNAP), that through its phosphomonohydrolytic activity produces inorganic phosphate (Pi), from adenosine-5'-triphosphate (ATP) and inorganic pyrophosphate (PPi) and ectonucleotide pyrophosphatase/phosphodiesterase 1 (NPP1), an ATP phosphodiesterase, producing adenosine-5'-monophosphate (AMP) and PPi. OBJECTIVES: In this study is evaluating the synergically ATP hydrolysis made by NPP1 and TNAP incorporated into liposomes, correlating kinetics and *In vitro* mineralization assays, and determinate the calcium-phosphate based minerals quality. MATERIALS AND METHODS: Liposomes composed by 1,2-dipalmitoylphosphatidylcholine (DPPC) and carrying TNAP and/or NPP1 were prepared using different lipid/enzyme molar ratios. Non-stationary state kinetics assays of ATP hydrolysis were performed in the absence/presence of Suramin, a NPP1 inhibitor. Turbidimetry assay was used to evaluate the potential of the proteoliposomes in the *In vitro* propagation of calcium-phosphate minerals. ATR-FTIR was used to investigate the chemical composition of the precipitated minerals. DISCUSSION AND RESULTS: NPP1-containing proteoliposomes exhibited both phosphomonohydrolase and pyrophosphohydrolase activities as revealed by the presence of ADP and AMP, respectively, as by-products in all the assays. The presence of Suramin reduced by 65% and 75% the amount of ADP and AMP, respectively, formed after 48 hours of reaction. The presence of TNAP in NPP1-containing proteoliposomes decreased Suramin inhibition. ATR-FTIR suggest the octacalcium-phosphate-based hydroxyapatite and calcium-deficient hydroxyapatite minerals in the presence and absence, respectively, of Suramin at the *In vitro* mineralization assays. CONCLUSION: ATR-FTIR as revealed PPi precipitation at the *In vitro* mineralization assay using NPP1-proteoliposomes. Taken together, the results prove that NPP1:TNAP-proteoliposomes support the propagation of CaP, mimicking the enzymatic processes of MVs. Different proportion of the enzymes in the presence and absence of a NPP1 inhibitor can be used to elucidate the complex balance of Pi/PPi homeostasis in biomineralization.

Keywords: NPP1, mineralization, proteoliposomes
Supported by: FAPESP (2021/13140-1)

X.16 - Yeasts maintain membrane order under ethanol stress

Natalia Wilke [1]
[1]Depto de Quimica Biologia Ranwel Caputto, Centro de Inv en Quimica Biologica (CONCET). (Cordoba, Argentina)

INTRODUCTION: Yeasts support different environmental conditions, including stress situations. Given the broad applications of yeast in food industry, adaptation of yeast to stress conditions is an active research area. It has been reported that membrane fluidity is affected by environmental stresses, and thus, regulation of membrane biophysical properties may be a key point for yeast adaptation. OBJECTIVES: To understand how yeast membranes react, and eventually adapt, to the presence of exogenous ethanol. MATERIALS AND METHODS: S. Cerevisiae strains: BY4741 (acclimated and non-acclimated to high ethanol levels); BY4741-erg6Δ; baker´s yeast. The Generalized Polarization (GP) of the fluorescent probe Laurdan (sensitive to dipolar relaxation of water molecules close to the membrane) was used for membrane characterization. DISCUSSION AND RESULTS: GP of unstressed cells was 0.2-0.3 for all strains, indicating high order, such as that for liquid-ordered membranes. The similar value found in erg6Δ yeast suggest that the absence of ergosterol is buffered by other lipids, probably by its precursor Zymosterol. In the presence of low ethanol amounts (20%v/v, GP decreased abruptly, and cells were no longer viable, suggesting that cells maintain the GP value while alive. In line with this, the GP value of liposomes or of death yeasts changed continuously with ethanol. BY4741 yeast acclimated to high ethanol levels showed similar behavior, but with higher initial GP values (GP= 0.4), indicating an increase in the membrane order. CONCLUSION: Maintaining a constant and high GP value is crucial for cell viability. Cells adapt membrane composition in order to keep them with a certain order.

Keywords: membrane adaptation, water structure, membrane order
Supported by: FONCYT, SECYT-UNC

A

Abdalian, S.B.; *SP.15.01*
Abdelhay, A.; *U-14*
Abrahão, G.D.; *R-07*
Abrahão, J.; *B-15, B-34*
Abrahão-Souza, P.C.; *M-01*
Abrahim, M.; *E-38*
Abrahim-Vieira, B.A.; *D-24*
Abreu L.; *B-17*
Abreu, B.O.; *F-48*
Abreu, E.; *S-04*
Abreu, E.L.; *K-49*
Abreu, J.S.S.; *F-83*
Abreu, L.; *A-01*
Abreu, L.A.; *B-50*
Abuchery, B.E.; *M-05*
Acosta, A.C.; *K-49*
Adolpho, L.F.; *SM.03.04*
Adriani, P.P.; *F-27, F-30, K-68*
Adur, M.A.; *S-12*
Affonso, R.; *F-20, F-35*
Afonso, C.C.S.; *U-15, N-06*
Afonso, L.G.; *K-32*
Agner, G.C.; *R-10*
Agnes, J.P.; *K-12*
Aguiar, K.S.A.; *F-23*
Aguiar, S.B.; *E-01*
Aguiar, T.W.A.; *T-03, T-20*
Aguiar, W.S.; *F-05, O-03, O-07*
Ahmad, S.; *D-54*
Ahmed, Z.; *E-18, E-62*
Aidar, I.N.; *X-07*
Aires, A.L.; *T-03, T-20*
Aires, R.; *P-18, U-24*
Aires-Lopes, B.; *K-28*
Aisenbrey, C.; *P-05, P-15*
Alarta, C.L.S.; *S-12*
Alberici, L.C.; *B-31, B-38*
Albernaz, F.P.; *E-26, E-43*
Albuquerque, M.C.P.; *T-20*
Albuquerque, M.C.P.A.; *T-03*
Alencar A.L.F.; *M-01*
Alencar, L.M.R.; *C-06, C-08, F-21, L-01*
Alencar, L.M.R.; *SP.11.04*
Alexandrino-Jr, F; *G-01, G-11*
Alisson-Silva, F.; *J-05, J-06*
Almada, J.C.; *P-17*
Almazloum, A.A.; *D-15*
Almeida M.; *K-53*
Almeida V.D.; *K-60*
Almeida, A.P.; *F-14, F-77, F-81*
Almeida, B.R.; *K-58*
Almeida, C.J.L.R.; *M-18*
Almeida, C.P.; *R-14*
Almeida, C.V.; *M-27, P-19*
Almeida, F.; *O-09*
Almeida, F.C.L.; *D-42, E-11, E-24, E-30, E-37, E-42, E-54, E-57, E-87, T-14, V-01*
Almeida, I.O.; *H-01*
Almeida, L.; *G-35*
Almeida, L.; *SP.18.03*
Almeida, L.H.O.; *M-27, M-33, P-13*
Almeida, L.R.; *E-04*
Almeida, M.S.; *E-45*
Almeida, M.S.; *V-01*
Almeida, R.; *K-03*
Almeida, R.M.; *G-05*
Almeida, R.S.; *U-27*
Almeida, V.D.; *K-09, K-70*
Almeida-Oliveira, F.; *A-10*
Altei, W.F.; *K-37*
Alvarenga, E.S.L.; *A-03, A-26*
Álvarez, C.; *C-16*
Álvarez, L.; *E-89*
Alves S.A.S.; *M-01, M-24, K-18*
Alves, A.P.S.; *B-34*
Alves, C.; *E-86*
Alves, E.S.F.; *P-09*
Alves, J.N.; *K-42*
Alves, J.R.; *D-18*
Alves, L.C.; *A-16*
Alves, L.P.S.; *R-05*
Alves, M.H.M.E.; *F-50*
Alves, R.R.V.; *A-16*
Alves, W.A.; *SP.02.04*
Alves, W.S.; *R-07, R-10*
Alves-Filho, J.C.; *M-18*
Amaral, A.T.-Do; *D-33, D-47*
Amaral, L.A.; *SP.04.02*
Amaral, M.J.; *V-04*
Amaral, M.S.; *U-10*
Ambrosio, L.; *K-43*
Amgarten, D.A.; *M-21*
Amorim, F.G.; *P-18*
Amorim, G.; *B-52*
Amorim, G.C.; *B-57, E-42, E-91, E-93, E-96, F-68, F-86, M-31*
Amorim, M.S.N.; *C-08*
Anastácio, J.M.; *F-07, F-41*
Andia, D.C.; *K-35*
Andrade, A.L.; *J-04*
Andrade, A.V.; *F-75, F-79*
Andrade, C.M.; *K-09*
Andrade, C.M.; *K-60*
Andrade, G.C.; *E-54*
Andrade, M.F.; *K-09*
Andrade, M.F.; *K-60*
Andrade, M.M.S.; *G-13*
Andrade-Oliveira, V.; *G-09*
Andreassa, E.C.; *B-09*
Andrilli, L.H.S.; *C-03, X-15*
Andrilli, L.H.S.; *SM.03.04, SP.03.04*
Andrioli, L.P.; *O-26*
Andriolo, B.V.; *D-38*
Angelo, A.B.S.; *E-12, E-94*
Angelo, R.M.; *D-65*
Annunciato, I.; *D-31, T-10*
Anobom, C.D.; *E-37, E-43, E-54, E-81, H-01, O-04*
Antonenko, Y.N.; *X-02*
Antonin, V.S.; *D-65*
Antonio, L.M.; *E-46*
Antonio-Santos, J.; *B-46*
Antunes, B.; *F-85*
Antunes, F.; *A-32*
Antunes, T.F.S.; *R-04*
Aoki, P.H.B.; *F-53*
Aquino, P.F.; *M-08*
Aragão, A.Z.B.; *E-41, E-46*
Araki, K.; *G-31*
Arantes, E.C.; *A-02*
Arantes, G.M.; *D-39*
Araújo Jr., J.P.; *D-35*
Araujo, A.; *E-36*
Araujo, A.A.S.; *T-09*
Araujo, A.P.U.; *E-50, E-66*
Araújo, A.S.; *D-22, U-05*
Araújo, D.L.; *J-07*
Araujo, D.R.; *F-43*
Araújo, G.A.T.; *O-16*
Araujo, G.G.; *E-22*
Araujo, G.R.; *E-30*
Araújo, H.D.A.; *T-03, T-20*
Araújo, N.; *O-24*
Araújo, N.V.P.; *SP.09.03*
Araujo, S.M.; *T-08*
Araujo, S.M.S.; *G-29*
Araújo, T.S.; *E-77*
Araújo, W.L.; *F-19*
Arcanjo, A.; *A-19*
Arcanjo, A.; *B-50*
Arcanjo, A.F.; *B-19*
Archiolli, I.; *K-26, K-54, K-59*
Arend, L.B.; *D-37*
Argibay, J.D.R.; *E-43*
Argüello, J.M.; *E-88*
Armentano, G.M.; *K-01*
Armijos, M.J.G.; *G-16*
Arni, R.K.; *E-20*
Arrido, S.S.; *F-45, P-07*
Arruda, D.C.; *B-10, F-07, F-29, F-41, F-55*
Arruda, E.; *F-22*

Arruda, E.; *SP.04.02, SP.18.03*
Arruda, L.; *U-26*
Arruda, V.M.; *B-06*
Asai, C.N.; *K-42*
Assis, A.L.E.M.; *K-53*
Assis, D.M.; *O-18*
Assis, L.H.C.; *M-13*
Assis, L.H.P.; *B-14, B-24*
Assis, R.I.F.; *K-35*
Assis-De-Lemos, G.; *C-12, E-25*
Assunção, M.A.S.; *F-48*
Astakhova, K.; *D-09*
Atella, G.C.; *A-03, A-26*
Atella, M.O.; *K-08*
Atiba, F.A.; *G-34*
Atum, A.L.B.; *U-04*
Augusto, B.O.; *J-07*
Augusto, B.S.; *E-61*
Augusto, O.; *S-06*
Ávila, A.R.; *B-09*
Ávila, D.; *K-45*
Ávila, D.S.; *T-15*
Ávila, M.C.; *F-36*
Azeredo, F.J.; *G-11*
Azevedo, P.N.; *O-09*
Azevedo, R.D.S.; *B-32*
Azevedo, S.L.C.; *E-38*
Azevedo, V.A.C.; *F-33, M-03*
Azevedo-Reis,; *A.-29*
Azzoline, A.E.C.S.; *B-31*

B

Bachega J.F.R.; *D-60*
Bagnato, V.S.; *B-07, C-07*
Bahia, A.C.; *A-15*
Baima, P.T.S.; *E-56*
Bajay, M.M.; *F-69*
Balok, F.R.M.; *T-08*
Baptista, M.S.; *I-04, I-09, I-12, X-02*
Baptista, M.S.; *SP.12.04*
Baptista, S.N.; *K-24*
Barbosa, A.N.; *D-66*
Barbosa, C.P.; *K-03*
Barbosa, G.M.; *E-30*
Barbosa, J.C.J.; *K-67*
Barbosa, L.A.O.; *N-13*
Barbosa, L.R.S.; *C-04, E-65, E-83, F-01, G-20*
Barbosa, M.L.C.; *E-55*
Barbosa, R.L.; *A-08*
Barbosa, R.M.R.; *F-48*
Barbugli, P.A.; *U-08*
Barcellos, G.; *G-11*
Bari, S.E.; *D-63*
Barletta, A.B.; *A-25*
Baroni, M.P.M.A.; *C-06*
Barrantes, F.J.; *SP.07.04*
Barreto, A.S.; *T-09*
Barreto, L.V.; *G-25*
Barros, B.C.S.C.; *O-14*
Barros, C.A.; *E-25*
Barros, J.M.; *B-55*
Barros, K.; *O-02*
Barros, M.H.; *B-01, B-05, B-47*
Barros, M.R.A.; *U-06*
Barros, R.; *V-05*
Barroso, I.G.; *A-24*
Bartkevihi, L.; *E-37*
Bassani, J.W.M.; *D-15*
Bassani, R.A.; *D-15*
Basseres, D.S.; *K-20, K-51, U-02*
Basso, L.G.M.; *C-11*
Bastos, A.B.M.; *F-76*
Bastos, B.M.A.; *F-89*
Batah, S.S. ; *SP.04.02, SP.18.03*
Batista, A.C.C.A.; *M-17*
Batista, A.C.S.; *O-12*
Batista, B.S.; *F-21*
Batista, F.A.H.; *E-05, E-49, E-51*
Batista, J.C.B.; *SP.14.04*
Batista, J.J.; *T-03, T-20*
Batista, K.L.; *T-17*
Batista, M.P.; *M-14*
Batista, M.R.B.; *C-11*
Batista, P.R.; *SP.22.03*
Batista, R.; *N-08*
Bauer, D.; *B-02, B-28*
Baumart, G.J.; *B-43, N-05*
Bavier, A.C.D.; *E-43*
Bayer-Santos, E.; *M-25*
Bazán, J.M.N.; *T-17*
Beatriz, K.; *O-08*
Becerra, A.; *G-35*
Becerra, A.; *SP.18.03*
Becerra, A.M.; *SP.04.02*
Bechinger, B.; *P-05, P-15*
Becker, D.H.; *F-69*
Beheshti, A.; *SP.14.01*
Behrens, L.M.P.; *K-03, M-22*
Beirão, B.C.B.; *S-12*
Bekish, A.V.; *X-02*
Belchor, M.N.; *D-31, T-04, T-10, T-19*
Belizário, J.E.; *K-68*
Bellini, A.; *F-01*
Bellini, M.H; *Q-01, Q-05, U-23*
Bellissimo, N.O.A.; *U-13*
Benetti, T.R.S.; *A-34*
Bentes, P.N.M.; *D-66*
Bentley, M.V.L.B.; *K-43*
Bento, A.A.; *J-08*
Bergado, G.; *C-16*
Bernabé, R.; *A-11*
Bernardes, L.G.; *T-13*
Bernardi, M.M.; *U-24*
Bernardino, R.; *S-04*
Berthomieu, P.; *F-49*
Bertoldi, E.R.M.; *K-51*
Bertolini, G.R.F.; *F-59*
Bertolini, M.C.; *U-08, U-18*
Bertolino, G.M.; *K-43*
Berzoti-Coelho, M.G.; *U-20*
Beserra, S.S.; *C-09, T-09*
Bessas, N.C.; *I-08*
Bevilaqua, V.R.; *E-27, H-07*
Bezerra F.C.; *F-14*
Bezerra F.F.; *E-07*
Bezerra Filho, C.M.; *I-11*
Bezerra, C.F.; *F-81*
Bezerra, C.M.; *K-09*
Bezerra, E.A.; *R-02*
Bezerra, F.C.; *F-77*
Bezerra, J.; *N-02*
Bezerra, L.C.A.; *E-55*
Bezerra, M.A.C.; *U-05*
Bezerra, P.R.; *D-42, E-42*
Bezerra, S.A.; *K-08*
Bianchini M.C.; *B-21*
Bicev, R.N.; *E-84*
Biedermann, F.J.; *D-37*
Bila, N.M.; *P-14*
Bilsland, E.; *M-19*
Binato, R.; *U-14*
Birbrair, A.; *U-24*
Bisch, P.M.; *G-08*
Bishop, A.J.R.; *K-23*
Bittencourt, G.H.; *F-31*
Bittencourt, M.F.; *D-02*
Bleicher, L.; *D-27, D-60*
Bolais-Ramos, L.; *O-18*
Bolean, M.; *SP.03.04*
Bolean, M.; *X-15*
Bolzani, V.; *SP.06.02*
Bonfá, I. S.;
Bonfá, I.S.; *A-33, T-11*
Bonilla, C.; *F-34*
Bonomo, J.H.; *K-17*
Bonturi, C.R.; *R-03, R-06, R-11, T-19, T-21*
Bottini, M.; *SM.01.02*
Bonzoumet, M.; *U-26*
Bordallo, H.N.; *SP.02.04*
Bordon, K.C. F.; *A-02*

Borges, B.C.; *T-12*
Borges, C.S.; *E-12, E-94*
Borges, D.C.; *E-38*
Borges, G.N.R.; *H-02*
Borges, I.B.; *S-34*
Borges, J.C.; *E-01, E-17, E-35, E-33, E-40, E-51, E-56 E-65, E-71, E-72, E-73, E-83*
Borges, M.H.; *G-07*
Borges, T.S.; *F-89*
Boroni, M.; *K-33, K-55*
Boroni, M.; *SP.09.01*
Borrego-Varillas, R.; *D-26*
Bortolotto, V.C.; *T-08*
Bottini, M.; *SP.03.04*
Bottini, M.; *X-15*
Bourgard C.; *M-19*
Bozza, F.; *K-22*
Bozza, P.; *K-22*
Bracht, A.; *B-40, T-18*
Bracht, L.; *B-40*
Braga, C.A.; *K-61*
Braga, D.L.; *O-01*
Braga, V.L.A.; *D-42, E-26*
Bragança, R.; *SP.18.03*
Brambilla, M.P.; *T-02*
Brancini, G.T.P.; *O-26*
Branco, S.J.S.C.; *F-23*
Brandao, A.; *SP.21.02*
Brandao, M.M.; *M-07*
Brandão, T.A.S.; *E-58*
Brandão, W.F.; *B-57*
Brandao, W.F.; *F-68*
Brasil, M.C.O.A.; *G-23*
Brayner, F.A.; *A-16*
Braz, A.S.K.; *E-19*
Braz, G.; *A-11*
Breitkreitz, M.C.; *F-46, G-22, G-17*
Bressan, G.C.; *F-39, G-30, K-33, K-55*
Brezesinski, G.; *D-10*
Briard-Bion, V.; *M-03*
Brígido, M.C.; *R-13*
Brito, B.A.; *V-02*
Brito, C.A.A.; *K-07*
Brito, C.D.; *F-74, O-11*
Brito, G.; *B-33*
Brito, I.A.A.; *M-11*
Brito, N.F.; *A-12*
Brito, N.F.; *A-31*
Brito, R.V.L.; *B-46*
Brito, T.F.; *M-11*
Brito, T.G.S.; *T-22*
Brondino, C.D.; *S-27*
Bronze, E.; *K-20*
Brunetta, H.; *O-25*
Buchet, R.; *SM.01.01, SP.03.01*
Budel, M.L.T.; *G-24*
Bueno-Janice, J.C.; *S-08*
Burton, G.; *B-02*
Bustos, A.H.; *D-09*

C

Cabo, G.C.M.; *A-12, A-31*
Cabral Filho, P.E.; *A-16, I-02, F-57, I-06, I-11, J-01, M-12*
Cabral, B.C.A. ; *K-67, K-71*
Cabral, F.; *I-03*
Cabral, F.V.; *F-44, I-01*
Cabral, K.M.S.; *E-26*
Cabral, M.C.; *A-28*
Cabral, V.A.; *D-50*
Cabrejos, D.A.L.; *E-64*
Cabrera, M.P.; *F-50, I-06, I-11*
Cabrera, P.M.; *J-01*
Cabrera, V.I.M.; *S-17, S-21, S-23*
Cacemiro, M.C; *K-43*
Caetano, C.C.S.; *G-35*
Caetano, C.C.S.; *SP.04.02*
Caetano, C.S.; *SP.18.03*
Caetano, M.M.; *I-08*
Caetano, M.M.M.; *K-33*
Calcagno, T.G.; *E-38*
Caldeira, I.D.S.; *K-68*
Caldeira, M.M.; *D-42, M-23*
Calderano, S.G.; *M-05*
Calderon, L.; *D-11*
Calegari-Silva, T.C.; *U-26*
Calixto, C.; *B-17, B-19*
Calixto, C.C.; *B-33, B-50*
Calixto, R.D.; *SM.03.04*
Calixtro, J.D.; *N-10*
Calvacante, G.A.; *K-60*
Camacho, S.A.; *F-53*
Camacho-Pereira, J.; *K-08*
Camandona, V.L.; *K-19*
Camara, G.L.; *E-13*
Camargo, J.B.; *D-53*
Camargo, M.L.M.; *N-03*
Camargo, N.S.; *S-14*
Campagnaro, B.P.; *P-18*
Campanella, J.E.M.; *U-08, U-18*
Campanini, E.B.; *M-07*
Campo, V.L.; *SP.06.01*
Campos Da Paz, M.; *F-80, F-82*
Campos, E.; *A-11*
Campos, E.L.S.; *O-16*
Campos, E.V.R.; *F-43*
Campos, F.M.B.; *R-12*
Campos, F.V.; *G-07, O-19*
Campos, L.M.B.; *F-18, F-24*
Campos, R.; *C-14*
Campos, R.M.S.; *K-42*
Canavan, F.S.; *D-66*
Cândido, M.F.; *F-17*
Candido, T.S.; *U-08*
Canellas, L.P.; *R-07*
Cano, M.I.N.; *E-39, M-13, M-26*
Capece, L.; *D-63*
Cappato, J.S.; *S-02*
Caramori, S.S.; *B-23*
Cardili, L.; *K-46*
Cardoso, A.M.; *M-11*
Cardoso, F.F.; *E-61*
Cardoso, G.D.; *E-31*
Cardoso, I.A.; *A-02, C-11*
Cardoso, I.C.A.; *E-54*
Cardoso, M.I.C.; *Q-02*
Cardoso, S.C.; *C-17, J-08*
Cardoso, S.C.; *K-44*
Cardoso-Lima, R.; *L-01*
Carli, S.; *O-26*
Carlos, R.M.; *F-60, D-36, K-04, X-10, X-13*
Carmos, M.E.G.; *I-10*
Carneiro, D.A.A.; *C-17*
Carneiro, L.T.; *U-11*
Carneiro, P.G.; *E-77, E-85*
Carneiro, R.F.; *J-04*
Carneiro-Ramos, M.S.; *K-01*
Carrera, I.L.; *K-45*
Carretero, G.; *X-04*
Carretero, G.P.B.; *E-49, G-31, X-07*
Caruso, I.P.; *D-42, E-08, E-18, E-29, E-42, E-59, E-62, E-87, K-02, T-14*
Caruso, M.B.; *O-06*
Caruso-Neves, C.; *C-01, G-32, K-10, K-18, K-32, K-56, M-01, M-24*
Caruso-Neves, C.; *SP.18.01*
Carvalho Jr., F.A.; *D-27*
Carvalho, A.E.; *K-09, K-60, K-70*
Carvalho, C.; *U-26*
Carvalho, C.P.S.; *R-02*
Carvalho, C.R.O.; *K-64*
Carvalho, E.M.; *T-17*
Carvalho, F.V.; *F-46, G-17, G-22*
Carvalho, L.B.; *F-50, N-06, U-15*
Carvalho, L.E.D.; *N-13*
Carvalho, R.F.; *O-25*
Carvalho, R.S.; *U-14*
Casadei, B.R.; *G-20*

Casalinho, A.L.F.; *E-26, E-43*
Cason, F.A.P.; *M-17*
Cassali, G.D.; *F-28*
Cassiano, C.S.S.; *F-33*
Castanho, M.; *G-02*
Castanho, M.A.R.B; *SP.20.03*
Castelletti, C.H.M.; *F-02*
Casteluci, G.; *E-08, E-29*
Castilho, R.F.; *B-07, C-07, U-01*
Castilo, I.D.; *E-55*
Castro Faria Neto, H.C.; *SP.15.03*
Castro -Jr, C.; *P-16*
Castro, D.H.O.; *F-85*
Castro, É.; *B-51, K-13, K-25, K-40*
Castro, F.A.; *K-43*
Castro, G.F.; *G-32*
Castro, J.C.; *E-80*
Castro, R.C.; *SP.18.03*
Castro, R.D.; *F-74, F-76, G-20*
Castro, S.B.R.; *G-37*
Castro-Junior, C.J.; *N-01*
Catanho, M.; *E-38*
Caurio, A.C.; *T-15*
Cavalcante, A.G.; *K-70*
Cavalcante, G.A.; *K-09*
Cavalcanti, M.I.S.; *D-43*
Cavini, I.A.; *E-66, E-69*
Celej, S.; *V-06*
Cerchiaro, G.; *G-26, N-09*
Cerf, N.; *Q-08*
Cerhiaro, G.; *N-03*
Cerullo, G.; *D-26*
Cesar, G.C.; *M-24*
César, M.C.C.; *F-41*
Cesar, M.C.M.; *F-07, F-29, F-55*
Cestari, L.; *B-13*
Cestari, L.L.; *B-27*
Chagas, J.A.C.; *B-47*
Chagas, M.A.; *D-56*
Chagas, V.L.; *T-17*
Chagas-Santos, G.; *M-25*
Chaimovich, H.; *D-10, P-04, X-01, X-07, X-04*
Chain, M.O.; *M-32*
Chambergo, F.S.; *F-27, F-42, F-62, F-71*
Chame, D.F.; *M-10*
Chammas, R.; *K-46*
Chan, L.Y.; *P-12*
Chang, J.E.; *E-11*
Chao, L.; *C.-16*
Charles, J.E.; *G-14*
Chauhan, J.; *G-14*
Chaves, A.F.A.; *O-14*
Chaves, C.; *D-49*
Chaves, C.R.; *F-57*
Chaves, D.S.A.; *S-14, S-19, S-20*
Chaves, R.B.R.; *R-05*
Chaves, V.E.; *K-69, N-13*
Chaves-Filho, A.B.; *K-15, O-15, S-28*
Chávez-Olórtegui, C.; *F-34*
Chemes, L.B.; *E-89*
Cheohen, C.F. A. R; *D-38*
Chistyakof, D.V.; *X-02*
Christoffolete, M.A.; *B-13, B-27, B-37*
Chubatsu, L.S.; *O-16, O-20*
Ciambarella, A.E.M.; *V-01*
Ciancaglini, I.; *F-11*
Ciancaglini, P.; *C-03, F-17, X-15*
Ciancaglini, P.; *SM.03.04, SM.03.03, SP.03.04, SM.03.02*
Cilli, E.M.; *G-23, T-14*
Cino, E.A.; *E-26*
Clarke, J.; *K-08*
Clarke, J.H.R.; *E-55*
Claudino, R.C.C.; *F-36*
Clerici, S.P.; *SP.04.04*
Côco, L.Z.; *P-18*
Coelho, C.M.; *A-17*
Coelho, D.F.; *D-41*
Coêlho, D.F.; *F-10*
Coelho, F.R.; *S-06*
Coelho, L.C.B.B.; *I-06, M-12, T-03, T-20*
Coelho-De-Souza, A.N.; *SP.18.04*
Cogo, A.J.D.; *K-34, K-53*
Coitinho, J.B.; *E-78, G-07*
Colmenares, V.C.R.; *X-10*
Colombo, R.; *D-65*
Colpani Filho, C.L.; *K-52*
Cominal, J.G.; *K-43*
Comis Neto, A.A.; *B-21*
Conceição M.R.L.; *C-09*
Conceição, C.C.; *B-22*
Conceição, K.; *D-34*
Conceicao, M.G.; *E-16*
Conceiçâo, N.H.; *F-74*
Conceição, N.H.; *R-14*
Conceição. M.R.L.; *T-09*
Condori, E.; *E-50*
Conrado, R.; *F-19*
Consonni, S.R.; *SP.04.04*
Consuli, T.O.; *M-29*
Contreras, H.D.P.; *A-27*
Coque, A.A.; *U-24*
Coque, A.C.; *N-06*
Cordeiro, H.G.; *K-24*
Cordeiro, Y.; *E-55, V-04*
Cordova, J.A.; *D-63*
Córdula, C.R.; *F-02*
Correa, T.B.; *K-20*
Corrêa, T.B.; *K-51*
Corrêa, T.L.R.; *F-04, F-11*
Correa, T.S.; *F-80*
Corrêa-Castro, G.; *K-62*
Correia, L.I.V.; *P-18, T-12*
Correia, M.T.S.; *I-06, I-11, M-12, R-03*
Cortes, V.F.; *N-13*
Cortez, M.P.; *X-01*
Costa Dos Santos, G.; *K-22*
Costa Filho, A.J.; *E-47, E-79*
Costa, A.D.; *C-10*
Costa, A.D.; *C-15, T-09*
Costa, C.C.O.S.; *N-08, N-14*
Costa, C.C.R.; *D-31*
Costa, C.R.C.; *T-04, T-10*
Costa, G.A.; *K-53*
Costa, G.C.A.; *A-21, F-13, F-54*
Costa, H.M.O.; *B-30*
Costa, L.S.; *F-89*
Costa, M.; *D-12*
Costa, M.C.R.; *T-24*
Costa, M.F.D.; *N-11*
Costa, M.G.S.; *D-37*
Costa, M.R.; *U-24*
Costa, R.M.; *F-59*
Costa, S.G.; *A-07*
Costa, S.L.; *F-76, F-85, N-08, N-11, N-14*
Costa, T.R.; *T-07, T-12*
Costa, V.F.; *M-18*
Costa-Beber, L.C.; *B-43, K-05, K-21*
Costa-Beber, L.C.; *SP.04.03*
Costa-Filho, A.J.; *C-11, E-44*
Costa-Filho, A.J.; *SM.04.04*
Costa-Filho, R.N.; *R-02*
Cota, G.; *K-62*
Coto, A.L.S.; *E-35, E-72, E-73*
Coutinho De Oliveira, L.; *X-07*
Coutinho-Neto, M.D.; *D-03*
Couto, C.A.P.; *F-82*
Couto-Vieira, J.; *K-38*
Covali-Pontes, H.R.; *A-34, A-35*
Covas, D.T.; *K-43*
Cozachenco, D.; *N-10*
Craik, D.J.; *P-12*
Creczynski, T.; *U-12*
Cruz, C.H.B.; *D-41*
Cruz, C.R.C.; *T-19*

Cruz, D.R.R.; *R-09*
Cruz, F.T.; *E-78*
Cruz, J.S.; *C-10, C-15, T-09*
Cruz, J.S.; *SP.15.02*
Cruz, K.P.; *G-11*
Cruz, L.C.; *A-23*
Cruz, M.A.E.; *SP.03.04*
Cruz, T.A.; *S-20*
Cuccovia, I.M.; *D-10, G-31, P-04, X-01, X-04, X-07*
Cugnasca, B.S.; *G-31*
Cunha, A.F.; *K-17, M-07, U-05*
Cunha, A.R.; *D-04*
Cunha, E.S.; *S-24*
Cunha, F.A.S.; *B-32*
Cunha, F.Q.; *M-18*
Cunha, G.; *H-05*
Cunha, M.M.L.; *B-57, F-68, M-30, M-31*
Cunha, T.M.; *M-18*
Cury, S.S.; *O-25*
Cussiol, J.R.; *U-16, U-22, U-21*

D

D'muniz Pereira, H.; *E-69*
Da Costa, C.A.; *F-41*
Da Costa, C.L.R.; *A-15*
Da Cunha, F.F.M.; *F-07, F-55*
Da Cunha, F.M.; *U-21*
Da Poian, A.T.; *E-30, K-08*
Da Silva Aragão, R.; *B-46*
Da Silva, A.M.; *M-20*
Da Silva, A.N.R.; *D-58*
Da Silva, F.A.; *O-18*
Da Silva, G.D.S.; *B-55*
Da Silva, G.F.; *F-66, F-73, F-75*
Da Silva, G.F.; *F-87*
Da Silva, J.A.; *F-63*
Da Silva, J.G.; *G-37*
Da Silva, K.R.; *SM.03.03*
Da Silva, L.H.G.; *F-17*
Da Silva, M.L.; *E-09, G-06, Q-04*
Da Silva, M.S.; *M-05*
Da Silva, W.S.; *B-30*
Da Silva-Neto, P.V.; *K-30*
Da-Cruz, A.M.; *K-62*
Dahleh, M.M.M.; *T-08*
Dale, C.S.; *K-57*
Dam, M.N.; *F-49*
Damiano, G.S.; *B-35*
D'angelo, N.A.; *G-21*
Dantas, D.C.C.P.; *K-09*
Dantas, H.L.; *A-06*
Dantas, L.S.; *K-15*
Dántola, M.L.; *I-07*
Dardenne, L.E.; *SP.10.02*
Da-Silva, W.S.; *B-39, B-41*
Davey, N.E.; *E-89*
David, V.C.; *E-11*
Davilla, G. F. B.; *X-11*
De Almeida, S.P.; *E-75*
De Amorim, G.C.; *E-95*
De Andrade, A.V.; *F-73, F-87*
De Andrade, M.F.; *K-70*
De Araujo, D.R.; *F-06*
De Araujo, D.R.; *G-09, G-15*
De Araújo, W.M.; *U-11*
De Azevedo, J.M.; *V-01*
De Bem, A.F.; *SP.14.03*
De Camargo, P.; *N-05*
De Carli, A. M; *D-31*
De Carli, B.P.; *T-10*
De Carvalho, J.C.S.; *K-30*
De Castro, D.R.; *F-89*
De Castro, D.R; *F-85*
De Castro, R.D.; *O-11, R-14*
De Cena, G.; *D-34*
De Felice, F.G.; *N-10*
De Freitas-Fernandes, A.; *E-69*
De Jesus, B.A.P.; *F-66, F-73, F-87*
De Jesus, B.C.; *U-04*
De Jong, G.; *F-26*
De León Esperón, L.; *C-16*
De Lima, E.O.V.; *E-16*
De Lima, G.B.; *K-58*
De Lima, L.F.S.; *M-02*
De Lima, M.E.; *P-16*
De Magalhães M.T.Q.; *D-27, E-70, E-85, O-12, P-03, P-09*
De Matos, L.P.; *U-04*
De Melo, J.D.; *T-01*
De Melo, L.D.B.; *M-32*
De Melo, M.T.; *SM.03.02*
De Melo,; *F-12*
De Mesquita, J.F.; *D-24, D-58*
De Moraes, L.L.F.; *T-10*
De Moura, L.D.; *F-46*
De Oliveira, C.S.; *E-17*
De Oliveira, J.; *B-43, K-16, K-21*
De Oliveira, J.; *N-05*
De Oliveira, L.C.; *D-27*
De Oliveira, M.A.; *T-10*
De Oliveira, R.F.; *G-27*
De Paula, A.M.; *D-26, F-18, F-24, F-28*
De Paula, A.M.; *SP.12.01*
De Paula, D.R.; *SP.09.03*
De Paula, E.; *F-46, G-12, G-17*
De Prat Gay, G.; *SP.13.01*
De Pretto, L.R.; *I-05*
De Souza, A.O.; *F-19*
De Souza, A.R.; *D-40, G-03*
De Souza, J.C.; *B-14*
De Souza, L.F.; *S-13*
De Souza, M.R.D.P.; *B-42*
De Souza, R.C.; *A-05*
De Souza, R.C.; *D-05*
De Souza, R.F.; *E-60*
De Souza, R.S.C.; *SP.17.02*
De Souza, V.V.; *X-07*
De-Carli, B.P.; *D-31*
Delai, R.M.; *M-17*
Del-Bem, L.E.; *O-11*
Delfino, G. V.; *X-06*
Delft, F.V.; *G-18*
Delgobo, M.; *S-30*
De-Melo, L.D.B.; *U-25*
Denadai, Â.M.L.; *F-72*
Denardin, C.; *B-21, T-01*
Denardin, E.L.G.; *T-15*
De-Oliveira Paulo, S.L.; *K-57*
Di Mascio, P.; *S-09, S-10, S-16, S-24, S-28, S-29*
Dias, D.A.; *K-34*
Dias, D.P.M.; *K-69*
Dias, F.A.; *A-27*
Dias, G.; *B-28*
Dias, M.M.G.; *B-08*
Dias, R.V.R.; *D-19, E-08, E-29*
Dias, S.C.; *P-09*
Dias, W.B.; *J-05*
Dias, W.B.; *SP.18.01*
Dias, W.D.; *J-03*
Dias-Baruffi, M.; *K-30*
Díaz Navarro, O.; *C-16*
Diego A. Leonardo; *E-69, E-80*
Dinamarco, T.M.; *E-03, E-68, F-26,*
Diniz, J.F.; *C-06*
Diniz, L.; *K-15*
Diniz, L.; *O-15*
Diniz, L.A.; *G-13*
Diogo, I.L.; *N-13*
Do Amaral, M.J.; *V-01*
Do Carmo, D.R.; *G-10*
Do Valle, V.B.; *K-30*
Dobroff, A.S.; *F-29*
Dolci, I.; *E-23, G-28*
Domingos, I.F.; *U-05*
Dominguez Antty, J.H.; *E-91*
Domont, G.B.; *A-09, O-04*
Donato, M.; *X-08, X-09*
Donatti, L.; *B-42*
Doretto-Silva, L.; *G-09*

Dorighello, G.G.; *B-14, B-24*
Dorneles, M.L.A.; *L-01*
Dornelles, P.H.; *S-04*
Dorta, D.J.; *E-03, E-68*
Dos Reis, Y.A.; *B-04*
Dos Santos Jr; *B-31*
Dos Santos, A. A.; *G-31*
Dos Santos, C.C.; *C-06*
Dos Santos, R.S.; *B-39*
Dreyer, T.R.; *E-21*
Dreyfuss, J.L.; *J-07*
Duarte, E.L.; *X-05*
Duarte, J.C.; *F-38*
Durán, N.; *K-28*
Durand T.; *B-02*
Durco, A.O.; *C-10, T-09*
Dutra, B.A.; *T-24*
Dutra, M.R.; *K-62*

E

Echeverri, L.M.S.; *F-73, F-75, F-79, F-87*
El Bacha, T.; *B-02, B-28*
El-Bacha, P.; *K-22*
Elias, G.B.; *B-08*
Elifio-Esposito, S.; *G-27*
Elio, A.C.; *D-57*
Elizeu, R.S.Jr.; *R-08*
Emery, F.; *G-14*
Engel, E.E.; *F-17*
Enoki, T.A.; *SP.19.05*
Enoki, T.A.; *X-12*
Epifanio, N.M.M.; *S-14, S-19, S-20*
Eriksson, L.; *M-19*
Espindola, F.S.; *S-32*
Esteves, M.E.A.; *G-06*
Estrin, D.A.; *D-63*
Evanelista, N.N.; *F-47*
Evangelista, R.S.; *G-30*
Evaristo, J.A.M.; *O-23*

F

Fabiano, K.C.; *K-15*
Fabri, L.M.; *P-10, Q-02, Q-03*
Fabri, V.M.R.; *E-70*
Fabro, A.T.; *SP.04.02*
Faça, V.M.; *F-22, K-26, K-54. K-59*
Façanha, A.R.; *K-34, K-38, K-53*
Faccin, J.P.M.; *C-05*
Faccioli, L.H.; *K-30*
Fagundes, T.M.; *U-25*
Faierstein, G.B.; *F-48*
Falchetti, M.L.B.; *K-12, S-30*
Farah, C.S.; *E-22, M-09, M-25*
Farah, S.C.; *E-28*
Faria, A.V.S.; *K-36*
Faria, H.O.F.; *K-15*
Faria, R.L.; *K-15, T-23*
Farias, H.R.; *B-43, K-05, K-16, K-21, N-05*
Farias, H.R.; *SP.04.03*
Farias, J.O.; *SP.09.01*
Farias, S.O.; *U-17*
Favarin, B.Z.; *X-15*
Fedosova, N.; *Q-08*
Feitosa, L.N.T.; *E-26*
Felicios, A.R.; *F-79*
Félix, A.S.; *P-02*
Felix, M.; *A-06*
Felix, R.G.S.; *E-12, E-94*
Felix, R.R.; *U-21*
Felix, W.P.; *R-09, T-16*
Féliz, A.G.; *B-32*
Feltran, G.S.; *K-35*
Fenerich, B.A.; *K-26*
Fenuchi, M.C.M.; *B-37*
Fernades, L.A.; *F-87*
Fernandes, A.P.M; *B-12, K-30*
Fernandes, B.V.; *S-15*
Fernandes, C.; *S-05, S-31*
Fernandes, C.A.H.; *E-39*
Fernandes, C.J.C.; *SP.03.02*
Fernandes, F.; *SM.04.01*
Fernandes, F.L.; *K-09, K-70*
Fernandes, F.L.; *K-60*
Fernandes, G.A.; *T-04*
Fernandes, G.M.; *A-18*
Fernandes, J.; *B-52, B-54, F-83*
Fernandes, L.A.; *F-73, F-75, F-79*
Fernandes, M.E.S.; *A-32*
Fernandes, M.F.; *O-24*
Fernandes, M.F.; *SP.09.03*
Fernandes, M.M.L.; *A-35*
Fernandes, M.P.; *B-03, B-11, B-29, B-49*
Fernandes, P.M.B.; *R-04*
Fernandes, P.O.; *D-56*
Fernandes, R.A.; *F-03*
Fernandes, R.S.; *G-28*
Fernandes, R.S.; *SP.07.02*
Fernandes, T.; *K-09*
Fernandes, T.A.A.M.; *K-60*
Fernandes, T.A.M.; *T-07, T-12*
Fernandes, T.M.; *K-30*
Fernandes-Siqueira, L.O.; *B-55*
Fernandez, G.L.; *F-76, F-85, F-89*
Fernandez, L.G.; *F-74, O-11, R-14*
Fernandez, M.A.; *A-05, D-46, E-87*
Fernandez, M.A.; *F-59*
Ferrari, G.D.; *B-31*
Ferraz, L.S.; *B-10*
Ferraz, M.V.F.; *K-07, F-09, F-10*
Ferreira, A.F.; *F-23*
Ferreira, A.M.T.; *A-35*
Ferreira, A.M.T.; *M-14*
Ferreira, A.N.; *F-32*
Ferreira, A.S.; *F-53*
Ferreira, C.; *A-24, B-28*
Ferreira, C.C.; *F-69*
Ferreira, C.S.; *B-02*
Ferreira, C.S.; *P-06*
Ferreira, F.S.; *B-43*
Ferreira, G.; *H-04*
Ferreira, G.C.; *F-13, K-58*
Ferreira, G.F.; *G-37*
Ferreira, G.F.; *K-66*
Ferreira, I.C.C.; *S-32*
Ferreira, J.E.; *O-05*
Ferreira, L.; *M-17*
Ferreira, L.A.; *P-16*
Ferreira, L.C.A.; *N-01*
Ferreira, L.F.; *P-02*
Ferreira, L.S.; *Q-01*
Ferreira, L.S.; *Q-05*
Ferreira, M.R.A.; *T-03*
Ferreira, M.S.; *D-32, G-19*
Ferreira, P.; *E-53*
Ferreira, R.; *E-38*
Ferreira, R.S.; *G-13, G-25, G-33*
Ferreira, S.; *K-06*
Ferreira, S.A.O.; *T-03*
Ferreira, S.C.; *P-01*
Ferreira, S.T.; *N-10*
Ferreira, V.S.; *R-08, R-10*
Ferreira, W.S.; *G-08*
Ferreira-Almeida, I.; *A-15, A-30*
Ferreira-Da-Silva, F.W.; *SP.18.04*
Ferreira-Halder, C.V.; *K-24, K-28, K-36*
Ferreira-Halder, C.V.; *SP.04.04*
Ferreira-Júnior, J.R.; *E-52, K-19*
Ferretti, G.D.S.; *E-25*
Ferrins, L.; *G-14*
Ferro, D.M.; *F-20*
Ferro, E.A.V.; *T-12*
Ferro, J.C.; *A-06*
Ferroni, F.M.; *S-27*

Festuccia, W.F.; *K-40*
Festuccia, W.T.; *B-51, K-13*
Festuccia, W.T.L.; *K-25*
Fetter, B.Z.; *J-07*
Fidelis, E.M.; *T-01, N-07*
Fietto, J.L.R.; *F-39, G-30, K-33, K-55*
Fietto, L.R.F.; *SP.20.01*
Figueira, A.C.M.; *B-08, E-05, E-49*
Figueiredo, L.S.; *O-25*
Figueiredo, R.C.; *T-22*
Figueiredo, R.C.B.Q.; *S-08*
Figueiredo, S.G.; *O-19*
Figueiredo-Pontes, L.L.; *K-43*
Filho, A.B.C.; *B-51*
Filho, A.C.; *K-25*
Filho, R.M.; *P-18*
Filipak Neto, F.; *S-12*
Fiorotti, H.B.; *G-07, O-19*
Firsov, A.M.; *X-02*
Fitz, A.F.R.; *M-17*
Florenzano, F.H.; *X-07*
Foguel, D.; *E-15, E-53*
Fomich, M.A.; *X-02*
Fonseca, A.S.; *SP.12.02*
Fonseca, B.B.; *M-31*
Fonseca, G.L.; *K-20*
Fonseca, G.L.; *U-02*
Fonseca, G.R.; *F-18*
Fonseca, G.R.; *F-24*
Fonseca, J.V.M.; *S-20*
Fonseca, N.P.; *K-26*
Fonseca, R.C.; *T-24*
Fontana, N.A.; *E-79*
Fontes C.F.L.; *Q-03*
Fontes, A.; *A-16, I-02, I-06, I-11, J-01, M-12, S-08*
Fontes, A.; *F-57*
Fontes, E.P.B.; *CF.04.*
Fontes, M.R.M.; *E-21, E-39, E-61*
Fontes, V.S.; *M-26*
Fontes-Cal, T.C.M.; *O-25*
Forbes, G.L.; *SP.15.01*
Forchhammer, K.; *O-16*
Foressi, N.N.; *V-06*
Formiga, F.R.; *F-48*
Fortes, G.N.; *U-20*
Forti, F.L.; *K-14, K-51*
Forti, F.L.; *SP.04.05*
Fortuna, V.F.; *U-27*
Fossey, M.A.; *E-87, K-02, T-14*
Fraceto, L.F.; *F-43*
Fraceto, L.F.; *SP.02.02*
Frachini, E.; *N-02*
Fragoso, M.S.I.; *B-09, E-06*
Franchini, K.G.; *SP.13.04*
Franco, D.F.P.; *S-03*
Franco, G.F.; *F-38*
Franco, G.R.; *M-10*
Franco, L.V.R.; *B-01, B-05, B-47*
Franco, M.D.S.; *I-08*
Franco, M.K.K.D.; *G-15*
Franco, M.S.F.; *X-02*
Franco, O.L.; *F-49, P-09*
Franklin, P.F.C.; *D-06*
Freire, K.A.; *F-61*
Freire, K.F.; *F-61*
Freire, M.C.L.C.; *F-09*
Freire, M.C.L.C.; *SP.07.02*
Freire, M.H.O.; *V-04*
Freire, R.V.M.; *SP.19.04*
Freitas De Lima, F.; *G-12*
Freitas G.P.; *SM.03.04*
Freitas, A.Z.; *I-05*
Freitas, B.L.; *F-42, K-64*
Freitas, C.D.P.; *P-09*
Freitas, C.D.T.; *F-52*
Freitas, D.B.M.; *D-43*
Freitas, E.; *F-59*
Freitas, F.Z.; *U-18*
Freitas, J.S.; *T-24*
Freitas, R.O.; *SP.23.03*
Freitas, V.F.; *SP.24.01*
Frenedoso Da Silva, R.; *F-01*
Frézard, F.; *G-05, G-29*
Froes, T.Q.; *G-14*
Frohlich, N.; *K-03*
Fronzes, R.; *SP.11.01*
Fuhler, G.M.; *SP.03.02*
Fujiwara, R.T.; *G-05*
Fukada, S.Y.; *SM.03.03*
Furnham, N.; *G-18*
Furtado, A.A.; *E-64*
Furtado, A.T.F.; *S-14*
Fuzo, C.A.; *K-30*

G

Gabriel-Junior, E.A.; *K-48*
Gaio, P.; *G-33*
Galeano, R.M.S.; *F-90*
Galhardo, R.S.; *M-25*
Galizio, N.C.; *F-05, F-40, O-07, O-08, O-17*
Gallo, G.; *A-17*
Galvão, V.N.; *A-12*
Gama-Almeida, M.C.; *K-22*
Garay-Malpartida, H.M.; *K-68*
Garbelotti, C.V.; *O-26*
Garbocci, V.; *A-25*
Garcia, A.P.V.; *F-28*
Garcia, C.S.; *M-14*
Garcia, L.M.D.; *SP.05.01*
Garcia, M.L.C; *K-04, X-10, X-13*
Garcia, W.; *E-04*
Garçon, D.P.; *P-10, Q-02, Q-03*
Garratt, R.C.; *E-32, E-50, E-64, E-69, E-80*
Garrito, G.I.M.; *U-03*
Garrone, N.; *E-89*
Gaspar, J.M.; *K-52*
Gasparini, P.; *D-30*
Gasparotto, J.; *M-22*
Gava, L.M.; *E-33, E-71*
Gawriljuk, V.O.; *SP.07.02*
Gazolla, P.A.R.; *A-05*
Generoso, W.C.; *F-36*
Genta, F.; *A-07, T-06*
Gerbelli ; *SP.02.04*
Gerbelli, B.B.; *D-61*
Gerhardt, E.C.M.; *O-16*
Gerlach, R.F.; *K-30*
Geronimo, G.; *F-46, G-17, G-22*
Giachini, L.F.; *F-66*
Giannesi, G.C.; *F-14, F-77, F-78, F-81, F-90*
Gil, M.L.S.; *E-44*
Gilio, G.R.; *K-25, K-40*
Gimenes, M.L.; *F-59*
Gimenes, S.N.; *P-18*
Gismene, C.; *E-20*
Giudice, M.; *E-33*
Giudice, M.D.; *E-71*
Glattard, E.; *P-15*
Glezer, I.; *K-15*
Glienke, N.N.; *F-78*
Gobauto-Costa, R.; *O-01*
Godoy, A.S.; *E-23, G-28*
Godoy, A.S.; *SP.07.02*
Godoy, B.R.B.; *D-17*
Goedert, A.C.; *O-16*
Goes, C.P.; *K-57*
Goes, V.A.; *B-02*
Goes, V.A.; *B-28*
Góis, M.M.; *U-09*
Golfetto, L.; *K-12*
Gomes, A.A.; *F-73, F-87, F-75, F-79*
Gomes, A.A.S.; *D-35*
Gomes, A.A.S.; *U-08*
Gomes, A.L.V.; *B-11*
Gomes, A.M.O.; *D-42, E-26, E-43, M-23*
Gomes, B.J.S.; *N-01*
Gomes, B.M.; *F-62, F-71*

Gomes, C.; *O-12*
Gomes, C.P.; *U-04*
Gomes, D.A.; *G-16*
Gomes, D.E.B.; *G-19*
Gomes, D.S.; *O-06*
Gomes, F.A.; *O-22*
Gomes, H.; *A-11*
Gomes, H.; *B-17*
Gomes, K. De S.; *P-03*
Gomes, M.E.; *B-22*
Gomes, M.J.M.; *E-72, E-73*
Gomes, R.H.M.; *D-55, D-62*
Gomes, R.L.; *P-15*
Gomes, R.N.; *G-26*
Gomes, R.N.; *N-03*
Gomes, T.C.; *F-19*
Gomes-Filho, S.M.; *K-51*
Gomes-Neto, F.; *E-54*
Gomez, M.V.; *N-01*
Gonçalves, D.B.; *F-65, F-84, F-88, F-63, M-28*
Gonçalves, G.G.A.; *A-16*
Goncalves, J.; *F-55*
Gonçalves, J.M.; *S-25*
Gonçalves, L.F.A.; *K-38*
Gonçalves-Machado, L.; *O-02*
González Flecha, F.L.; *E-88*
Gonzalez, P.J.; *S-27*
Goriainov, S.V.; *X-02*
Gosti, F.; *F-49*
Goulart, L.R.; *S-32, P-18*
Gozzer, L.T.; *K-37*
Granato, M.J.G.; *B-06*
Grandini, G.S.; *D-29*
Granjeiro, J.M.; *SP.03.03*
Granjeiro, P.A.; *F-63, F-65, F-67, F-84, F-88, M-28*
Gravina, F.; *O-16*
Greer, A.; *SP.12.04*
Grego, K.F.; *E-16, F-05, F-56, O-03, O-07, O-08, O-13, O-21*
Grillo, D.C.N.; *S-03*
Grupenmacher, A.T.; *J-07*
Gualberto, S.A.; *T-24*
Guarneri, A.A.; *SP.08.01*
Guatimosim, S.; *O-23*
Guedes-Silva, T.C.; *A-15, A-27*
Guedon, E.; *M-03*
Guerra, J.F.C.; *B-06*
Guerra-Duarte, C.; *F-34*
Guerrero, T.N.; *O-06*
Guevara, L.A.; *S-27*
Guidolin, A.F.; *O-27*
Guima, S.S.; *SP.17.04*
Guimaraes, A.C.R.; *D-06*
Guimarães, A.C.R.; *E-38, G-18*
Guimarães, G.R.; *K-33, K-55*
Guimarães, G.R.; *SP.09.01*
Guimarães, N.C.A.; *F-78*
Guimaraes, P.P.G.; *G-16*
Guimarães, P.P.G.; *G-29*
Guimarães, R.R.; *G-31*
Guma, F.C.R.; *K-39, K-50*
Guma, F.C.R.; *SP.04.03*
Guma, F.T.C.R.; *B-43, K-05, K-21*
Gutierrez, C.O.; *M-27, P-13*
Guzzo, C.R.; *E-22, E-82*

H

Haenni, E.; *SP.19.04*
Hamley, I.W.; *E-34*
Hamley, I.W.; *SP.02.04*
Hampton, M.; *S-13*
Hartfelder, K.H.; *SP.08.02*
Hasenkamp, L.; *K-51*
Hatakeyama, D.M.; *F-56, O-17, O-03, O-21*
Heberl, F.A.; *X-12*
Heidrich, V.; *K-51*
Hemerly, A.S.; *SP.16.02*
Henriques, B.; *T-06*
Hernández-González, J.E.; *D-07, E-20*
Herrera, C.T.; *S-04*
Herrerias, T.; *B-42*
Hespanhol, J.T.; *M-25*
Hilario, H.O.; *F-38*
Hilhorst, H.W.M; *O-11*
Hoch, N.C.; *K-51*
Hoffmann, L.; *K-65, K-67, K-71, M-29*
Holanda, G.C.R.; *F-02*
Holanda, V.N.; *T-22*
Holtz, E.; *K-22*
Honório, K.M.; *D-65*
Hoppe, J.B.; *N-04*
Horn Jr., A.; *S-05, S-31*
Hornink, G.G.; *H-06*
Hotta, C.T.; *SP.16.01*
Hottum, H.M.; *S-05*
Hottum, M.H.; *S-31*
Houston, J.P.; *SP.24.02*
Hözer, R.M.; *N-04*
Hueck, G.S.; *E-60*
Huergo, L.F.; *O-16*

I

Ibrahim, S.P.; *A-04*
Icimoto, M.Y.; *E-34*
Iijima, T.S.; *D-47*
Inada, N.M.; *B-07, C-07*
Inague, A.; *D-33, K-15, O-01*
Ingerslev, L. R; *O-01*
Isasi, J.; *F-34*
Ishimoto, A.; *SP.18.03*
Ishimoto, A.Y.; *G-35*
Islam, Z.; *D-51*
Itri, R.; *C-02, D-04, D-18, X-02, X-08, X-09, X-11, X-14, SP.12.04*
Ivarsson, I.; *E-89*

J

Jacobowski, A.C.; *P-11, S-11*
Jager, M.; *E-53*
Jan, G.; *M-03*
Jaramillo, M.L.; *F-34*
Jardin, J.; *M-03*
Jayme, S.B.; *S-09*
Jekabson, R.; *U-21, U-22*
Jesus, A.P.; *F-79*
Jesus, B.A.P.; *F-75*
Jesus, I.C.G.; *O-23*
Jesus, T.S.R.; *O-11*
Joviano-Santos, J.V.; *C-10, C-15, Q-07*
Juliano, M.A.; *F-13*
Julião-Silva, L.S.; *K-69*
Júnior, D.F.G. Dos S.; *U-09*
Junior, E.R.S.; *R-10*
Junior, L.A.S.; *T-13*
Junqueira, M.; *O-04*
Junqueira, M.R.; *A-09*
Justino, A.B.; *S-32*
Justo, G.Z.; *K-28*
Justulin Junior, L.A.; *K-35*

K

Kagami, L.; *K-45*
Kamienski, C.; *H-04*
Kanaan, S.H.; *S-04*
Kashima, S.; *K-43*
Kava, E.; *E-47*
Kavazoi, V.K.; *F-56, O-17, O-13*
Kawano-Dourado, L.; *V-05*
Kazmirczak, R.M.; *K-21*
Kazura, J.W.; *CF.03.*
Kelly, J.; *E-53*
Ketzer, L.A.; *B-55, B-56, F-86*
Kim, S.; *X-04*

King R.; *M-19*
Kinoshita, K.; *SP.12.03*
Kinoshita, K.; *X-14*
Klein, C.P.; *N-04*
Kleiz-Ferreira, J. M.; *E-45*
Kliousoff, A.; *D-30*
Kliousoff, A.; *D-48*
Klippel, A.H.; *M-19*
Knapp, K.; *D-52*
Kobal, M.B.; *F-53*
Koehbach, J.; *P-12*
Koenigkam-Santos, M.; *SP.04.02*
Kolatkar, P.R.; *D-51*
Kolb, H.C.; *K-43*
Konnai S.; *B-48*
Koopman, E.O.; *B-20*
Kotani, P.O.; *F-20*
Kotova, E.A.; *X-02*
Kowaltowski, A.J.; *SP.21.04*
Kramar, J.; *E-89*
Krieger, J.E.; *Q-07*
Kümmerle, A. E.; *E-55*
Kümmerle, A.E.; *S-03*
Kussi, F.; *S-12*

L

Lacerda, C.D.; *X-07*
Ladeira, T.Q.; *F-33*
Ladeira, Y.F.X; *E-74*
Laet-Souza, D.; *M-10*
Lago, J.H.G.; *S-22, S-23, S-26*
Lagranha, C.J.; *B-03, B-29, B-49*
Lai, C.; *B-28*
Lam, E.W.; *U-11*
Lamim, V.B.; *A-31*
Lamy, M.T.; *X-05*
Lanfredi, G.P.; *F-22*
Lanna, E.; *N-08*
Lara, L.S.; *O-06*
Lara-Ribeiro, A.C.; *O-18*
Latge, C.; *E-15*
Latini, A.; *K-11, K-35, K-52, N-06, U-12, U-15, U-24, V-05*
Lawrence, N.; *P-12*
Lazaro, C.M.; *B-18*
Le Loir, Y.; *M-03*
Leal, A.B.C.; *G-32*
Leal, T.S.S.; *F-59*
Leal-Cardoso, J.H.; *SP.18.04*
Leandro; *V-06*
Lee, K.S.; *K-42*
Lehmann, L.T.; *T-05*
Leidens, D.; *K-12*
Leite, A.C.; *F-27*
Leite, A.C.R.; *B-18, B-32*
Leite, B.H.S.; *D-41*
Leite, I.K.O.; *N-01*
Leite, V.B.P.; *D-08, D-19, D-52, D-53*
Leite, V.B.P.; *SP.22.01*
Leite, W.A.; *P-11*
Leite; B.H.S.; *F-16*
Lemes, R.M.R.; *J-02*
Lemos, F.; *B-48*
Lemos, M.D.T.B.; *B-03*
Lemos, M.T.D.; *B-49*
Lemos, R.P.; *D-27*
Lencina, J.S.; *A-33, T-11*
Lencina, J.S.L.; *A-22*
Lenz, G.; *SP.24.04*
Leonardi, B.F.; *B-51, K-13*
Leone, F.A.; *P-10, Q-02, Q-03*
Lessa, R.T.; *E-45*
Levada, A.J.L.; *E-61*
Lião, L.M.; *P-09*
Libardi, S.H.; *E-33, E-40, E-71*
Liberalino, J.P.S.; *K-09, K-60*
Lie, K.C.M.; *R-03*
Ligterink, W.; *O-11*
Lillo, C.; *F-51*
Lima, A.N.; *D-48*
Lima, B.S.; *T-09*
Lima, C. N.; *J-01*
Lima, C.; *E-53*
Lima, C.Z.G.P.A.; *F-23*
Lima, D.C.A; *D-13*
Lima, D.S.; *D-46*
Lima, E.O.V.; *O-03, O-17*
Lima, E.V.; *K-08*
Lima, F.; *O-16*
Lima, F.S.; *SP.19.03*
Lima, G.M.A.; *SP.07.02*
Lima, J.V.A.; *I-06, J-01*
Lima, J.Y.; *B-09, E-48*
Lima, K.A.; *K-12, K-23, S-30*
Lima, K.B.R.; *E-37, E-54*
Lima, L.C.; *A-34, A-35*
Lima, L.M.T.R.; *E-14, O-12*
Lima, M.A.; *J-02*
Lima, M.E.G.; *A-19*
Lima, M.M.; *G-05*
Lima, M.V.; *T-20*
Lima, N.; *M-02*
Lima, R.; *F-43*
Lima, R.G.; *I-08*
Lima, R.M.; *H-09*
Lima, R.S.; *K-15*
Lima, R.T.; *M-23*
Lima, T.E.; *F-10, K-07*
Lima, V.; *K-45*
Lima, V.L.M.; *T-22*
Lima, W.K.R.; *F-23*
Limache, D.E.S.; *M-25*
Lima-Filho, J.L.; *F-02*
Lima-Oliveira, D.; *B-46*
Linda Hong; *SP.19.04*
Linden, R.; *V-04*
Lins, R.; *F-16*
Lins, R.D.; *D-41, F-09, F-10, J-05, K-07*
Lins, R.D.; *SP.10.03*
Lira, R.B.; *X-07*
Lira-Nogueira, M.C.B.; *B-11*
Livio, D.F.; *F-63, F-88*
Llontop, E.; *E-82, M-25*
Llontop, E.E.; *E-22, E-28*
Llorente Álvarez, F.; *C-16*
Lobo, Y.J.G.; *D-64*
Logullo, C.; *A-01, A-11, A-19, A-32, B-16, B-17, B-33, B-48, B-50*
Logullo, C.; *B-19*
Lopes, H.B.; *SM.03.04*
Lopes, A.L.; *J-06*
Lopes, A.L.; *S-10*
Lopes, A.M.; *G-21*
Lopes, C.; *B-19*
Lopes, C.F.B.; *B-38*
Lopes, D.O.; *F-84*
Lopes, D.O.L.; *F-33*
Lopes, D.S.; *T-12*
Lopes, E.S.; *B-15, B-34*
Lopes, J.C.; *E-07*
Lopes, J.L.S.; *X-09*
Lopes, L.F.; *I-04*
Lopes, L.; *SP.18.03*
Lopes, R.M.; *B-10, B-35*
Lopes, R.M; *K-27, Q-06*
Lopes, U.G.; *U-26*
Lopes, V.K.; *O-26*
Loponte, H.F.B.R.; *J-05*
Lorente, C.; *I-07*
Lossavaro, P.K.M.B.; *T-11*
Lotierzo, M.C.G.; *G-20*
Loureiro, B.M.; *F-89*
Loureiro, M.B.; *F-76, F-85*
Loureiro, S.E.; *F-14*
Lourenço, I.O.; *E-87*
Lourenço, M.V.C.; *N-10*
Lourenco, M.V; *SP.05.03*
Lourenço, T.C.; *E-34, E-84*
Lovering, L.; *M-25*
Lucena, D.M.S.; *J-05*
Lucena, M.N.; *A-34, A-35*
Luchiari, A.C; *SP.05.02*

Lucía, B.Z.; *G-31*
Luciane Passaglia; *SP.17.01*
Lujan, B.P.; *E-52*
Luz, B.S.R.; *M-03*

M

Macambira, S.G.; *D-28, U-27*
Macedo, A.A.; *F-70*
Macedo, A.M.; *F-38*
Macedo, A.M; *M-10*
Macedo, G.H.R.; *T-17*
Macedo, L.; *P-11*
Macedo, M.L.R.; *M-27, M-33, P-13, P-19, S-11*
Macedo-Silva, A.; *A-23*
Machado, A.M.; *K-37*
Machado, C.R.; *F-33, F-38, M-10*
Machado, F.S.; *G-33*
Machado, G.; *A-06*
Machado, G.C.; *K-53*
Machado, I.P.; *F-43*
Machado, J.A.; *A-32*
Machado, L.A.; *D-06*
Machado, L.A.; *G-18*
Machado, L.E.S.; *U-03*
Machado, L.E.S.F.; *U-13, U-17*
Machado, L.E.S.F; *S-06, U-19*
Machado, T.R.; *D-07*
Machado-Santeili, G.M.; *G-10*
Madrid, R.R.M.; *F-64*
Magalhães, C.M.; *N-14*
Magalhães, J.T.; *M-28*
Magalhães, L.B.M.; *F-79*
Magalhães, L.M.O.; *K-36*
Magalhães, M.L.B.; *F-66, F-73, F-75, F-87*
Magalhães, M.Q.T.; *E-77*
Magalhães, Y.T.; *K-14, K-51*
Magalhães, Y.T.; *SP.04.05*
Magne, D.; *SP.03.01*
Magro, A.; *D-30*
Magro, A.J.; *D-35, D-44, D-55, D-62*
Mahdizadeh, S.J.; *M-19*
Mahnke, L.C.; *F-50*
Maia, F.D.; *E-03, E-68*
Maia, M.L.S.; *A-28*
Maia, R.C.; *U-11, U-14*
Maia, Y.C.P.; *S-32*
Maidana, R.L.B.R.; *G-18*
Majerowicz, D.; *A-10, A-23, B-45*
Maklouf, G.R; *SP.09.01*
Malavazi, I.; *M-07, U-05*
Malheiros, B.; *G-20*
Malizia-Motta, C.; *V-01*
Malizia-Motta, C.L.F.; *E-14, V-03*
Malmegrim, K.C.R.; *K-43*
Malospirito, C.C.; *B-08*
Maltarollo, V.G.; *D-56*
Mancini, M.C.S.; *U-09*
Maniglia ; *SM.03.02*
Maniglia, B.C.; *F-12*
Manzato, V.M.; *A-17, F-13, F-54*
Manzi, A.S.; *T-10*
Manzoni, C.; *D-26*
Maraes, J.; *A-11*
Marchetti, C.R.; *F-77, F-90*
Marchi, F.A.; *E-19*
Marchi, R.C.; *F-60, X-13*
Marciano, C.L.; *F-14, F-77, F-81*
Maria Engler, Ss; *F-42*
Maria-Engler, S.S.; *B-10*
Mariano, X.M.; *P-16*
Mariano, X.M.; *P-16*
Marins, M.F.; *X-08*
Mariutti, R.B.; *E-20*
Mariz, C.A.; *K-07*
Mark Gontsarik; *SP.19.04*
Marques, A.G.; *A-18*
Marques, B.O.S.; *A-01*
Marques, I.L.; *C-15*
Marques, L.C.S.; *E-81*
Marques, L.P.; *C-09*
Marques, L.P.; *C-10, Q-07*
Marrink, S.J.; *P-04*
Martinez, G.R.; *S-24*
Martinez, P.A.; *U-07*
Martinez, R.R.M.; *F-37*
Martínez-Fernández, L.; *D-26*
Martinho, A.C.C.; *I-08*
Martins Jr, D.C.; *E-19*
Martins, D.; *F-02*
Martins, D.A.P.; *U-05*
Martins, D.M.; *E-58*
Martins, G.H.; *E-46*
Martins, I.B.S.; *D-07, D-22, D-32*
Martins, L.A.; *E-53*
Martins, L.A.M.; *B-43, K-39, K-50*
Martins, L.A.M.; *K-05*
Martins, L.C.; *D-57, E-76*
Martins, L.F.; *M-16, M-21*
Martins, L.F.; *SP.17.04*
Martins, L.M.; *F-55*
Martins, L.M.O.S.; *I-09*
Martins, M.A.; *G-32*
Martins, M.M.; *E-78*
Martins, M.T.; *X-07*
Martins, R.; *A-19*
Martins, R.; *B-17, B-48*
Martins, R.; *B-19*
Martins, R.B.; *F-22*
Martins, R.K.; *K-39, K-50*
Martins, R; *SP.04.02*
Martins, V.C.; *S-02*
Martins, W.K.; *I-12*
Martins, Y.P.M.; *B-48*
Martins.lbs; *E-36*
Martins-Duarte, E.S.; *P-03*
Marzolo, M.P.; *SP.18.01*
Masaki, R.; *R-01*
Massafera, M.P.; *S-09, S-28, S-29, O-22*
Massirer, K.B.; *M-19*
Masson, A.P.; *F-22, K-26, K-54, K-59*
Masui, D.C.; *F-14, F-78, F-77, F-81, F-90*
Mathews, P.D.; *F-37, F-64*
Matias, T.A.; *I-10*
Matos, A.R.; *G-37*
Matos, C.O.; *E-01*
Matos, C.O.; *E-24*
Matos, P.A; *I-10*
Matsamur, L.S.; *P-02*
Matsamura, L.S.; *P-06*
Matteo, T.M.V.; *S-18*
Mattos, D.; *S-26*
Mattos, J.C.; *D-30*
Mattos, L.M.M.; *S-05, S-18, S-19, S-31*
Maurastoni, M.; *R-04*
Mayrinck, J.V.R.; *F-68*
Mazepa, E.; *S-24*
Mcnamara, J.C.; *P-10, Q-02, Q-03*
Mead, L.; *M-25*
Mebarek, S.; *SM.01.01, SP.03.01*
Medei, E.; *C-13*
Medeiros, E.; *V-05*
Medeiros, M.H.G.; *K-15, S-10, S-16, S-28*
Medeiros, N.M.; *S-01*
Meichtry, L.B.; *T-08*
Meira, H.G.R.; *F-63, F-88*
Meira, M.R.; *T-24*
Meirelles, P.L.G.; *E-10*
Meleiro, L.P.; *O-26*
Melendez, M.E.; *P-18*
Melfior, L.; *K-52*
Mello, J.D.; *N-07*
Mello, L.R.; *F-25, E-34, E-84*
Melo, A.C.; *A-31*
Melo, A.C.A.; *A-12*

Melo, A.F.; *F-67*
Melo, A.F.; *F-84*
Melo, A.M.M.A.; *T-03, T-20*
Melo, F.A.; *D-19, E-08, E-18, E-29, E-57, E-59, E-62*
Melo, G.S.; *V-03*
Melo, I.S.; *F-19*
Melo, J.R.S.; *F-57*
Melo, L.D.; *R-10*
Melo, M.T.; *K-71*
Melo, N.M.Q.E.; *B-40*
Melo, P.A.L.; *D-59*
Melo, R.R.; *F-36*
Melo, V.S.; *F-62, F-71*
Melo-Braga, M.N.; *G-16*
Mendes, F.; *F-70*
Mendes, F.C; *K-33*
Mendes, J.H.L.; *R-13*
Mendes, L.F.S.; *C-11, E-44, E-79, F-47*
Mendes, Y.C.; *F-23*
Mendes-Giannini, M.J.S.; *P-14*
Mendonça, B.S.; *U-11, U-14*
Mendonça, L.; *D-05*
Mendonça, L.; *D-14*
Mendonça, T.C.; *F-46, G-17, G-22*
Menegassi, V.; *K-11*
Meneghetti, M.C.Z.; *J-02*
Menêses-Neto, M.B.; *K-65*
Menezes, L.B.; *S-31*
Menna-Barreto, R.F.S.; *M-32*
Meotti, F.C.; *S-13*
Merkulova, A.A; *SP.15.01*
Mertins, O.; *F-37, F-64*
Mesquita, G.P.; *F-23*
Mesquita, R.D.; *A-15, A-27*
Mesquita, R.D.; *B-04*
Meyrelles, S.S.; *P-18*
Mezzacappo, N.F.; *B-07, C-07*
Michalichyn, A.C.; *S-12*
Michel, D.A.G.R.; *P-01*
Micheletto, M.C.; *F-47*
Miguel W.M.; *K-63*
Miguel, M.; *S-04*
Milan, H.F.M.; *D-15*
Milan, J.L.; *CF.01.01*
Millán, J.L.; *SP.03.04*
Millán, J.L.; *X-15*
Minari, K.; *E-83*
Miranda, T.B.; *U-24*
Miranda, V. De J.; *F-49*
Mitsutake, H.; *G-22*
Miyamoto, S.; *B-51, D-33, D-47, K-15, K-25, O-01, O-15, S-28, T-23*
Monesi, N.; *A-14, A-18, O-26*
Montanari, J.; *F-51*
Monteiro, C.; *E-53*
Monteiro, J.V.; *K-42*
Monteiro, R.A.; *R-05*
Monteiro, T.M.; *A-32*
Montes, M.; *Q-08*
Montovaneli, G.F.; *F-31, R-12*
Moraes, A.H.; *D-56, E-63, E-76*
Moraes, B.C.; *K-57*
Moraes, C.M.; *Q-02, Q-03*
Moraes, C.V.; *U-05*
Moraes, D.; *O-01, O-25*
Moraes, J.; *B-17*
Moraes, L.A.B.; *B-44*
Moraes, L.L.F.; *D-31, T-19, T-04*
Moraes, M.C.; *P-10*
Moraes, M.N.; *B-51*
Moraes, R.K.; *SP.04.03*
Moraes-Santos, P.V.; *C-15*
Moraes-Vieira, P.M.; *B-18*
Moraes-Vieira, P.M.M; *SP.09.01*
Moraes-Vieira, P.M; *SP.14.02*
Morais, B.P.; *F-22*
Morais, H.L.O.; *G-05*
Morais, L.P.; *T-07, T-12*
Morais, S.B. De; *E-48*
Morais-Zani, K.; *E-16, F-05, F-40, F-56, O-03, O-07, O-08, O-13, O-17, O-21*
Morales, M.M.; *SP.01.04*
Morales-Vicente, D.A.; *U-10*
Morante, G.C.; *B-37*
Moreira, C.A.; *D-21*
Moreira, E.; *F-16*
Moreira, E.G.; *D-41*
Moreira, G.A.; *K-33, K-55*
Moreira, J.C.F.; *M-22*
Moreira, J.C; *K-03*
Moreira, L.; *B-19*
Moreira, L.G.; *F-53*
Moreira, M.F.; *A-03, A-09, A-26, A-28*
Moreira, T.; *E-81*
Morelli, A.P.; *U-09*
Moreno, M.F.; *K-25*
Moreno-Loaiza, O.; *C-13*
Moretti, E.H.; *O-15*
Morgon, N.H.; *D-40*
Mori, M.A.; *O-01, O-25*
Mori, M.A.; *SP.01.03*
Mori, M.A.S.; *SP.09.01*
Mori, R.L.; *F-25*
Mori, R.M.; *C-11*
Moritz, M.N.O.; *E-72, E-73*
Moro, C.R.; *S-04*
Mortara, L.; *D-10, X-04, X-01*
Mortara, R.A.; *F-41*
Mortari, M.R.; *A-22, A-33, P-12*
Mota, D.C.A.M.; *C-11*
Mota, F.J.T.; *O-20*
Motta, M.C.M.; *M-05*
Moura, L.D.; *G-22*
Moura, M.A.; *B-56*
Moura, M.J.N.; *E-12, E-94*
Moura, P.M.; *D-20*
Moura, S.S. B; *E-26, E-43*
Moura, T.C.; *E-40*
Mourão P.A.S.; *E-07*
Moura-Silva, J.; *A-10*
Mousovich-Neto, F.; *O-01, O-25*
Mroczek, J.; *SM.03.01*
Mukhina, T.; *D-10*
Mukhopadhyay, S.; *SP.13.02*
Muller, J.A.I.; *A-22, A-33, P-12*
Muller, M.E.; *K-52*
Müller-Santos, M.; *G-24, O-20, R-01*
Munhoz, V.H.O.; *P-05, P-15*
Muniz, J.R.C.; *E-04*
Muniz, M.I.; *D-09*
Murakami, M.T.; *E-75*
Murakami, M.T.; *F-04, F-11*
Mustafá, Y.; *U-26*

N

Nadalini, M.T.; *K-17*
Nader, H.B.; *J-02*
Nader, M.; *D-01*
Nakagawa, A.L.; *D-11*
Nakaie, C.R.; *E-02, E-34*
Nakamura, A.M; *SP.07.02*
Nakao, L.S.; *S-12, U-07*
Nakaya, H.T.I; *SP.09.02*
Naliatto, N.; *P-07*
Napoleão, T.H.; *A-16, I-11, M-12*
Nardelli, S.C.; *B-09, E-06*
Nascimento, A.F.Z.; *E-75*
Nascimento, A.F.Z; *SP.23.04, SP.07.02*
Nascimento, A.M.; *N-13*
Nascimento, E.G.C.; *K-09*
Nascimento, E.G.C.; *K-60*
Nascimento, F.D.; *K-27*
Nascimento, G.N.; *T-24*
Nascimento, J.S.; *A-16*
Nascimento, M.H.M.; *F-06*
Nascimento, M.L.S.; *O-06*
Nascimento, M.S.; *T-24*

Nascimento, N.B.; *B-43, K-16, N-05*
Nascimento, P.R.; *F-89*
Nascimento, P.V.P.; *B-45*
Nascimento, R.; *S-29*
Nascimento, R.M.; *C-12*
Nascimento, R.P.; *F-76, F-85*
Nascimento, R.P.; *N-08, N-14*
Nascimento, T.S.; *A-03, A-09, A-26*
Nascimento-Sales, M.; *B-27, B-37*
Navarro, D.M.A.F.; *A-16*
Navarro, R.; *E-67*
Navegante, G.; *G-23*
Neri, E.A.; *Q-07*
Neri, G.V.A.; *A-28*
Nespoulous, C.; *F-49*
Nestal De Moraes, G.; *U-11, U-14*
Neta, L.C.S.; *F-76, F-85, F-89*
Neto, A. ; *M-32*
Neto, A.N.; *U-25*
Neto, C.D.M.; *D-59*
Neto, J.B.; *G-18*
Neto, L.R.S.; *G-18*
Neto, R.; *N-10*
Netto, L.E.S.; *S-17, S-21 S-22, S-23, S-26*
Neundorf, A.K.A.; *B-42*
Neves, C.G.; *U-02*
Neves, N.; *E-25*
Neves, R.N.; *K-12, K-23, S-30*
Neves, V.B.S.; *U-27, D-28*
Neyra Recky, J.R.; *I-07*
Nicastro, G.G.; *M-25*
Niero, L.; *K-11*
Nirello, V.D.; *O-24*
Nirello, V.D.; *SP.09.03*
Nobre, L.R.; *M-08*
Nobre, P.A.; *K-48*
Nociti, F.H.; *SM.01.04*
Nogueira De Faria, B.E.; *F-24*
Nogueira, A.J.P.; *X-03*
Nogueira, C.L.; *A-19*
Nogueira, C.L.; *B-22*
Nogueira, C.T.; *F-13*
Nogueira, C.W.; *T-08*
Nogueira, F.C.S.; *A-09, O-04, O-23, R-07*
Nogueira, F.T.S; *SP.16.03*
Nonato, M.C.; *C-11, E-36, E-44, G-14, G-34, G-36*
Noske, G.D.; *E-23, G-28*
Noske, G.E.; *SP.07.02*
Nunes, A.C.C.; *K-37*
Nunes, C.J.; *N-08, N-14*
Nunes, L.E.C.; *R-04*
Nunes, L.O.; *P-05, P-06, P-15*
Nunes, L.O.C.; *G-23*
Nunes, R.D.; *A-30*
Nunes, R.O.; *R-07, R-08, R-10*
Nunes, T.K.; *K-52*
Nunes, V.A.; *F-30, F-42, K-64, K-68*
Nunes-Da-Fonseca, R.; *B-16, J-03*
Nunes-Oliveira, M.; *A-06, A-08, A-15*

O

Ohashi, K.; *B-48*
Okamoto, D.N.; *F-13*
Okorokova-Façanha, A.L.; *K-34, K-38, K-53*
Oliva, G.; *E-23, G-28*
Oliva, G.; *SP.07.02*
Oliva, M.L.V.; *I-11, R-06, R-11, T-19, R-03, T-21*
Olivares, F.L.; *R-07*
Oliveira Jr., P.N.; *C-02, D-04*
Oliveira Junior, N.L.; *R-09*
Oliveira M.F.; *B-04*
Oliveira M.T.; *B-04*
Oliveira S.E.; *B-55*
Oliveira S; *SP.18.03*
Oliveira, A.C.; *D-42, E-26, E-43, M-23*
Oliveira, A.F.; *R-10*
Oliveira, A.M.; *B-41*
Oliveira, A.N.; *P-18*
Oliveira, A.P.; *T-09*
Oliveira, B.C.D.; *M-13, M-26*
Oliveira, B.T.M.; *O-09*
Oliveira, C.C.; *U-06*
Oliveira, C.F.R.; *M-27, M-33, P-13, P-19*
Oliveira, C.G.; *E-44, E-79*
Oliveira, C.J.L.; *B-22*
Oliveira, C.S.; *E-71*
Oliveira, C.V.N.; *D-43*
Oliveira, D.M.P.; *E-37, O-04*
Oliveira, D.M.P.; *H-01*
Oliveira, F.; *P-02*
Oliveira, F.G.C.; *F-38*
Oliveira, H.C.; *E-21, E-39*
Oliveira, H.C.F.; *B-18, B-24, B-14*
Oliveira, I.B.; *D-42*
Oliveira, I.N.; *D-31, T-04*
Oliveira, I.S.; *A-02*
Oliveira, I.M.; *SP.04.02*
Oliveira, J.; *SP.04.03*
Oliveira, J.A.; *R-07*
Oliveira, J.L.; *C-03*
Oliveira, J.L; *SP.02.03*
Oliveira, J.P.B.; *F-52*
Oliveira, J.R.; *K-39, K-50*
Oliveira, J.R.; *T-21*
Oliveira, L.C.; *D-19*
Oliveira, L.C.; *E-08*
Oliveira, L.F.B.; *E-18*
Oliveira, L.S.; *A-31*
Oliveira, L.S.; *G-07*
Oliveira, M.A.; *D-31, F-63, F-88, S-17, T-04*
Oliveira, M.A.; *S-21, T-19, S-22, S-23, S-26*
Oliveira, M.A.B.; *A-35*
Oliveira, M.A.S.; *B-15, B-34*
Oliveira, M.C.; *K-43*
Oliveira, M.F.; *A-15, A-20*
Oliveira, M.S.; *K-52*
Oliveira, P.L.; *A-08, A-13, A-15, A-20, A-27, A-29, A-30*
Oliveira, P.S.L.; *E-05, E-49*
Oliveira, R.B.; *G-13*
Oliveira, R.C.; *K-35*
Oliveira, R.G; *SP.11.02*
Oliveira, R.J; *SP.22.02*
Oliveira, S.; *G-35*
Oliveira, S.B.; *Q-01, Q-05*
Oliveira, S.C.; *E-70*
Oliveira, S.D.; *E-35, E-72*
Oliveira, T.E.; *B-51*
Oliveira, T.E.; *K-13, K-25*
Oliveira, T.R.P.; *B-46*
Oliveira, V.H.; *S-28*
Oliveira, V.R.S.; *K-57*
Oliveira, W.A.; *B-11*
Oliveira, W.F.; *I-06, J-01*
Oliveira, W.F.; *I-11, M-12*
Oliveira. L.A.S.; *K-61*
Oliveira-Marques, G.H.; *K-69*
Oliveita, T.M.; *F-72*
Olivera-Nappa, A.; *SP.07.03*
Oliviera, E.L.; *T-24*
Onuchic, J.N.; *D-52*
Orives, K.G.R.; *U-18*

Orlandi, C.B.C.; *P-14*
Ortiz, N.; *F-20*
Oseliero Filho, P.L.; *D-61*
Oseliero Filho, P.L; *SP.02.04*
Osorio, R.P.; *S-02*
Otenio L.I.; *K-02*
Otvos, L.P.; *U-19*
Outeiro, T.F.; *D-58*
Owens, R.J.; *G-18*
Oyamada, I.; *E-95*

P

Pace, P.; *S-13*
Pachane, B.C.; *K-37*
Pacheco Da Silva, M.F.; *G-12*
Pacheco, L.S.; *B-03*
Padovani, C.T.J.; *M-14*
Pádua, T.A.; *K-18*
Paduel, L.D.; *F-40*
Padueli, L.D.; *F-05*
Paes, M.C.; *A-03*
Paiva, C.E.; *S-32*
Paiva, I.M.; *M-18*
Paiva, P.M.G.; *A-16, M-12, R-03*
Paiva, S.C.; *E-39*
Paiva, S.I.S.; *K-33*
Paiva-Silva, G.O.; *A-06, A-08, A-13, A-15, A-20*
Paixao, R.R.; *X-13*
Palermo J.; *D-63*
Palhano, F.; *E-53*
Palma, C.S.; *K-54*
Palma, L.M.; *H-02*
Pando, L.A.; *T-02, T-13*
Pando, S.C.; *T-02, T-13*
Pane A. ; *SP.08.03*
Pane, A.; *M-11*
Panessa, G.M.; *U-16, U-21, U-22*
Pankievicz, V.C.S; *SP.17.03*
Pantaleão, S.Q.; *D-44, E-19*
Pantano, S.; *E-90*
Pantano, S.; *SP.07.01*
Papoian, G.; *D-08*
Parisotto, E.B.; *S-11*
Parize, E.; *O-16*
Parizi, L.F.; *A-01*
Park, P.; *P-04*
Parra, J.A.A.; *P-03*
Parra, M.F.; *E-19*
Parreira, A.G.; *F-65, F-88, F-63, M-28*
Pascoal, A.C.R.F.; *F-08*
Pascutti, P.G.; *D-16, D-07, D-32, G-19, D-05*
Passos, A.O.; *M-05*
Passos, G.F.; *K-08*
Pastrello, B.; *D-23*
Pato, G.T.G.; *O-05*
Patricio, B.F.C.; *G-01, G-08*
Patricio, B.F.De C.; *G-11*
Patricio, M.R.; *B-44*
Patriota, L.L.S.; *I-11*
Patrocinio, A.O.T.; *I-10*
Paula, E.; *G-22*
Paula, L.; *K-62*
Paula, M.B.M.; *B-46*
Paulino, A.C.; *J-06*
Paulino, P.G.S.B.; *J-01*
Pavan, I.C.B.; *U-09*
Pavão, M.S.; *K-44*
Pavão, M.S.G.; *J-08*
Paz, G.; *B-52*
Pazin, W.M.; *X-13*
Peabody, D.S.; *E-26*
Peçanha, F.H.; *E-93*
Pedro, R.P.; *D-19, E-08, E-18, E-59, E-62*
Pedrosa, F.O.; *O-16, O-20, R-01, R-05*
Pedroza, B.S.; *M-23*
Peixoto, A.S.; *B-51*
Peixoto, A.S.; *K-13, K-25, K-40*
Peixoto, H.R.; *C-01*
Pelentir, G.F.; *E-27*
Pellegrina, D.V.S.; *K-51, O-05*
Peluffo, R.D.; *SP.19.01*
Peluso, A.A.; *O-01*
Penna, B.R.; *E-07, E-81*
Peppelenbosch, M.P; *SP.03.02*
Perahia, D.; *D-12, D-30, D-35*
Perahia, D.; *SP.10.01*
Peralta, R.M.; *B-40, T-18*
Pereira, A.A.; *E-35*
Pereira, A.C.C.; *H-03*
Pereira, A.E.S.; *F-43*
Pereira, A.K.H.; *E-12, E-94*
Pereira, A.P.R.; *M-27*
Pereira, A.R.; *B-11*
Pereira, C.S.; *D-39*
Pereira, D.M.C.; *B-42*
Pereira, D.R; *T-20*
Pereira, F.S.; *F-87*
Pereira, F.S.O.; *T-15*
Pereira, G.; *F-57*
Pereira, G.A.L.; *F-57*
Pereira, G.A.L.; *I-06*
Pereira, G.C.; *K-42*
Pereira, G.R.C.; *D-24, D-58*
Pereira, H.; *E-53*
Pereira, H.; *E-80*
Pereira, H.D.; *SP.07.02*
Pereira, H.D.M.; *E-64*
Pereira, H.J.V.; *F-32*
Pereira, J.C.T.; *A-26*
Pereira, J.O.S.L.; *G-01*
Pereira, L.C.; *F-39, G-30*
Pereira, L.F.; *G-27*
Pereira, L.M.B.; *D-36*
Pereira, M.D.; *S-05, S-18, S-19*
Pereira, M.D.; *S-20, S-25, S-31*
Pereira, M.S.; *E-58*
Pereira, S.T.; *I-05*
Pereira, V.A.R.; *D-42*
Péres, L.E.P.; *SP.16.03*
Peres, R.A.S.; *G-32, K-10*
Perez, A.S.; *B-07*
Perez, A.S.; *C-07*
Pérez, C.A.; *K-44*
Perry, G.T.; *H-05*
Persinoti, G.F.; *F-36*
Peruchetti, D.B.; *C-01, G-32, K-10, K-18, K-32, K-56*
Peruchetti, D.B; *SP.18.01*
Pesquero, J.B.; *U-04*
Pessoa, E.V.M.; *K-40*
Pessoa, N.M.; *K-40*
Pessoa, R.B.G.; *M-12*
Pessoa-Nascimento, M.; *B-45*
Petri, D.F.S.; *N-02*
Petry, F.S.; *N-04*
Petta, T.; *B-44*
Philot, E.; *D-21*
Philot, E.A.; *D-30, D-44, E-67*
Piccirillo, E.; *D-33*
Pieretti, J.C.; *K-01*
Pikula, S.; *SM.01.01, SM.03.01*
Pimenta, A.M.; *P-03*
Pimenta, A.M.C.; *G-16*
Pimenta, B.V.; *F-64*
Pimenta, D.C.; *O-19*
Pimentel, V.E.; *K-30*
Pimienta, B.V.; *F-37*
Pinhal, M.A.S; *SP.06.04*
Pinheiro A.A.S; *SP.15.04*

Pinheiro, A.A.S.; *C-01, G-32, K-10, K-32, K-56*
Pinheiro, A.A.S.; *K-18, M-01*
Pinheiro, A.A.S.; *M-24*
Pinheiro, A.A.S; *SP.18.01*
Pinheiro, A.S.; *E-14, V-02*
Pinheiro, A.S.; *K-18*
Pinheiro, A.S.; *M-24*
Pinheiro, A.S.; *V-01*
Pinheiro, A.S.; *V-03*
Pinheiro, A.S; *SP.11.03, SP.15.01*
Pinheiro, F.C.; *T-08*
Pinheiro, G.; *E-24*
Pinheiro, I.O.; *F-48*
Pinheiro-Aguiar, R.; *E-57*
Pinho, A.M.V.F.; *S-07*
Pinto, B.A.S.; *C-08*
Pinto, G.D.A.; *B-02*
Pinto, I.F.D.; *K-15*
Pinto, S.; *O-01*
Pinton, S.; *B-21, K-49, N-07, T-01*
Pires, D.C.; *S-02, S-19, S-31*
Pires, M.R.; *U-16, U-21*
Pizzol, S.U.; *C-02*
Poiato, G.E.H.; *K-15*
Polizelli, L.G.; *B-34*
Pollard, P.; *SP.11.01*
Polloni, L.; *T-07, T-12*
Polo, C. ; *SP.23.02*
Polycarpo, C.; *M-02*
Ponte, C.G.; *C-17*
Pontes, F.L.D.; *O-16*
Porcionatto, M.A; *SP.05.01*
Possa, L.; *K-03*
Possato, G.; *E-41*
Pougy, K.C.; *E-14*
Prado A.J.M.; *S-22*
Prado, F.M.; *S-09, S-10, S-16, S-28, S-29*
Prado, V.C.P.; *F-60*
Prado-Souza, L.F.L.; *B-10, B-35, K-27, Q-06*
Prata, B.A.; *F-35*
Prazeres, G.B.; *A-16*
Pretti, M.A.; *SP.09.01*
Prieto, M.J.E; *SM.04.01*
Prieto, P.; *SP.21.01*
Prigol, M.; *T-08*
Procópio, A.; *X-07*
Puccia, R.; *S-22*
Puntel, R.L.; *B-21*
Purificação Junior, A.F.; *F-09*

Q

Queiroz, D.D.; *S-25*
Queiroz, M.I.C.; *B-18*
Quezado De Magalhães, M.T.; *D-60*
Quina, F.H.; *I-09*
Quines, C.B.; *B-21, N-07, K-49, T-01*
Quiroz, R.C.N.; *D-30*
Quispe, R.L.; *F-34*

R

Rabelo, N.G.; *F-67, F-84*
Rade, L.L.; *F-36*
Rafaela, S.F.; *D-57*
Raíssa-Oliveira, B; *G-16*
Ramalho, S.R.; *M-33*
Ramos Jr, S.L.; *E-51*
Ramos, A.F.; *F-66, F-73, F-87*
Ramos, A.P.; *C-03, X-15*
Ramos, A.P.; *E-55, F-12*
Ramos, A.P.; *F-17*
Ramos, A.P.; *SM.03.02*
Ramos, A.P.; *SP.03.04*
Ramos, C.H.I.; *E-01, E-24, E-33, E-39, E-41, E-46, E-65, E-71*
Ramos, F.S.; *N-11*
Ramos, G.S.; *G-05, G-29*
Ramos, I.; *A-06*
Ramos, I.B.; *A-26*
Ramos, J.A.; *K-65, K-67, K-71, M-29*
Ramos, J.M.O.; *B-43, K-50, K-05*
Ramos, J.O.M.; *K-39*
Ramos, L.F.C.; *H-01, O-04*
Ramos, L.S; *F-39*
Ramos, M.V.; *F-52*
Ramos, M.V.; *R-02*
Ramos, P.I.P.; *D-28*
Ramos, P.W.C.; *A-12*
Ramos, S.; *K-72*
Ramos, S.L.; *K-52*
Ramos, V.S.; *Q-06*
Rangel, *E-26*
Rangel-Yagui, C.; *G-21*
Ranquine, L.; *B-28*
Raposo, B.L.; *I-02*
Ratajczak, M.Z; *CF.06.*
Raucci, A.R.; *H-04*
Ray, P.; *F-75, F-79*
Rebouças, J.S.; *F-48, I-02, S-08*
Recoulat Angelini, A.; *E-88*
Reddy, S.N.; *X-04*
Regatieri, C.V.S.; *J-07*
Regis, T.S.A.; *B-45*
Rego, L.G.M.R.; *K-34*
Rêgo, P.A.O.D.; *K-70*
Reigado, G.R.; *F-30, F-42*
Reis, A.A.A.S.; *F-74, R-14*
Reis, E.M.; *K-51, O-05*
Reis, K.C.P.; *F-67, F-84*
Reis, L.A.; *A-15*
Reis, L.A.; *F-28*
Reis, M.; *F-86*
Reis, P.V.M.; *S-16, S-28*
Reis, T.P.; *D-23*
Reis, W.F.; *J-04*
Renon, J.P.R.C.; *B-26*
Rentz, T.; *B-14, B-18*
Resende, A.C.; *B-42*
Resende, B.C.; *F-33*
Resende, J.M.; *P-05, P-15*
Resende-Lara, P.T.; *D-12*
Rezende Júnior, C.R.G.; *I-08*
Rezende, G.S.; *M-07*
Rezende, H.K.; *S-15*
Rezende, M.; *K-66*
Rhana, P.; *C-10, T-09*
Ribeiro, B.R.M.; *F-18, F-24, F-28, G-10*
Ribeiro, E.H.P.; *N-12*
Ribeiro, F.A.; *H-09*
Ribeiro, F.C.; *N-10*
Ribeiro, G.F.; *K-61*
Ribeiro, G.S.; *E-55*
Ribeiro, I.R.; *F-01*
Ribeiro, J.F.F.; *F-57*
Ribeiro, J.R.P.; *F-85, F-89*
Ribeiro, L.; *J-03*
Ribeiro, L.F.C.; *A-05, F-59*
Ribeiro, L.N.M.; *G-22*
Ribeiro, M.; *I-01*
Ribeiro, M.S.; *F-44, I-03, I-05*
Ribeiro, N.B.S.; *G-32*
Ribeiro, O.R.; *E-63*
Ribeiro, P.R.; *F-74, F-76, N-11, O-11, R-14*
Ribeiro, R.A.; *F-63*
Ribeiro, R.T.; *F-57*
Ribeiro, S.M.; *I-02*
Ribeiro, T.N.; *SP.05.01*
Ribeiro-Filho, H.V.; *E-05, E-49, K-57*
Ricachenevsky, F.K.; *D-37*
Ricci, M.F.; *C-15*
Riger, C.J.; *S-02, S-03, S-14, S-19, S-20*
Rios, D.L.; *F-33*

Riske, K.A.; *F-64, X-04, X-07*
Rivas, A.V.; *M-17*
Rivas, M.G.; *S-27*
Rizzatti, S.; *K-11*
Rizzi, A.C.; *S-27*
Rocco, P.R.M.; *SP.01.01*
Rocha T.S.; *B-04*
Rocha, A.; *H-05*
Rocha, A.; *SP.18.02, SP.04.01*
Rocha, A.M.F.; *E-73*
Rocha, B.M.; *B-39*
Rocha, G.J.M.; *F-04*
Rocha, H.V.A.; *G-01, G-11*
Rocha, L.; *U-26*
Rocha, T.S.; *E-32*
Rocha, V.F.; *F-17*
Rocha, V.M.; *R-06, R-11*
Rodovalho, V.R.; *M-03*
Rodrigues Da Silva, G.H.; *B-25, F-46, G-17, G-22*
Rodrigues, B.C.; *J-03*
Rodrigues, C.F.B.; *F-56*
Rodrigues, C.G.; *D-43*
Rodrigues, J.A.; *F-48*
Rodrigues, J.C.F.; *B-55, R-04*
Rodrigues, L.F.C.; *E-65*
Rodrigues, L.V.; *P-09*
Rodrigues, M.Q.R.B.; *F-67, F-84*
Rodrigues, M.S.; *K-16*
Rodrigues, M.S.; *N-05*
Rodrigues, R.S.; *P-18, T-07*
Rodrigues, S.D.; *K-06*
Rodrigues, S.P.; *F-31*
Rodrigues, S.P.; *R-04, R-12, R-13*
Rodrigues, T.; *B-10, B-35, F-41, Q-06, T-21*
Rodrigues, T.; *K-02*
Rodrigues, T.; *K-27*
Rodrigues, T.S.; *G-35*
Rodrigues, T.S.; *SP.18.03*
Rodrigues, V.M.; *P-18, T-07*
Rodrigues, V.M.; *T-12*
Rodrigues-Ribeiro, L.; *O-23*
Rodriguez, M.A.; *G-14*
Roggero, A.; *D-31, T-04, T-10, T-19*
Rojas, V.B.; *A-08, A-30*
Roldão, A.P.; *E-05, E-49, K-57*
Roman, E.A.; *E-88*
Roman, E.K.B.; *F-04, F-11*
Roman-Campos, D.; *C-09, C-10, Q-07, T-09*
Romanelli, M.A.; *O-06*
Romanello, K.S.; *U-05*
Romano, M.P.; *S-06*
Romão, S.; *B-42*
Romero, J.G.; *SM.04.03*
Ronsein, G.E.; *O-10, O-22, S-09, S-10, S-28, S-29*
Rosa, A.L. ; *SM.03.04*
Rosa, D.S.; *F-57*
Rosa, H.V.D.; *SP.07.02*
Rosa, R.S.L.; *E-09*
Rosa, T.S.; *T-05*
Rosário, M.E.F.; *E-96*
Rossi, A.D.; *D-16*
Rossi, F.P.N.; *M-21*
Rossmann, M.; *SP.17.04*
Rossoni, L.V.; *S-04*
Rozental, S.; *M-31*
Ruas, J.S.; *U-01*
Ruas, M.M.; *E-14*
Ruiz, N.Q.; *U-09*
Ruller, R.; *F-14, F-77, F-78, F-81*
Rumi, G.A.; *SM.04.02*
Russo, L.C.; *K-51*

S

Sá, E.J.R.B; *R-09*
Sa, E.J.R.B.; *T-16*
Sá, J.M.; *T-14*
Sá, K.; *SP.18.03*
Sá, K.S.G.; *SP.04.02*
Sá, K.S.G.; *G-35*
Sá, R.A.; *A-16*
Sá, Y.A.P.J.; *G-32*
Sabino, A.P.; *O-12*
Sachetto-Martins, G.; *E-14, V-03*
Sacocchi, I.C.R.; *G-14*
Safranchik, M.; *E-89*
Saiki, F.A.; *O-27*
Saito, A.; *K-33, K-55*
Saito, C.B.; *D-66*
Saito, C.P.B.; *F-70*
Saito, D.; *D-66, F-70*
Saito, V.I.; *F-43*
Sala, F.A.; *E-32*
Saladino, G.C.R.; *E-66*
Salardani, M.; *K-46*
Salbego, C.G.; *H-05, N-04*
Saldanha, J.; *A-13*
Salentinig, S.; *SP.19.04, SP.02.01*
Sales, L.G.P.; *K-70*
Sales, M.; *B-13*
Sales, M.V.S.; *B-32*
Salinas, R.K.; *X-07*
Salles, T.S.; *A-28*
Salmaso, C.J.L.; *B-12, B-36*
Salnikov, E.; *P-05*
Salnikov, E; *P-15*
Salvador, G. H. M; *E-39*
Salvador, M.J.; *F-08*
Salvego, C.A.; *F-44*
Sampaio, A.F.A.; *B-22*
Sampaio, M.L.C.; *A-01*
Sampaio, T.B.; *N-07*
Sampaio, T.B.; *T-01*
Sanches, F.S.; *N-11*
Sanches, L.; *O-05*
Sanches, M.N.; *D-08, D-19, D-52, D-53*
Sanna, P.L.S.; *N-06, U-15*
Sant'anna, S.S.; *O-03*
Santamaria, A; *S-11*
Santana, A.K.C.; *F-07*
Santana, A.M.; *H-09*
Santana, B.M.; *F-44*
Santana, L.C.; *R-14*
Santana, S.G.; *I-02*
Santana-Silva, M.C.; *E-96*
Sant'anna, C.B.; *B-57*
Sant'anna, S.S.; *E-16, F-05, O-08, O-21*
Santiago, F.; *Q-08*
Santillan, J.A.V.; *E-64*
Santoro, A.L.; *O-01*
Santos D.S.; *A-25*
Santos P.C.; *F-76*
Santos P.H.G.; *D-60*
Santos, A.; *F-69, G-20*
Santos, A.C.S.; *F-86*
Santos, A.J.R.; *E-74*
Santos, A.K.; *G-29*
Santos, A.M.; *H-03*
Santos, A.M.C.; *E-78*
Santos, A.R.S.; *O-16*
Santos, A.T.; *B-30*
Santos, A.T.F.P.; *P-14*
Santos, A.Y.I.; *U-24*
Santos, B.G.; *N-04*
Santos, B.M.; *F-31*
Santos, B.P.O.; *E-70, E-85*
Santos, B.P.O.; *P-03, P-09*
Santos, B.S.; *F-57, I-06*
Santos, B.S.; *S-08*
Santos, C.C.; *F-76*
Santos, C.C.A.; *E-12, E-94*
Santos, C.Dos; *F-49*
Santos, C.L.; *F-57*
Santos, C.M.; *F-35*

Santos, C.P.; *F-89*
Santos, D.; *D-01*
Santos, D.A.; *B-09*
Santos, D.C.; *B-19*
Santos, D.C.; *B-22*
Santos, D.E.S.; *D-25*
Santos, D.G.T. Dos; *B-23*
Santos, D.M.P.O.; *E-54, E-81*
Santos, D.W.; *S-32*
Santos, E.S.; *U-01*
Santos, F.L.E.; *E-33, E-71*
Santos, G.D.C.; *F-23*
Santos, H.L.; *N-13*
Santos, I.; *N-09*
Santos, J.C.C; *B-32*
Santos, J.F.; *F-42*
Santos, J.F.; *K-64*
Santos, K.; *E-63*
Santos, K.S.; *E-74*
Santos, L.; *K-03*
Santos, L.H.S.; *P-09*
Santos, L.L.D.; *S-32*
Santos, L.L.S.; *F-33*
Santos, L.O.; *K-33, K-55*
Santos, L.O.; *SP.09.01*
Santos, L.S.; *E-82*
Santos, L.V.; *F-04*
Santos, M.B.; *H-05*
Santos, M.C.; *D-65*
Santos, M.C.; *S-23*
Santos, M.C.O.S.; *R-14*
Santos, M.F.; *S-15*
Santos, M.R.V.; *T-09*
Santos, N.R.M.; *I-11*
Santos, P.C.; *F-85*
Santos, R.C.M.; *J-05*
Santos, R.R.O.; *B-54*
Santos, R.S.; *K-15*
Santos, S.A.A.; *K-35*
Santos, S.B.; *I-02*
Santos, T.L.; *P-05, P-15*
Santos, V.C.; *G-13*
Santos, V.C.; *G-33*
Santos, V.F.; *D-25*
Santos, V.V.; *G-11*
Santos-Filho, N.A.; *G-23*
Santos-Jr, A.P.; *M-16, M-21*
Santos-Junior, O.H.; *B-03, B-49*
Santos-Miranda, A.; *C-10, C-15*
Santos-Miranda, A.; *Q-07*
Santos-Oliveira, J.R.; *K-62*
Santos-Oliveira, R.; *C-06*
Santos-Oliveira, R.; *F-21, L-01*
Sá-Nunes, A.; *SP.08.04*
Saraiva, F.M.S.; *A-03*
Saraiva, G.K.V.; *X-07*
Saraiva, G.N.; *K-08*
Saraiva, L.S.; *E-57*
Sardi, J.C.O.; *P-19, M-33, P-13*
Sarkis, G.; *F-70*
Sarmento, J.O.; *Q-07*
Sarmento-Neto, F.J.; *I-02*
Sarmento-Neto, J.F.; *S-08*
Sarmiento, C.A.R.; *E-64*
Sartori, J.; *D-06*
Sasaki, S.D.; *K-58*
Sastre, G.; *K-59*
Sastre, G.S.; *K-54*
Savall, A.P.; *K-49, N-07*
Savall, A.S.P.; *T-01*
Sawaya, A.C.H.F.; *F-08*
Sbano, A.; *D-02, G-04*
Scarpa, I.; *E-61*
Scarpassa, J.A.; *F-77*
Scavassa, B.; *D-34*
Scharfstein, J.; *M-24*
Schechtman, D.; *E-05, E-49, K-57*
Scheffer, D.L.; *K-11*
Schelk, V.; *Q-04*
Schiess, G.H.A.; *D-20*
Schleger, I.C.; *B-42*
Schmaier, A.A.; *SP.01.02*
Schmaier, A.H.; *K-32, K-56*
Schmaier, A.H; *SP.15.01*
Schmitt, L.O.; *K-52*
Schmitz, F.; *B-43*
Schneck, E.; *D-10*
Schneedorf, J.M.; *SP.20.02*
Schneider, A.H.; *M-18*
Schons, T.; *K-16*
Schons, T.; *N-05*
Schwartz, J.Z.; *E-06*
Scomparim, G.; *A-17*
Scott, A.L.; *D-11, D-21, D-30, D-44, E-67, H-04*
Scott, A.L.; *SP.22.04*
Sculaccio, S.A.; *E-64*
Seabra, A.B.; *F-44, K-01*
Seabra, V.A.V.; *M-09*
Sebinelli, H.G.; *C-03, X-15*
Sebinelli, H.G; *SP.03.04, SM.03.04*
Segat, B.B.; *S-05*
Sehr, P.; *E-89*
Seixas, F.A.V.; *A-05, D-46, E-87*
Selistre-De-Araujo, H.; *SP.24.03*
Selistre-De-Araujo, H.S.; *K-37*
Sellani, R.; *K-48, O-09*
Sena Jr, D.M.; *SP.19.02*
Sepulveda, A.F.; *G-15*
Serain, A.F.; *F-08*
Seraphim, T.V.; *E-35, E-51, E-83*
Sergeeva, M.G.; *X-02*
Seribelli, A.A.; *G-35*
Serino-Silva, C.; *F-05, F-56, O-08, O-17, O-21*
Serpa, G.B.; *K-10*
Serrano, S.M.T.; *O-14*
Serrão, V.H.B.; *E-51*
Serrato, R.V.; *H-08*
Setubal, J.C.; *M-20*
Setubal, J.C.; *SP.17.04*
Severino, M.B.; *U-09*
Severo, V.R.; *E-06*
Sgro, G.G.; *E-05, E-22, E-28, E-49, M-04, M-09*
Sharko, O.L.; *X-02*
Sharrocks, A.D.; *U-11*
Shchepinov, M.S.; *X-02*
Shiburah, M.E.; *M-26*
Shimizu, M.H.M.; *S-12*
Shmanai, V.V.; *X-02*
Sigurdardottir, S.; *M-19*
Silva Jr, J.A.; *U-04*
Silva Junior, F.P.; *G-18*
Silva Vaz Jr., I.; *A-21, F-54*
Silva, A.A.R.; *D-43*
Silva, A.B.A.; *K-26*
Silva, A.C.; *K-45*
Silva, A.C.P.; *A-28*
Silva, A.G.G.; *E-12*
Silva, A.H.M.; *E-13, E-74, E-77*
Silva, A.L.T.; *J-02*
Silva, A.L.T.S.; *A-16*
Silva, A.M.; *M-21*
Silva, A.M.; *SP.17.04*
Silva, A.M.R.P.; *X-14*
Silva, A.R.; *A-16*
Silva, A.R.M.; *O-10*
Silva, A.S.; *E-15, M-08*
Silva, A.S.; *F-69*
Silva, A.T.F.; *S-32*
Silva, B.D.L.; *H-02*
Silva, B.G.; *T-06*
Silva, B.M.S.; *M-18*
Silva, B.P.; *K-25*
Silva, C.; *A-04*
Silva, C.A.S.; *B-49*
Silva, C.C.; *F-23*
Silva, C.P.S.; *I-09*
Silva, C.R.; *I-05*
Silva, C.V.; *T-24*
Silva, E.P.; *K-35, U-24*
Silva, E.Q.G.; *F-39*
Silva, E.R.; *E-02, E-34, E-84, F-25, F-15, M-06*

Silva, E.S.; *E-23*
Silva, E.S.; *G-28*
Silva, F.A.A.; *A-21*
Silva, F.A.A.; *F-13*
Silva, F.F.; *F-84, F-67*
Silva, F.F.F.F.; *G-11*
Silva, F.N.; *F-87*
Silva, F.S.; *F-79*
Silva, G.; *A-15*
Silva, G.M.T.; *I-09*
Silva, G.R.; *F-82*
Silva, G.S.M; *B-29*
Silva, H.C.; *J-04*
Silva, I.S.; *K-19*
Silva, J.A.; *F-65, F-67, F-84, F-88*
Silva, J.A.; *M-28*
Silva, J.C.; *M-18*
Silva, J.L.; *E-25, E-26, E-43, E-86, M-23*
Silva, J.L.; *SP.21.03, SP.13.03*
Silva, J.N.; *B-33, B-50*
Silva, J.P.C.; *O-14*
Silva, J.P.M.O.; *U-05*
Silva, J.R.; *H-07*
Silva, K.N.; *H-03*
Silva, L.C.N.; *F-23*
Silva, L.C.N.; *T-17*
Silva, L.G.; *D-45*
Silva, L.G.S.; *U-09*
Silva, L.M.; *F-21*
Silva, L.S.; *A-32*
Silva, L.S.; *F-23*
Silva, L.S.; *K-18*
Silva, M. L; *D-02*
Silva, M.A.A.; *G-35*
Silva, M.C.; *F-74, F-76, F-85*
Silva, M.C.; *F-89*
Silva, M.G.; *F-18, F-24*
Silva, M.J.B.; *T-12*
Silva, M.L.; *D-38*
Silva, M.L.; *G-04*
Silva, M.M.; *O-26*
Silva, M.N.; *I-12*
Silva, M.O.; *K-25*
Silva, M.R.S.; *R-08*
Silva, M.R.S.; *R-10*
Silva, N.D.; *A-01*
Silva, N.M.A.; *A-02*
Silva, N.M.S.; *E-56*
Silva, N.S.M.; *E-01*
Silva, N.S.M.; *E-17*
Silva, N.X.; *A-07*
Silva, P.M.R.; *G-32*
Silva, R.; *K-67, K-71*
Silva, R.A.; *K-35*
Silva, R.A.; *N-06, U-12, U-15, U-24*
Silva, R.A.; *SP.03.02*
Silva, R.G.P.; *M-30*
Silva, R.N.; *B-11*
Silva, R.S.F.; *M-32*
Silva, R.T.; *K-08*
Silva, S.; *F-02*
Silva, S.F.; *M-19*
Silva, S.M.; *F-90*
Silva, S.W.; *N-05*
Silva, T.B.V.; *T-18*
Silva, T.M.; *K-69*
Silva, T.N.L.; *M-28*
Silva, V.D.; *F-76*
Silva, V.D.A.; *N-11*
Silva, V.D.A.; *N-14*
Silva, V.F.; *K-42*
Silva, Y.M.; *O-04*
Silva-Aguiar R.P.; *SP.18.01*
Silva-Aguiar, R.P.; *C-01, G-32, K-10, K-18, K-32, K-56, M-24*
Silva-Alves, K.S.; *SP.18.04*
Silva-Filho, S.E.; *T-11*
Silva-Freitas, M.L.; *K-62*
Silva-Gabrielle, A.G.; *E-94*
Silva-Jr, A.; *F-39*
Silva-Neto, P.V.; *B-26, B-36, B-44, K-48, O-09*
Silveira, G.P.M.; *F-05, O-07, O-13, O-21*
Silvestre, D.H.; *D-38*
Silvestrini, V.C.; *K-26, K-54, K-59*
Simabuco, F.M.; *U-09*
Simas, A.L.O.; *F-78*
Simões, B.P.; *K-43*
Simões, R.P.; *D-30, D-55, D-62*
Simonin, V.R.S.; *H-02*
Sinigaglia, R.C.; *T-21*
Siqueira, C.M.; *E-06*
Siqueira, F.R.; *K-68*
Siqueira-Santos, E.S.; *B-07*
Sividanes, G.N.; *S-21*
Slott, S.; *D-09*
Smith, T.K.; *I-03*
Soares, I.N.; *E-26*
Soares, J.B.; *A-09*
Soares, L.; *K-62*
Soares, L.A.L.; *T-03*
Soares, M.A.; *K-44*
Soares, M.A.K.; *B-47*
Soares, M.A.K.M.; *B-05*
Soares, M.R.; *R-07*
Soares, T.; *D-01*
Soares, T.A.; *D-04, D-13, D-25, F-45*
Soares-Paulino, A.A.; *B-35*
Sodre, P.T.; *D-03*
Solano, A.; *U-12*
Solano, A.F.; *U-15*
Soliz, I.A.F.; *F-49, F-58*
Somacal, S.; *B-43*
Somensi, N.; *K-12*
Sone, A.P.; *F-59*
Soñora, M.; *E-90*
Sorgi, C.A.; *B-44, B-12, B-26, B-36, K-30, K-48, O-09*
Sorgine, M.H.; *A-25*
Sotero-Martins, A.; *A-28*
Soto, C.; *C-16*
Sousa Jr., I.P.; *C-12*
Sousa, A.C.; *K-05, K-39, K-50*
Sousa, G.S.; *E-25*
Sousa, L.O.; *U-01*
Souza Junior, D.R.; *O-10*
Souza Junior, L.A.; *T-02*
Souza, A.B.; *M-17*
Souza, A.B.C.; *E-02*
Souza, A.N.; *P-08*
Souza, A.R.; *D-23*
Souza, A.V.; *S-32*
Souza, C.; *G-03*
Souza, D.C.A.; *E-96*
Souza, D.S.; *C-09, C-10*
Souza, D.S.; *T-09*
Souza, D.V.; *F-89*
Souza, E.M.; *G-24, O-16, O-20, R-01, R-05*
Souza, F.P.; *D-42*
Souza, F.P.; *E-87*
Souza, F.P.; *K-02*
Souza, F.P.; *T-14*
Souza, G.A.; *O-16*
Souza, I.C.C.; *K-05*
Souza, J.; *K-11, U-12, U-15, V-05*
Souza, J.R.R.; *K-55*
Souza, K.L.; *S-34*
Souza, K.R.; *P-05, P-08, P-15*
Souza, L.A.; *G-30*
Souza, L.C.M.; *R-14*
Souza, L.E.A.; *E-84*
Souza, L.E.A.; *M-06*
Souza, L.J.; *N-08*
Souza, M.C.; *K-18*
Souza, M.C.; *X-05*
Souza, M.E.O.; *T-15*
Souza, M.L.; *G-25*
Souza, N.J.; *E-05*
Souza, N.J.; *E-49*

Souza, R.F.; *M-25*
Souza, S.O.; *I-02*
Souza, T.A.B. De; *E-48*
Souza, T.A.C.B.; *B-09*
Souza, T.A.C.B.; *E-06*
Souza, T.H.S.; *S-08*
Souza, T.L.F.; *E-26*
Souza, U.B.; *K-41*
Souza, V.C.; *E-54*
Souza, V.D.; *F-85*
Souza, V.L.; *B-29*
Souza, V.S.; *F-07*
Souza, W.R.; *F-84*
Souza-Lopes, T.; *U-10*
Spegiorim, G.C.; *B-38*
Spelta, G.I.; *D-32*
Speziali, M.G.; *F-65*
Staats, A.; *K-11*
Stefani, H.A.; *B-35*
Stefani, L.C.M.; *F-69*
Steffens, M.B.R.; *O-16*
Steinberg, P.Y.; *SM.04.02*
Steiner, A.A.; *O-15*
Stelling, M.P.; *J-08, K-44*
Stellingm, M.P.; *C-17*
Strzelecka-Kiliszek, A.; *SM.01.01, SM.03.01*
Suely, G.F.; *G-07*
Suguihiro, N.M.; *R-13*
Sulzbach, S.L.; *N-05*
Sunnerhagen, P.; *M-19*
Suzuki, E.; *K-58*

T

Tabanez, M.; *N-09*
Taborda, D.O.; *F-28*
Tahara, E.B.; *F-38, M-10*
Tahira, A.C.; *U-10*
Takahashi, D.; *F-76, F-85, F-89*
Takiya, C.M.; *K-10*
Talachia Rosa, L.; *SP.11.01*
Tamashiro, J.; *B-13*
Tamashiro, J.A.; *B-27*
Tanaka, A.M.T.; *O-13*
Tanaka, A.S.; *A-17, F-13, F-54*
Tanaka, A.S.; *A-21*
Tanaka-Azevedo, A.M.; *E-16, F-05, F-40, F-56, O-03, O-07, O-08, O-17, O-21*
Taniwaki, N.N.; *M-26*
Tanouye, F.T.; *D-18*
Tarabal, V.S.; *F-65*
Taruhn, L.F.; *K-52*
Tasima, L.J.; *E-16, O-03, O-17, O-21*
Tavares, C.P.; *M-04*
Tavares, J.; *R-13*
Tavares, L.P.; *F-20*
Tavares, M.P.S.; *A-10*
Tavares, T.S.; *H-02*
Tedesco, J.A.; *E-08, E-29*
Teixeira D.E.; *K-18, M-01*
Teixeira, A.B.S.; *R-06, R-11*
Teixeira, C.E.; *SP.09.01*
Teixeira, C.S.; *T-17*
Teixeira, D.E.; *G-32, K-10, K-32, K-56*
Teixeira, D.E.; *M-24*
Teixeira, E.H.; *J-04*
Teixeira, J.A.; *F-50*
Teixeira, K.K.L.; *U-05*
Teixeira, L.; *K-22*
Teixeira, L.F.S.; *U-23*
Teixeira, O.; *E-36*
Teixeira, O.; *E-44*
Teixeira, R.R.; *A-05*
Teixeira, R.R.; *G-30*
Teixeira, S.C.; *T-12*
Teixeira-Fonseca, J.L.; *C-09*
Teixeira-Fonseca; *C-14*
Tejada, A.H.; *E-17*
Teles, J.S.; *N-11*
Teles, J.S.S.; *F-78*
Teles-Ferreira, D.C.; *D-26*
Terra, W.R.; *A-24*
Tersariol, I.L.S.; *K-27, Q-06*
Tessarollo, N.G.; *SP.09.01*
Thaumaturgo, M.A.; *J-05*
Thiemann, O.H.; *E-32*
Thomas, A.H.; *SP.12.04*
Thomas, A.M.; *SP.17.04*
Tiburcio, M.A.; *K-04*
Tilelli, C.Q.; *K-72*
Tiukova, I.; *M-19*
Todeschini, A.R.; *J-03, J-05, J-06, E-07*
Todo Bom, M.A.; *G-24*
Toffoli-Kadri, M.C.; *A-22, A-33, P-12, T-11*
Tognoni, G.; *E-59*
Toledo, N.E.; *SP.09.01*
Tolentino, H.; *K-11*
Toleto, K.A.; *F-53*
Tomazelli, C.A.; *B-51*
Tomazelli, C.A.; *K-25*
Tomazini, L.F.; *B-15, B-34*
Toniolo, E.F.; *K-57*
Toro, D.M.; *B-26, B-36, B-44, K-30, K-48, O-09*
Torquato, R.J.S.; *A-17, A-21, F-13, F-54*
Torres, A.G.; *B-02*
Torres, A.H.F.; *F-45*
Torres, H.; *C-04*
Torres, P.H.M.; *D-05*
Torres, P.H.M.; *Q-04, D-49, D-16*
Torres, P.H.M.; *SP.10.04*
Torres, T.T.; *O-26*
Torres-Bonfim, E.; *D-11*
Torres-Bonfim, N.E.S.M.; *D-30*
Torres-Huaco, F.; *F-34*
Torricillas, M.S.; *E-83*
Tótoli, G.C.; *SM.03.03*
Tótoli, G.G.; *C-03*
Tótoli, G.G.C.; *SM.03.04*
Toyama, M.H.; *D-31, T-04, T-10, T-19*
Tozatto, G.F.; *M-23*
Tozetti, I.A.; *M-14*
Traina, F.; *K-26*
Trapé, A.A.; *B-26*
Treebak, J.T.; *O-01*
Trinca, V.; *A-14, O-26*
Trindade, A.T.T.; *K-17*
Trindade, V.M.T.; *H-05*
Trindade, V.M.T.; *N-04*
Trostchansky, A.; *K-11*
Trovatti, E.; *F-45*
Truzzi, D.R.; *S-01, S-07*
Trzan, I.F.L.; *O-15*
Tsai, S.M.; *D-66*
Tseng, Y.H.; *SP.09.04*
Tsubone, T.; *X-11*
Tsubone, T.M.; *I-08, I-10*
Turnes, B.L.; *K-11*
Tuyen Dao, T.; *SM.04.01*
Tytgat, J.; *E-45*
Tzagoloff, A.; *B-01*

U

Uceda-Campos, G.; *SP.17.04*
Uceda-Campos; *M-20*
Ueira-Vieira, C.; *P-18*
Uhl, M.; *B-16*
Uliana, J.V.C.; *A-14, A-18, O-26*
Ulrich, H.; *N-02*
Uno, M.; *K-46*
Urbanski, A.H.; *O-16*
Uyemura, S.A.; *U-01*
Uzan, T.J.; *D-22*

V

Valadares, A.S.S.; *E-57*
Valadares, V.S.; *E-76*
Valente, A.P.; *E-07, E-13, E-63, E-74, E-81, K-22*
Valente, A.P.C.; *E-43*
Valente, V.; *G-23, Q-06*
Valerio, H.P.; *S-24*
Vallejos, V.M.R.; *G-05, G-29*
Valsecchi, W.; *Q-08*
Valverde, F.; *SP.16.03*
Valverde, V.F.S.; *M-31*
Van Der Eerden, B.C.J.; *SP.03.02*
Van Der Vegt, N.; *D-10*
Vargas, A.P.; *K-26, K-54, K-59*
Vargas, B.S.; *S-16, S-28*
Vargas, D.M.; *K-50*
Vargas, J.A.; *E-80*
Vargas, S.; *S-17*
Vargas, T.S.; *G-07*
Vargas-Rechia, C.G.; *R-01*
Varga-Weisz, P.D.; *O-24*
Varga-Weisz, P.D.; *SP.09.03*
Vasconcellos, B.M.; *A-09, A-26, A-28*
Vasconcelos, A.A.; *E-11*
Vasconcelos, C.M.L.; *C-10*
Vasconcelos, M.A.; *J-04*
Vaso, C.O.; *P-14*
Vasques, J.; *E-53*
Vasques, L.B.; *S-19, S-25*
Vasques, L.H.; *D-55, D-62*
Vassallo, D.V.; *S-04*
Vaz Jr, I.; *A-01, A-11*
Vaz Jr, I.S.; *B-17*
Vaz, I.; *A-32*
Vaz-Busoli, F.A.; *F-13*
Vellardo, N.; *B-19*
Veloso, S.I.; *U-12*
Velri, H; *D-54*
Vendruscolo, E.C.G.; *S-15*
Verano-Braga, T.; *G-16*
Verano-Braga, T.; *O-18, O-23*
Veras, F.P.; *M-18*
Veras, P.S.T.; *G-11*
Vercesi, A.E.; *B-07, B-14, B-18, C-07*
Verçoza, B.R.F.; *R-04*
Vergani Junior, C.A.; *O-01*
Verjovski-Almeida, S.; *U-10, U-20*
Verli, H.; *D-17, D-37, D-50*
Verli, H.; *SP.06.03*
Verly, R.M.; *P-01, P-02, P-05, P-06, P-08, P-15*
Vernhes, E.; *SP.11.01*
Vettorazzi, C.B.M.; *K-10*
Viana, C.E.; *T-08*
Viana, I.; *F-16*
Viana, I.F.T.; *F-09, F-10, K-07*
Vicente, E.F.; *P-14*
Vicente, M.H.; *SP.16.03*
Vicente, R.M.; *F-17*
Vidueiros, J.P.; *F-40*
Viegas, R.G.; *D-08, D-52, D-53*
Vieira, D.S.C.; *K-12*
Vieira, E.C.; *K-49*
Vieira, H.G.S.; *M-10*
Vieira, T.C.R.G.; *C-12, E-25, E-86*
Vieira, T.S.; *B-51, K-25*
Vieites, I.M.; *C-12*
Vigato, A.A.; *F-06, F-43*
Vignoli Muniz, G.S.; *X-05*
Vignoni, M.; *SP.12.04*
Vigor, C.; *B-02*
Vilches, M.L.; *F-51*
Vilela, A.F.L.; *B-12, B-26, B-36, B-44, K-48, O-09*
Vilela, T.N.; *G-07*
Villard, D.R.; *S-34*
Villela, D.C.; *P-02*
Villela-Nogueira, C.A.; *K-67, K-71, M-29*
Vinces, T.C.; *E-82*
Vinolo, M.A.R.; *O-24*
Vinolo, M.A.R.; *SP.09.03*
Virgens, C.B.S.; *F-74*
Virgilio-Da-Silva, J.V.; *B-18*
Visani, V.; *F-02*
Visnardi, A.B.; *E-82*
Vitoria, T.K.; *E-78*
Viviani, L.G.; *D-33, D-47*
Viviani, V.; *F-08*
Viviani, V.R.; *E-27, H-07*
Vogel, C.I.G.; *O-27*
Vollrath-Kumpgdee; *G-15*

W

Waddington-Cruz, M.; *E-53*
Walter-Nuno, A.B.; *A-06, A-13, A-15, A-20*
Wang, X.; *I-09*
Ward, R.J.; *O-26*
Weber, G.; *D-09, D-20, D-45*
Weissmüller, G.; *G-08*
Welbert, J.; *T-06*
Weremiejczyk, L.; *SM.03.01*
Wiggers, G.A.; *S-04*
Wilke, N.; *X-16*
Winterbourn, C.C.; *S-13*
Wolf, L.D.; *F-04*
Wolynes, P.G.; *D-52*
Wondrak, G.T.; *I-04*
Wu, H.; *D-08*
Würtele, M.; *A-17*
Wyze, A.T.S.; *B-43*

X

Xavier, L.S.; *K-07*
Xavier, L.S.S.; *F-10*
Xavier, V.P.S.; *T-13*
Ximenes, P.B.; *K-07*
Ximenes, V.F.; *D-23*
Ximenes, V.F.; *D-40, G-03*

Y

Yamada, C.A.O.; *E-85*
Yi, R.C.F.; *X-07*
Yoguim, M.I.; *D-23, D-40*
Yokaichiya, F.; *G-15*
Yonamine, D.K.; *E-03, E-68*
Yoneda, J.S.; *SP.23.01*
Yoneyama, K.A.G.; *P-18*
Yoshinaga, M.; *O-01*
Yoshinaga, M.Y.; *B-51, K-15, K-25*

Z

Zagmignan, A.; *F-23, T-17*
Zago Ribeiro, L.; *J-07*
Zambalde, E.P.; *U-09*
Zamboni, D.S.; *CF.02, SP.04.02, SP.18.03*
Zamboni, D.S.; *G-35*
Zambuzzi, W.F.; *K-35*
Zambuzzi, W.F.; *SP.03.02*
Zamuner, S.R.; *U-04*
Zanata, S.M.; *K-06, U-07*
Zancarano, H.S.; *K-12, K-23, S-30*
Zanelli, ; *M-19*
Zanin, T.B.N.; *F-69*
Zanoelo, F.F.; *F-14, F-77, F-78, F-81, F-90*
Zanotto-Filho, A.; *K-12, K-23, S-30*
Zanphorlin, L.M.; *F-36*
Zatz, M.; *U-10*
Zeidler, J.D.; *K-08, F-86*
Zelanis, A.; *K-41, K-46*
Zeng, P.; *SP.15.01*
Zimmer, E.R.; *SP.05.04*
Zingali, R.B.; *E-45, E-11, R-13, O-02, O-06, R-07*

Keywords Index

A

Article Processing Charge; *SP.21.02, SP.21.03*
A2 milk; *F-73*
A549 cells; *K-58*
A9; *F-04*
AAA+ ATPase; *E-51*
Abiotic stress; *F-74*
ACE2; *F-75, C-01, D-22*
ACE-2; *D-06*
Acemannan; *F-37*
Acetylation; *D-08, B-08*
Acetyl-COA; *B-15*
Ache; *SP.02.04*
Ácidos graxos ômega 3; *B-51*
Active learning; *H-03*
Active principles; *F-85*
Acute toxicity; *P-10*
Acyltransferases; *B-36*
Adaptable binder; *SP.07.01*
Adipocyte; *O-25*
Adriamycin; *K-10*
Aedes aegypti; *SP.08.04, F-48, A-09, A-15, A-17, B-19, A-25, T-06, A-06, A-08, A-28, B-50*
Aedes aegypti cell; *B-22*
Aerobic exercise; *C-15*
Affinity assays; *E-21*
AFM; *C-08, G-08*
Aggregation; *SP.13.03, D-21, E-55*
Aging; *D-15, A-15, B-21, O-01*
Agro-industrial residues; *F-78*
Ahpc; *S-17*
Ahr-pathway; *A-30*
Aki; *O-06*
Akt oncogenic kinase; *U-11*
Albizia inundata; *T-16*
Albumin endocytosis; *C-01, K-32*
Albumin overload; *SP.18.01*
Albuminuria; *K-56*
Alcohol; *SP.05.02*
Alcoholism; *SP.05.02*
Aldehydes; *S-28*
Aldose redutase; *B-13*
Aldrin; *C-09*
Alkenes; *F-36*
Allantoin; *B-52, B-54*
Allosteric inhibitors; *D-07*
Allotopic gene expression; *B-01*
Alpha-synuclein; *K-61, E-55, D-58*
Alternative model; *T-15*
Alzheimer; *B-20*
Alzheimer's disease; *N-03, C-08, N-10, SP.05.03, SP.18.02, B-31, N-04*
Amburana cearensis; *N-11*
American trypanosomiasis; *K-70*
Amidation; *P-02*
Amino acid absorption; *A-24*
Amp; *P-09*
Amphotericin B; *G-05, G-11*
Ampk; *B-16*
Amydetes vivianii; *E-27*
Amylase; *F-81*
Amyloid fibrils; *E-86*
Amyloid formation; *SP.13.02*
Amyloid-beta; *K-04*
Amyloidosis; *E-53, C-12*
Amyloids; *F-15, SP.13.03, K-61, E-25*
Amyloid-β; *D-52*
Amyotrophic lateral sclerosis; *K-15, D-24, S-28*
ANC1; *E-44*
Angiogenesis; *J-07, G-27, T-12*
Animal feed; *F-78*
Animal model; *B-32*
Ant venom; *P-18*
Antarctic yeast; *F-38*
Antartic; *E-64*
Antibiotics; *F-72, X-05*
Antibody; *E-13*
Antibody characterization; *E-85*
Antibory library; *F-73*
Anticancer peptides; *G-16*
Anticarsia gemmatalis; *O-04*
Antifreeze protein; *F-38*
Antifungal activity; *P-14*
Antifungal agents; *K-66*
Antifungal peptides; *P-07*
Antifungals; *P-19*
Antigens; *A-21, F-54*
Anti-inflammatory; *F-21*
Antimicrobial; *M-28, X-07, F-61, J-04*
Antimicrobial activity; *G-37, F-08*
Antimicrobial nanomaterials; *SP.02.01*
Antimicrobial peptides; *P-01, P-15, X-09, F-49, P-04, P-06, M-06, P-14, D-34, F-58*
Antimicrobial resistance; *M-27, Q-04*
Antimicrobials; *P-19*
Antineoplasic; *F-01*
Antioxidant activity; *S-25*
Antioxidant capacity; *S-32*
Antioxidants; *S-11, F-30, S-34, S-03, S-31, S-02, S-14*
Antiphage immune system; *M-20*
Antipsychotic drugs; *E-12*
Antipsychotics; *E-94*
Antitumoral; *F-83*
Anti-vegf; *J-07*
Antivenom; *O-08, F-40*
Antiviral; *D-02, G-04, D-56*
Anxiety; *K-72*
Apigenin; *N-14*
Apoptosis; *U-25, SP.04.03*
Applications lipopeptides; *F-88*
Aptamers; *F-09*
Arabidopsis; *V-03, E-31, V-02*
Arboviruses; *F-57, A-28*
Arginine; *SP.19.01*
Aromatase/cyclase; *E-76*
Arrhythmia; *C-10*
Arsenic; *B-40*
Arthropods; *B-48*
Articaine; *B-25*
Artificial intelligence; *D-16*
Ascidians; *J-08*
Ascomycota strain; *F-11*
Aspergillus fumigatus; *U-01*
Aspergillus sp; *F-78*
Astrocytes; *SP.18.02, N-05*
Atgrp2; *V-03, E-14*
Atgrp7; *E-31, V-02*
Atherosclerosis; *SP.03.01, B-18*
Atomic force microscopy; *F-52*
Atorvastatin; *N-13*
Atp quantification; *H-07*
Atrial fibrillation; *C-13*
Autism spectrum disorder; *K-52*
Autismo; *N-12*
Autonomic nervous system; *K-69*
Autophagy; *SM.01.02, U-09, K-27, A-06, B-20*
Autophagy-associated cell death; *I-12*
Aviculture; *F-69*
Azospirillum brasilense; *O-16*

B

Bacillus thuringiensis infection; *O-04*
Bacteria; *M-03, M-07, M-12*
Bacterial outer membrane; *D-01*
Bacterial resistance; *P-13*
Bacteriophages; *M-09, M-20*
Bacth; *M-09*
Balteatide; *F-61*
Bam; *SP.11.02*
Barbatimão; *F-76*
Base J; *M-13*
Base modification; *M-02*
Beer fermentation; *B-57*
Bees; *SP.08.02*
Beetle; *B-45*
Benzalkonium chloride; *F-72*
Benzocaine; *G-12*
Beta-catenin; *SP.03.02*
Beta-thalassemia; *U-05*
Betulinic acid; *X-06*
Bh4 pathway; *K-52*
Bicarbonate buffer; *S-06*
Bifunctional enzymes; *F-71*
Bioactive peptides; *P-09, P-02*
Bioactive products; *T-17*
Bioactive scaffolds; *SM.03.02*
Biochemical and biophysical characteriza; *C-03*
Biochemical machinery; *SM.01.02*
Biochemistry; *H-09*
Biochemistry education; *H-03, H-06*
Biochemistry teaching; *H-02*
Bioenergetic; *CF.03*
Biofilm; *M-31, F-65*
Bioinformatics; *D-32, U-27, K-71, U-03, O-25*
Biological activity; *A-35, SP.06.01*
Biological conflicts; *M-25*
Biological control; *F-90, F-14*
Biological mineralization; *F-17*
Biological phase transitions; *SP.13.02*
Biological samples; *H-07*
Biological systems; *SP.11.04*
Bioluminescence; *F-08*
Biomarker; *M-29, SP.03.03, K-67, K-11*
Biomaterial; *F-45, O-12*
Biomaterials; *F-03, F-17*
Biomechanics; *SP.11.04, C-08*
Biomembranes; *SP.11.02, SP.12.03, SP.12.04*
Biomimetic medium; *P-01*
Biomimetic membrane; *P-05*
Biomineralization; *SP.03.04*
Biomolecular interaction; *E-37*
Biomolecule interaction; *I-10*
Biomolecules; *I-07, D-26*
Biophotonics; *F-24*
Biophysics; *SP.13.04*
Bioprocessos; *F-26*
Bioprospection; *T-01, F-08*
Biopsies; *SP.12.01*
Biorremediation; *F-47*
Biostimulation; *R-07*
Biosurfactants; *F-88*
Biotechnology; *K-71, R-01, O-03, O-07*
Biotin-painted; *SP.22.02*
Biotinylation; *SP.22.02*
Birefringence; *F-24*
Bisphenol-A; *B-39*
Blood brain barrier; *M-24*
Blood cancer; *S-30*
Bmnpv; *A-05*
Bmp; *X-11, D-04*
Bombyx mori; *A-05*
Bone mineralization; *SM.03.01*
Bone regeneration; *SM.03.04*
Bone-brain axis; *K-35*
Bothrops; *F-05*
Bothrops erythromelas; *O-21*
Bothrops jararaca; *O-06*
Bougainvillea glabra; *T-15*
Bovine serum albumin; *D-23*
Bradykinin; *SP.15.01, M-24*
Bradyrhizobium; *SP.17.01*
Bradysia hygida; *A-14, A-18*
Brain; *SP.09.02*
Brain oxidative damage; *S-11*
Brazilian biodiversity; *SP.06.02*
Breast cancer; *SP.24.02, K-23, F-45, U-14, G-22, S-32*
Breast cancer drug resistance; *U-11*
Breast milk; *O-09*
Breast tumor; *K-68*
Bromocresol purple; *D-23*
Brown adipose tissue; *SP.09.04*
Budding yeast; *U-22*

C

C. Elegans; *O-01*
C2c12; *B-37*
Ca2+; *SM.04.03*
Caatinga; *F-19*
Caco-2; *F-53*
Cafeteria diet; *N-13, K-69*
Caffeine; *U-15*
Calbicans; *M-30*
Calcium; *E-17, Q-06, U-08, E-56*
Callinectes danae; *Q-02*
Camellia sinensis; *T-18*
Cancer; *SP.12.01, SP.24.04, C-17, F-82, O-05, J-05, K-05, E-25, K-27, B-35, K-37, K-59, K-06, B-10, G-26, K-44, K-54*
Cancer hallmarks; *K-36*
Cancer stem cells; *U-02, K-20*
Cancer therapy; *F-80*
Candida albicans; *M-31, P-07, I-02*
Candida glabrata; *I-02*
Cannabinoids; *S-20*
Capsaicin; *B-55*
Capsid protein; *E-30*
Carbohydrates; *SP.06.01, SP.06.03, J-01*
Carbon metabolism; *B-09*
Carbon quantum dots; *F-51*
Cardiac ischemia-reperfusion; *O-18*
Cardiac remodeling; *C-15*
Cardiac sodium channel; *Q-07*
Cardiorenal syndrome; *K-01*
Cardiotoxicity; *Q-07, C-09, T-09*
Cardiovascular; *K-21*
Carnosine; *S-16*
Cartilage tissue; *F-06*
Casein micelles; *F-52*
Caseinase; *F-32*
Castor bean; *R-14, F-74*
Catalase; *T-05, F-27*
Catalytic mechanism; *E-48*
Catalytic sites of ace; *F-35*
Catechol-o-methyl-transferase; *E-78*
Cationic surfactant; *D-10*
Caudovirales; *M-21*
Cazymes; *O-26*
CD44; *G-23*
C-DI-GMP; *E-28, E-82*
Cedro beamline; *SP.23.01*
Cell; *SP.09.02*
Cell adhesion; *SP.24.03*
Cell aging; *X-14*

Cell culture; *O-14*
Cell cycle; *SP.24.04, F-41, E-89*
Cell death; *F-55*
Cell imaging; *M-06*
Cell mechanism; *C-06*
Cell migration; *SP.24.03, U-07, K-44*
Cell motility; *K-37*
Cell penetrating peptides; *D-34*
Cell signaling; *K-57*
Cell viability; *R-11, M-26*
Cell-penetrating peptide; *F-30, E-84*
Cellular development; *U-10*
Cellulosome; *E-04*
Centrolobium robustum; *J-04*
Cerebral ischemia; *N-11*
Cerebral malaria; *M-24*
Cervical intraepithelial neoplasia; *M-08*
Chagas disease; *SP.08.01, SP.08.03, SP.15.02, G-14, K-60, M-05, E-09, K-09, G-13, C-15, A-27, G-33, M-14*
Chaperones; *E-65, E-46*
Chemotherapy; *SP.06.04, T-22*
Chikungunya; *L-01, D-02*
Chikungunya virus; *F-09, B-11*
Children; *B-49*
Chimera; *F-49, E-50, F-58*
Chiral enhancement; *D-03*
Chitin; *A-16, A-26*
Chitin syntase; *A-03*
Chlamydomonas reinhardtii toxicity; *R-13*
Chloro-aluminum phthalocyanine; *F-46*
Cholesterol; *B-43*
Chromatin remodeling; *SP.09.03, U-18*
Chromosome level genome assembly; *O-26*
Chronic kidney disease; *K-10, S-12*
Chronic myeloid leukemia; *K-26*
Chronic restraint stress; *S-11*
Chronification; *D-28*
Circadian clock; *SP.16.01*
Circovirus; *D-35*
Circular dichroism; *E-03*
Cisplatin; *B-52, B-54*
Citrus canker; *M-04*
Clear cell renal carcinoma (rcc); *U-23*
Clostridium thermocellum; *E-04*
CMC; *F-63*
Coagulant; *F-32, F-40*
Coagulation; *T-02*
Coagulation cascade; *O-17*
Coagulopathology; *F-40*
Coagulopathy; *F-05*
Coarse-grained molecular dynamics; *E-90*
Cobalt; *F-21*
COCL2; *Q-02*
Coditioned medium; *K-21*
Cognitive deficit; *K-35*
Coiled-coils; *E-50*
Collaborative learning methodology; *H-02*
Collagen; *F-12*
Collagen fibres; *F-28*
Collective variables; *D-12*
Colonization; *R-05*
Colorectal cancer; *SP.04.04, J-06*
Combined paternity index; *O-27*
Combined therapy; *I-05*
Complex; *F-60*
Complex paternity investigation; *O-27*
Computational biology; *R-14, D-16*
Computational chemistry; *D-63*
Computational modeling; *D-27*
Computational tools; *SP.10.04*
Computer simulations; *P-04*
Condensation; *SP.13.01*
Confocal microscopy; *F-70, R-02*
Conformation; *E-70*
Conformational analysis; *D-50*
Conformational dynamics; *E-76*
Conformational sampling; *D-12*
Conopeptides; *O-19*
Contractility task-1; *C-14*
Conus; *O-19*
Coordination compounds; *S-25, S-31*
Copolymers; *X-07*
Copper; *D-59, G-26*
Copper chelator; *N-03*
Coronaviridae; *SP.07.04*
Coronavirus; *V-01, F-22*
Corynebacterium pseudotuberculosis; *F-33*
Coumarin; *E-29, E-59, T-14*
Coumarin 343; *G-03*
Covalent inhibitors; *G-14*
Covid; *E-85*
Covid-19; *CF.02, SP.01.01, SP.01.02, SP.01.03, SP.01.04, SP.07.02, SP.07.03, SP.07.04, SP.18.03, SP.20.02, U-03, F-75, G-25, SP.04.02, H-04, H-06, O-12, B-12, M-18, K-22, B-26, K-30, G-36, K-42, E-68*
COX-1; *D-44*
COX-2; *D-44*
CRISPR/CAS; *F-84*
CRISPR/CAS9-based genetic engineering; *F-11*
Crispr-cas9; *F-67, U-09, M-26*
Critical developmental period; *B-29*
Crotalus durissus; *T-19*
Crotalus durissus ssp; *E-16*
Crotalus durissus terrificus; *T-04*
Crotoxin B; *E-61*
Cruzain; *G-13, G-33*
Cryoem; *SP.11.01, E-22*
Cryo-em; *E-51*
Cryptococcus; *K-66*
Crystal structure; *E-69*
Crystallization; *E-61*
Crystallographic structure; *E-64, E-80*
Crystallography; *E-44*
Cubosomes; *F-01, F-37, C-04, G-20, F-64*
Cultivation; *T-24*
Curcuminoids; *K-34*
Currents; *SP.19.01*
Cutaneous leishmaniasis; *I-03*
Cuticle-degrading enzymes; *F-14*
Cyclodextrin; *T-09*
Cyp-D; *E-33*
Cysteine protease inhibitors; *G-13*
Cytokine storm; *SP.04.02*
Cytometry; *SP.24.02*
Cytosolic pla2; *D-31*
Cytotoxic activity; *G-37*
Cytotoxicity; *G-27, G-29, P-12*

D

DA; *E-67*
Damage in mitochondria and lysosomes; *I-12*
Day-neutral tomato; *SP.16.03*
De novo lipogenesis; *B-27*
Decarboxylase; *F-36*
Deep neural network; *D-55*
Degenerative diseases; *SP.14.04*
Delivery agents; *X-04*
Dendrimers; *G-10*
Dengue virus; *E-30, D-56*
Dental cementum; *SM.01.04*
Deoxyhypusine synthase; *M-19*
Depression; *M-22*
Dermal equivalent; *F-42*
Desenho racional de fármacos; *D-32*
Design experiment; *G-01*
Design of experiments; *G-17*
Design of pharmaceutical forms; *G-08*
Desmopressin; *E-02*
Deubiquitinase; *K-06*
Deuterium reinforced pufa; *X-02*
Development; *J-03*
DFT; *D-59*
Diabetes; *SP.09.04, E-02*
Diabetes mellitus; *SP.18.04, H-09*
Diabetic kidney disease; *K-32*
Diabetic nephropaty; *B-13*
Diagnosis; *F-79, F-39, F-02*
Diatomite; *F-20*
Didactic tool; *SP.20.03*
Dietary restriction; *O-01*
Digestion; *A-04*
Digital educational resource; *H-05*
Dihydroorotate dehydrogenase; *G-36*
Dihydropyrimidinones; *K-45*
Dilated cardiomyopathy; *O-23*
Dinitrosyl iron complex; *S-01, S-07*
Diphtheria and tetanus anatoxins; *D-61*
Disease severity; *K-07*
Disintegrin; *E-11*
Disintegrins, angiogenesis; *SP.24.03*
Disorder; *E-89*
Distance teaching; *SP.20.02*
Djc20; *E-35*
D-limonene; *T-09*
DLS; *G-20*
DMPEI; *F-65*
DNA; *SP.24.04, D-09*
DNA damage response; *U-21, U-16*
DNA damage signaling; *U-22*
DNA g-quadruplex; *E-18*
DNA hypermethylation; *U-12*
DNA methylation; *U-24*
DNA repair; *SP.12.02, F-33*
DNA vaccine; *F-16*
DNA -peptide complexes; *E-34*
DNAS; *M-25*
Docetaxel; *G-22*
Docking; *E-19, G-33*
Docking techniques; *D-62*
Docking with artificial intelligence; *D-62*
Dockthor vs; *SP.10.02*
Dodecyl phosphocholine; *X-01*
Doenças parasitárias tropicais; *D-32*
Domains; *X-03*
Doxorubicin; *F-46*
Dppc/dopc; *X-12*
Dppc/dppg; *SP.19.03*
Drop-in biofuels; *F-36*
Drosophila melanogaster; *B-04, B-38*
Drosophila metabolism; *B-04*
Drug; *G-02*
Drug delivery; *F-82, F-01, G-15, F-15, G-22*
Drug delivery systems; *G-11, G-17, G-12*
Drug delivery systems (DDS); *G-21*
Drug design; *SP.10.02, G-19, G-25, E-38*
Drug discovery; *M-19, G-28, G-36, D-46*
Drug repositioning; *G-04, M-14*
Drug screening; *G-35*
Drug targeting; *K-34*
Druggability; *D-49*
Dusp; *U-19*
Dynamics; *E-37, E-63*
Dynamics signature; *D-48*

E

E75; *A-23*
Early secretory pathway; *C-11*
Ecdysone; *A-27*
Ectatomma opaciventre; *P-18*
Education; *SP.20.01, SP.20.02, SP.20.03*
Effluents; *B-23*
Egfr; *J-06*
Egg white hydrolysate; *S-04*
Elapidae; *O-08*
Electrochemistry; *D-65*
Electrogenicity; *M-15*
Electron donors; *F-04*
Electron microscopy; *M-30, E-96, V-05*
Electron paramagnetic resonance; *S-01*
Electron transport chain; *D-39*
Electronic circular dichroism; *D-29, D-40*
Electrophysiology; *C-13*
Elp/ intein; *F-35*
Elvim; *SP.22.01*
Embryonic cells; *B-19, B-48*
Embryonic development; *A-14*
Embryotoxicity; *T-03*
Emergency remote teaching; *H-06, H-08*
Emt; *K-59*
Endocrine disruptors; *D-65*
Endocrine system; *E-94*
Endocytosis; *U-07*
Endophytes; *R-07*
Endoplasmic reticulum; *M-23*
Endoplasmic reticulum stress; *B-30*
Endosphere; *SP.17.04*
Endothelial cells; *SP.24.01*
Endotoxemia; *F-23*
Energetic metabolism; *B-56*
Energy cane straw; *F-77*
Energy control; *B-16*
Energy landscape; *D-53, D-08*
Energy landscape theory; *D-52*
Energy metabolism; *O-11*
Enterovirus; *C-12*
Envelope protein; *E-43*
Environment; *F-88*
Environmental contaminants; *K-49*
Enzimáticos; *C-05*
Enzymatic activity; *O-17*
Enzyme; *F-86, E-58, E-74*
Enzyme activity; *B-23*

Enzyme catalysis; *S-27*
Enzyme engineering; *E-27*
Enzyme purification; *F-50*
Enzymes; *A-34, T-06, B-42, E-54*
Epidemiological management; *SP.07.03*
Epigenetic modifications; *O-24*
Epigenetics; *SP.09.03, E-06, U-26*
Epirubicin; *F-64*
Epitope mapping; *E-77*
Eric allison philot; *E-67*
Erk; *K-40*
Erk/mapk pathway; *N-11*
Escherichia coli; *S-17*
Essential oil; *T-11, T-22, T-24, K-72*
Esterase; *A-31*
Estresse oxidativo; *N-12*
Ethnopharmacobotany; *F-85*
Ethnopharmacology; *F-76*
Eugenol; *G-30*
Evolution; *D-60*
EVS; *SM.03.03*
Excitability; *D-15*
Exercise; *B-20*
Exfoliative mechanism; *E-20*
Exosome isolation; *V-05*
Exossomes; *U-01, SM.03.03*
Experimental arthrits; *T-11*
Experimental biochemistry; *H-08*
Experimental burn wound; *F-59*
Extracellular vesicles; *SM.03.04, SP.03.03, SP.24.01, C-03, K-43, O-02*
Extradiol dioxygenase; *E-58*

F

Fam3b; *K-68*
Familial amyloidotic cardiomyopathy; *E-53*
Familial hypercholesterolemia; *K-16*
Fasting; *B-22*
FBLD; *G-18*
FDG-PET; *SP.05.04, SP.04.01*
Feeding habits; *A-18*
Fermentação; *F-26*
Fermentation; *F-68*
Ferroptosis; *D-33*
Fertility potential; *E-12*
Fetal bovine serum; *C-03*
Fiber formation; *D-52*
Filamentous fungi; *F-84*
Fimx-pilz-pilb complex; *E-28*
Fine particulate matter; *SP.04.03*
Fkbp12; *E-54*
Flash radiotherapy; *C-17*
Flavivirus; *E-43, E-81*
Flavoenzymes; *E-40*
Flavonoids; *F-89, T-08*
Flu; *M-17*
Fluorescence; *I-11, X-13, E-59, M-12, A-16*
Fluorescence spectroscopy; *E-21, E-18*
Fluorescent; *F-60*
Fluorine NMR; *E-57*
Flvcr; *A-20*
Folding funnel; *D-53*
Folding mechanism; *D-19*
Food colloids; *SP.02.01, SP.19.04*
Foxk2 transcription factor; *U-11*
Fragment screening; *G-34*
Frataxin; *D-14*
Free energy methods; *D-39*
Free energy of binding; *D-57*
Free energy perturbation; *D-57*
Friedreich's ataxia; *D-14*
Fruit juice; *F-23*
Functional prediction; *E-09*
Fungi; *S-22*
Fungo filamentoso; *F-67*

G

Gaba; *E-52*
Gain-of-function; *K-23*
Galleria mellonella; *S-05, P-13, S-18*
Gamma radiation stress; *M-10*
Gasto energético; *B-51*
Geminivirus-host; *CF.04*
Gene co-expression networks; *O-05*
Gene expression; *SP.16.02, U-05, U-04*
Gene expression regulation; *U-18*
Gene family; *R-14*
Gene knockout; *B-09*
Genes; *B-45*
Genes differentially expressed; *D-28*
Genetic polymorphisms; *K-60*
Genistein; *N-04*
Genome sequencing; *M-21*
Genomic stability; *SP.12.02*
Genomics; *M-20*
Genotoxic stress; *U-22*
Genotype BRS 188 paraguaçu; *O-11*
Gestation; *B-46*
Gestational diabetes mellitus; *B-28*
Gh43; *F-62*
Gixos; *SP.11.02*
Glial; *SP.05.01*
Glioblastoma; *Q-06, R-06*
Glioblastoma chemoresistance; *SP.04.05*
Glioblastomas; *K-14*
GLNB; *B-15*
GLNE; *B-34*
Glomerular injury; *M-01*
Gluconeogenesis; *B-22*
Glucose metabolism; *B-55, B-48*
Glutamine synthetase; *B-34*
Glutathione peroxidase 4 (GPX4); *D-33*
Glutathione s-transferase; *S-15*
Glutathiones; *B-06*
Gluthatione; *S-28*
Glycaemia; *B-46*
Glycine max; *SP.17.04*
Glycogen metabolism; *U-08*
Glycogen synthase kinase 3 (gsk3); *B-33*
Glycosaminoglycan; *E-13*
Glycosaminoglycans; *SP.06.04, E-25*
Glycosphingolipids; *J-05*
Glycosyl hydrolases; *F-71*
Glycosylase assay; *F-33*
Glyoxyl-agarose; *F-52*
Gold nanoparticles; *G-32*
Golgi reassembly; *SM.04.04*
GPCR; *SP.19.02*
G-PCR; *K-27*
Graphene oxide; *G-10*
Grasp65/55; *E-79*
Grasps; *SM.04.04*
Grasserie; *A-05*
GRB2; *K-65, E-29, E-57, E-59, E-08, E-18, E-62*
GRB2 protein; *D-19*
Green synthesis; *F-80*
Gromos; *D-17*
Gromos force-field; *D-13*
Guanosine; *F-03*
Gut microbiota; *O-18*
Guv; *X-08*
Guvs; *X-09*

H

H,k-ATPase; *Q-08*
HBMEC; *U-26*
HCV infection; *K-67*
HCV-NS5A; *K-65*
HDAC1; *U-26*
Head and neck carcinoma; *K-38*
Heart rate variability; *K-69*
Heat shock protein; *E-41*
Heat shock proteins; *SP.04.03, E-46*
Helicase; *SP.11.01*
Hematophagy; *A-13*
Hematopoiesis; *CF.06*
Heme- exporter; *A-20*
Heme transport; *A-13*
Hemicellulases; *F-71*
HEMKCA; *SM.04.03*
Hemocytes; *S-18*
Hep1; *E-73*
Heparin; *J-02*
Hepatic damage; *K-49*
Hepatic fibrosis; *K-39, K-50*
Hepatic steatosis; *B-06*
Hepatitis C; *M-29, K-71*
Hepatitis C virus; *E-26*
Hepatocarcinoma; *K-65*
Hepatocellular carcinoma; *K-40*
Hepatocite; *B-27*
Hepatocyte; *K-05*
HEPG2 cells; *B-30*
HER^{2+}; *S-32*
Herbaspirillum rubrisubalbicans m1; *R-05*
Herbaspirillum seropedicae; *O-20*
Heredity; *K-03*
Heterologous expression; *E-93, E-78*
Heterologous protein; *F-31*
Heterotypic coacervates; *V-06*
Heterotypic signalling; *K-41*
Hexameric rings; *E-96*
Hexokinase; *B-39*
Hexokinase ii; *B-41*
Hhep1; *E-01, E-72*
High molecular weight toxins; *A-02*
High school; *H-01*
High temperature of expression; *F-35*
High-density lipoprotein; *O-10*
High-performance liquid chromatography; *E-61*
High-resolution respirometry; *B-38*
Hipertrofia; *B-37*
Histone; *D-08*
Histone deacetylase; *E-06*
Histone modifications; *SP.09.03*
HIV; *M-08*
HNK; *N-10*
Hofmeister series; *X-01*
Holocellulose-degrading enzymes; *F-77*
Host restriction; *M-11*
Host-pathogen interaction; *D-28*
Hox genes; *A-14*
Hpv; *M-08, K-38*
Hrbc; *SM.04.03*
Hrsv; *T-14*
Hscb; *E-35*
Hsp27; *K-19*
Hsp40 type ii; *E-24*
Hsp90; *E-83*
Hspa1a; *E-17*
Hspa5; *E-56*
Hspas; *E-72*
Human; *K-17*
Human insulin; *K-04*
Human plasm samples; *B-12*
Human prion protein (prp); *G-34*
Human respiratory syncytial virus; *K-02*
Human serum albumin; *G-03, D-40*
Human trophoblast; *U-10*
Humicola; *F-81*
Huntington's disease; *K-19*
HUR, ATGRP2; *SP.11.03*
Hyaluronidase; *G-07*
Hybrid calculations; *D-60*
Hybrid polymer; *SM.04.01*
Hybrid systems; *F-43*
Hydrogels; *F-03, G-09, F-43*
Hydrogen bonds; *P-17*
Hydrolysis; *F-77*
Hydroperoxides; *S-09, S-17*
Hydroxyapatite; *CF.01.01*
Hydroxyl radical; *S-31*
Hydroxytyrosol; *N-09*
Hypercholesterolemia; *N-05, B-43, B-14*
Hyperglycemia, pregabalin; *P-16*
Hyperoxidation; *S-21*
Hyperspectral imaging; *F-24*
Hypertension; *S-04*
Hypohalous acids; *S-29*
Hypothyroidism; *C-10*
Hypoxia; *A-01, K-01, Q-05, U-23, B-26*

I

IAPP; *D-36*
IGF-1; *B-37*
IL-4; *K-10*
IMD pathway; *B-33*
Imidazole surfactant; *X-04*
Immune cells; *CF.06*
Immunoassays; *F-75*
Immunobiologics; *SP.08.04*
Immunodiagnosis; *F-73*
Immunoglobulin G; *F-66*
Immunoinformatics; *E-70*
Immunometabolic; *SP.14.02*
Immunometabolism; *B-24*
Importin alpha; *E-21*
Imune cells; *B-11*
In silico; *G-06, D-14, D-24, D-58*
In silico prediction; *D-34*
In vitro; *A-33*
In vivo assay; *P-13*
In vivo metabolomics; *B-57*
Inactivation/lysis; *M-28*
Incidence; *M-17*
Inclusion bodies; *F-66*
In-column refolding; *E-93*
Inflammasomes; *SP.04.02, SP.18.03, G-35*
Inflammation; *SP.01.01, SP.03.01, K-01, K-13, C-13, O-15, K-23, A-33, T-10, T-11, A-22, K-58*
Inflammatory diseases; *G-09*
Inflammatory pain; *K-11*
Infrared spectroscopy; *SP.23.03*
Inga capitata; *T-13*
Inhibition; *T-05*
Inhibitor screening; *S-26*
Inhibitors; *T-13, F-13, D-41, D-47, R-06, S-22, S-23*
Inibidor de protease; *F-67*
Innate immunity; *M-18*
Inosine; *D-20*
Inositol; *U-16*
Inositol pyrophosphates; *M-05, U-21*
Insect; *A-04, A-24*
Insecticide resistance; *A-18*
Insects; *SP.08.02*
Integrin; *E-11*
Integrin β3; *K-37*
Integrins, tumor microenvironment; *SP.24.03*
Interaction; *A-29, E-41, E-62*
Interdisciplinarity; *H-09*
Interdomain dynamics; *E-57*
Interfacial tension; *SP.19.04*

Interleukin-10; *K-09*
Intratumoral administration; *C-16*
Intrinsically disordered proteins; *SP.13.02, SP.22.01, D-48*
Invisible species; *E-30*
Iohexol; *F-83*
Iron; *M-15*
Iron oxide nanoparticle; *J-01, I-06*
Iron status; *K-42*
Iron transportation; *D-37*
Isolated mitochondria; *C-07*
Isolation protocol; *K-24*
Iturin; *F-63*

J

J-domain; *E-24*
Job's plot; *G-03*

K

K^+-phosphatase; *Q-02*
Kaempferol; *T-04*
Kallikrein-kinin system; *SP.15.04*
Kallikreins; *K-63*
Kazal-type inhibitor; *A-17*
Keratinocyte; *K-63*
Kidney function; *G-32*
Kidney injury; *M-01, O-06*
Kin protein; *E-87*
Kinase activity; *B-09*
Kinase inhibitor; *B-35*
Kinase/cyclin; *U-08*
Kinetic; *E-36*
Kinetic of inactivation; *Q-08*
Klebsiella pneumonia; *E-91*
Klebsiella pneumoniae; *E-95*
KRAS; *K-51*
Kunitz inhibitor; *P-11*
Kunitz-type inhibitor; *T-19*
Kynurenine-pathway; *A-30*

L

LAAO; *T-07, T-12*
Label-free proteomics; *R-07*
Laser; *B-07*
L-asparaginase; *F-31, E-48*
Lbraziliensis; *E-40*
LCDS; *F-47*
LDLR -/- mice; *K-16*
L-dopa; *I-07*
Lectin; *T-03, I-06*
Lectins; *R-09, T-16*
Leishmania; *M-32, M-13, E-39, T-22, G-30*
Leishmania amazonensis; *U-25, S-08*
Leishmania braziliensis; *S-08*
Leishmaniasis; *M-32, G-05, E-09, D-11, G-30*
Leptospira interragons; *E-82*
Leptospirosis; *F-39*
Let-60; *T-15*
Leukemia; *B-54*
Levofloxacin; *X-05*
Lidocaine; *B-25*
Ligand-residue binding network analysis; *E-67*
Ligands; *A-12*
Limyb; *CF.04*
Linear motif; *E-89*
Linezolid; *D-29*
Lipase activity; *B-12*
Lipase inhibition; *T-18*
Lipid; *X-03*
Lipid droplets; *K-48*
Lipid metabolism; *A-23, A-10, K-48*
Lipid nanocapsules; *G-12*
Lipid peroxidation; *N-07, X-02*
Lipid self-assemblies; *SP.19.04*
Lipid vesicles; *SM.04.01*
Lipidomics; *I-03, T-23*
Lipids; *N-04, B-44*
Lipopeptide; *D-03*
Lipopolysaccharide; *D-01*
Liposomes; *G-05, X-07, G-29, G-31, E-73*
Liquid-liquid phase separation; *V-01, V-04, V-06*
Liraglutide; *E-02*
Lkb1; *U-09*
Lmwptp; *SP.04.04, K-36*
Lmx1b; *K-17*
Lncrna; *SP.08.02, O-05, K-51*
Lncrnas; *K-20*
Long non-coding rnas; *U-10, U-20*
Longevity; *B-21*
Loxosceles laeta; *F-34*
Lpmos; *F-04*
Luciferase; *H-07*
Luminescence; *X-10, D-36*
Lunatin-1; *G-16*
Lung; *K-30*
Lung cancer; *K-12*
Lung compliance; *SP.01.01*
Lutzomyia longipalpis; *A-07*
Lysosomes; *D-04, X-11*
Lysozyme; *C-04, G-20*

M

Machine learning; *D-46*
Macrobrachium amazonicum; *P-10*
Macromolecular crystallography; *SP.23.04*
Macrophages; *K-48*
Macrophages; *SP.09.01, SP.14.02, B-14, B-24, B-36*
Mafld; *B-06*
Magnesium; *D-09, E-17*
Magnesium(ii); *F-60*
Magnetic composite; *F-50*
Magnetic field; *N-02*
Magre; *D-21*
Main protease; *D-07*
Main protease sars-cov-2; *F-13*
Major depressive disorder; *K-03*
Malaria; *CF.03., SP.15.03, SP.15.04, M-01, G-19*
Malaria acute kidney injury; *K-18*
Malathion; *D-31*
Maldi-tof /ms-ms; *E-85*
Malignant cells; *K-53*
Mammary tissue; *F-28*
Manganese; *K-44*
Marine sponge; *N-08*
Mass spectrometry; *S-09, K-15, T-23, O-10, O-14, S-16, F-22, O-22*
Maternal obesity; *B-02*
Mathematical modeling; *SP.07.03*
Matrix evesicles; *SP.03.04*
Matrix protein (m); *K-02*
Matrix vesicles; *CF.01.01, SM.01.02*
MCF-7; *K-54*
MD; *D-64*
Mead; *F-68*
Medicinal plants; *F-76, T-24*
Megalin; *SP.18.01*
Melanoma; *R-03, F-07, T-21, F-29, K-33, F-41, K-41, K-55, F-55, S-24, K-24, K-28, K-46, K-48*
Melatonin; *F-45*
Melatonina; *N-12*
Melittin; *G-23*
Membrane; *SM.01.01, SP.19.05, X-13, E-79, G-02*
Membrane adaptation; *X-16*

Membrane elastic properties; *X-14*
Membrane fission; *X-08*
Membrane fusion; *X-11*
Membrane model; *X-10*
Membrane order; *X-16*
Membrane proteins; *Q-04, E-47, E-88*
Membrane-active peptides; *P-03*
Membranes; *SP.19.03, X-05, X-12*
Memory improvement; *T-08*
Memory loss; *T-08*
Mercury toxicity; *B-18*
Mesenchymal stem cells; *SM.03.04*
Mesenchymal stem cells (msc); *F-42*
Mesenchymal stromal cells; *K-43*
Mesoionic compounds; *G-27*
Mesoscopic models; *D-09, D-20*
Metabolic diseases; *SP.09.04, SP.14.03*
Metabolic network modelling; *D-49*
Metabolic signals; *SP.05.03*
Metabolism; *SP.05.04, M-31, A-01, B-17, A-19, B-45, O-16, B-26, B-40, B-42, SP.04.01*
Metabolomic profile; *F-89*
Metabolomics; *O-09, K-22, B-28, B-44*
Metadynamics; *D-25, D-50*
Metal alloy; *F-21*
Metal biosensor; *E-27*
Metal complexes; *G-26*
Metal ligands; *F-25*
Metal-base-pair; *D-45*
Metallic nanoaggregates; *F-43*
Metalloenzymes; *S-27*
Metalloproteinases; *K-30*
Metal-mediated dna; *D-45*
Metalopeptides; *P-07*
Metal-organic frameworks; *F-25*
Metastasis; *K-68, F-07, F-29, K-33, U-02*
Metformin; *B-52, K-16*
Methadone; *N-01*
Methionine; *S-29*
Methylation; *F-86*
Methylene blue; *I-01, I-12*
Methylglyoxal; *I-04*
Metschnikowia australis; *F-38*
Micro reatores; *C-05*
Micro/nanotechnology; *SP.02.03*
Microbiology; *M-06*
Microbiome; *SP.17.02, D-66, SP.17.03*
Microbiota; *F-69, A-29, M-22, O-24, A-30*
Microcosmus exasperatus; *J-08*
Microdomains; *J-05*
Micro-extracellular vesicles; *O-09*
Microextraction; *B-44*
Microglia; *SP.14.03*
Microplus; *A-11*
Micro-rna; *K-60*
Micrornas; *K-67*
Microscopy; *R-04, A-16*
Micrurus altirostris; *E-45*
Midgut; *A-06, A-08*
Miltefosine-resistant parasites; *I-01*
Mimosa caesalpiniifolia; *R-09*
Mineralization; *CF.01.01, SM.01.01, SM.01.04, SP.03.02, X-15*
Miorex-complex; *B-47*
Mirnas; *U-01*
Mitochondria; *SP.14.01, SP.14.02, SP.14.03, SP.14.04, B-01, U-17, U-19, B-43, B-10, B-18, B-38*
Mitochondria-associated membrane; *B-14*
Mitochondrial bioenergetics; *B-07, B-32*
Mitochondrial biogenesis; *B-05*
Mitochondrial metabolism; *B-31*
Mitochondrial translation; *B-47*
Mitochondriopathies; *SP.14.04*
Ml365; *C-14*
Mnte-2-pyp5+; *S-08*
Modeling; *D-54*
Modified heparin; *J-07*
Molecular adjuvants; *F-16*
Molecular biology; *SP.13.04*
Molecular chaperones; *E-01, E-33, E-35, E-51, E-56, E-73, E-83, E-72*
Molecular docking; *D-23, D-43, D-16, S-26*
Molecular dynamics; *SP.10.01, SP.19.02, SP.19.03, SP.22.03, D-65, P-03, D-03, D-05, D-17, G-19, D-25, D-39, D-04, P-04, D-30, D-48*
Molecular dynamics simulations; *D-10, D-22, D-54*
Molecular modeling and dynamics; *E-36*
Molecular orbitals; *D-29*
Molecular simulations; *D-37*
Mononuclear cells; *A-22*
Monte carlo; *D-18*
Mosquito; *B-17*
Mpro; *E-23*
Mpro protease; *G-25*
Mptp; *T-01*
Mre11; *E-62*
Mrna translation; *N-10*
Mtorc1; *K-40*
Mtorc2; *K-13, K-25*
Multiphoton; *SP.12.01*
Multivulva; *K-45*
Mura; *D-54*
Muscle; *B-56*
Mycobacterium tuberculosis DHODH; *E-36*
Mycotoxins; *D-43*
Myocardium; *D-15, U-04*
Myofibroblasts; *K-39, K-50*
Myoglobine; *D-63*
Myosin va; *E-75*
Myrciaria dubia “camu-camu”; *E-80*
Myristoylation; *E-47*

N

Na^+/Ca^{2+}; *SP.15.02*
Nad(P)H; *SP.24.02*
Nad+ metabolism; *K-08*
Nafld; *K-13, K-25*
Nail-patella syndrome; *K-17*
Nanocomplexes; *E-84*
Nanocrystals; *M-12*
Nanomaterials; *SP.03.03*
Nanomechanical; *SP.11.04*
Nanomechanical properties; *L-01*
Nanoparticle toxicity; *R-13*
Nanoparticles; *SP.02.02, G-31, P-06, G-24*
Nanopesticides; *SP.02.02*
Nanostructure; *E-34*
Nanostructured lipid carriers; *G-01, G-17, F-46*
Nanostructured platform; *F-57*
Nanostructured systems; *C-04*
Nanotechnology; *F-82, G-01, F-48*
Nano-tomography; *SP.23.02*
Naphthoquinones; *M-32*
Native sad; *SP.23.04*
Natural compounds; *S-23, T-10, D-42*

Natural products; *SP.06.02, R-03, T-21, G-06*
Natural products for tumor chemotherapy; *K-53*
Natural transformation; *SP.11.01*
NAV1.5; *C-09*
NCX; *Q-06*
Negative staining; *E-32*
Neglected tropical diseases; *M-19*
Neoplasms; *SP.06.04*
Neopterin; *U-12*
Neurodegeneration; *SP.05.04, N-09*
Neurodegenerative diseases; *N-02, N-08, E-10*
Neuroepigenetics; *N-06, U-15, U-24*
Neuroinfection; *K-08*
Neuroinflammation; *SP.15.03, N-05, N-06*
Neuropathic pain; *P-16*
Neuroprotection; *N-13, N-14, N-08*
Neuroscience; *SP.13.04*
Neurotransmission; *U-12*
Neurotrophic factor; *E-15*
Neutralizing-antibody; *F-79*
Neutron reflectometry; *SM.04.02*
Neutrophils; *S-13, M-18*
New trends, bioproducts; *SP.06.02*
Nf-κb1; *U-23*
Nicotinic acetylcholine receptors; *E-45*
Nik1; *CF.04*
Nirmatrelvir; *E-23*
Nitric oxide; *S-01, S-07, F-44, K-52*
Nitrite; *P-10*
Nitrite reductase; *S-27*
Nitrogen; *SP.17.03*
Nitrogen-fixing; *SP.16.02*
Nlrp3; *CF.02*
NMR; *SP.06.03, SP.11.03, F-68, E-01, E-77, E-08, E-14, K-22, E-24*
NMR structure; *E-87*
No, H2o2; *A-33*
Nonalcoholic fatty liver disease; *B-27*
Nonlinear microscopy; *F-28*
Non-structural protein; *E-03*
Non-structural protein 4; *E-68*
Normal mode analysis; *D-30*
Normal modes; *SP.10.01, SP.22.04*
Normal-mode-based; *SP.22.03*
Nosocomial infections; *F-65*
NPP1; *X-15*
NRF2; *K-12*
NRF2/TXN1/NLRP3 AXIS; *U-15*
NS2B-NS3; *G-28*
NS2B-NS3 protease of zika; *A-17*
NS5; *D-05*
NSP2; *D-02*
NT157; *K-26*
N-terminal domain; *D-42*
Nuclear magnetic resonance; *B-57, P-03, P-05, E-63, E-74*
Nuclear XIAP; *U-14*
Nucleocapsid; *E-77*
Nucleocapsid protein; *V-01, E-42*
Nucleocapsid-like particles; *E-26*
Nucleotides; *F-25*
Number of acyl chains; *D-13*
Number of phosphorylation sites; *D-13*
Nutrients; *M-07*
Nutrition; *B-03, A-15*

O

Obesidade; *B-51*
Obesity; *B-03, O-25, T-18, K-42*
Obesogenic diet; *B-29*
Ocdocker; *SP.10.04*
Odorant binding protein; *A-12*
Odorant degrading enzyme; *A-31*
O-GLCNAC; *J-03*
O-glcnacylation; *SP.18.01*
Old yellow enzymes; *E-40*
Olfactory vectorization; *T-01*
Oligomerization; *C-11, E-47*
Oligomers; *E-55*
Omics; *SP.14.01*
Online classes; *H-08*
Online instruction; *H-03*
Ontogenetic; *O-13*
Ontogeny; *O-07*
Open access; *SP.21.01, SP.21.02, SP.21.04*
Open access journal; *SP.21.03*
Open science; *SP.21.03*
Opioids; *SP.19.02*
Oral biofilm; *F-70*
Oral candidiasis; *T-17*
Orange essential oil; *T-06*
Organic hydroperoxide; *S-21*
Organophosphate pesticide; *S-34*
Oriza sativa l; *R-10*
Osmoregulation; *Q-03*
Osmotic potential; *F-74*
Osteoblast; *SM.03.03*
Osteoblast matrix vesicles; *SM.01.01*
Osteoblast-to-osteocyte; *SP.03.02*
Osteocalcin; *K-35*
Osvit2; *D-37*
Overfed; *B-49*
Oxidants; *B-24*
Oxidative stress; *SP.12.03, S-34, S-05, U-05, T-07, B-11, S-19, S-25, B-29, B-49, I-04, T-12, S-12, S-18, S-20*
Oxidised fatty acids; *B-02*
Oximes; *K-49*
Oxylipidomics; *O-15*
Oxylipin; *K-15*

P

P53; *SP.04.05*
P53 tumor suppressor protein; *E-26*
P53; câncer, phase transitio; *SP.13.03*
Page4; *SP.22.01*
Pain; *K-57*
Palmitate; *K-64*
Panc; *S-14*
Pancreatic ductal adenocarcinoma; *U-02, K-20*
Papaya sticky disease; *R-04*
Paracoccidioides; *E-78*
Parkia pendula; *T-20*
Parkinson; *N-07*
Parkinson disease; *D-58*
Parkinson's; *I-07*
Parkinson's disease; *K-61, E-15*
Partial characterization; *R-09, T-16*
Particle size analyzer; *V-05*
Pathogenic fungus; *K-53*
Pathogenic yeasts; *M-27*
Pathogenicity; *M-15*
PDAC; *K-51*
PD-L1; *K-12*
PDT; *F-53*
Penicillium maximae; *F-19*
Pentose phosphate cycle; *H-05*
Pepck; *B-17*
Peptidase inhibitor; *P-11*

Peptide; *P-05, F-07, F-29, F-41, G-02, P-12*
Peptide design; *D-06*
Peptide self-assembly; *F-15*
Peptide solid-phase synthesis; *P-01*
Peptide structure; *P-09*
Peptide-hormone; *E-34*
Peptide-membrane interaction; *P-15, P-02, P-06, P-08*
Peptides; *SP.02.04, G-23, D-59, F-61*
Pep-X; *P-16*
Perimicrovilar membrane; *A-26*
Periodontits; *D-66*
Peroxides; *S-06*
Peroxinectin; *A-25*
Peroxiredoxin; *S-13, S-22, S-26, S-23*
Peroxirredoxin 2; *S-21*
Pest management; *SP.02.03*
Pesticides; *H-01*
Pesticides biosensors development; *SP.02.04*
PET; *E-64*
PETASE; *F-11*
Peyrard-bishop dna model; *D-45*
PGAM5; *U-17*
PH; *E-08*
PH responsive; *F-37*
Phage; *D-66, M-16*
Phage display; *F-13, F-54*
Phase; *X-03*
Phase separation; *SM.04.01, SP.13.01, V-03, V-02*
Phb; *R-05*
Phb depolymerase; *O-20*
Phenotype; *SP.01.01*
Phenotypic plasticity; *B-03*
Phosphatase; *U-19*
Phosphate; *U-16*
Phosphatidic acid; *E-86*
Phospholipase A2; *F-56*
Phospholipase A2 inhibitors; *F-56*
Phospholipase d (pld1); *E-93*
Phospholipases A2; *E-16*
Phospholipids; *B-36*
Phosphoproteomics; *O-23*
Phosphorylation; *SP.22.04*
Photoactivity; *I-08*
Photobiomodulation; *SP.12.02, B-07*
Photochemistry; *I-04*
Photodisinfection; *F-20*
Photodynamic inactivation; *I-02*
Photodynamic therapy; *I-03, I-09, X-02, X-06, I-08, I-10*
Photooxidation; *X-08*
Photo-oxidation; *C-02, X-06*
Photo-oxidative stress; *X-14*
Photoperiodic; *SP.16.03*
Photophysics; *I-09*
Photosensibilisation; *C-02*
Photosensitization; *SP.12.04, S-10*
Photosensitizer; *SP.12.03*
Photosensitizers; *I-08*
Photosynthesis; *CF.04*
Phototherapy; *I-05*
Photothermal therapy; *F-51*
Phylogenetics; *E-60*
Physical exercise; *B-31*
Physical training; *B-46*
Physical virology; *L-01*
Physico-chemical analysis; *G-15*
Phytobiotics; *F-69*
Phytocannabinoids; *S-19*
Phytopathogenic fungus; *F-90*
Phytopathology; *R-04*
Phytoremediation; *R-08, R-10*
Phα1β; *N-01*
Pi3k/akt; *B-41*
Pii proteins; *B-34, O-16*
Pilz; *E-82*
Pink-1; *E-71*
Pinx1; *E-39*
Pitanga; *B-21*
Placenta; *B-02, B-28*
Plamalogen; *T-23*
Plant; *SP.16.02, SP.17.02*
Plant defensins; *F-49, F-58*
Plant growth promoting bacteria; *S-15*
Plant lectin; *J-04*
Plant responses; *R-02*
Plant-insect interaction; *P-11*
Plants; *S-02*
Plasma kallikrein; *SP.15.01*
Plasmodium; *SP.15.03*
Plasmodium falciparum; *CF.03., SP.15.04, E-83*
Plasmodium sp; *K-18*
Platelet activating factor acetyl hydrol; *D-31*
Platelet aggregation; *E-11*
Plcg; *E-49*
Plcgamma; *E-05*
Plpro; *D-38*
Polarization; *H-04*
Polimeric biomaterials; *F-06*
Pollutants; *K-05*
Poly-3-hydroxybutyrate (phb); *O-20*
Polycythemia vera; *K-43*
Polyhydroxybutyrate; *G-24*
Polyketide; *E-76*
Polymer brush; *D-25*
Polymeric matrices; *SP.02.02*
Polymeric nanoparticles; *G-11*
Polymerization; *E-66*
Polymers; *T-17*
Polymersomes (ps); *G-21*
Polymorphonuclear; *K-70*
Porcine circovirus; *E-32*
Pore-forming peptides; *P-08*
Postpartum depression; *K-72*
PP2CM; *U-13*
PPAR gamma; *B-08*
PPTC7; *U-13*
Prediction; *D-21*
PRE-mRNA; *U-06*
Prenatal alcohol exposure; *U-04*
Preparations; *T-20*
Prion; *V-04, E-10, E-86*
Probiotic; *F-23, M-28*
Prognosis; *O-12*
Prolylcarboxypeptidase; *SP.15.01, K-56*
Propionate; *M-22*
Prostaglandin; *A-25*
Prostate cancer; *U-20*
Protchoir; *SP.10.04*
Protease; *D-41, R-06, F-32, F-50*
Protease inhibitors; *I-11, R-03, R-11, T-21*
Protease mpro; *D-30*
Proteases; *K-41*
Protein; *E-79, E-81, D-18, E-44, E-74*
Protein aggregates; *V-06*
Protein aggregation; *C-12*
Protein CAP; *D-35*
Protein corona; *SP.03.04*
Protein data bank; *SP.06.03*
Protein design; *D-41*
Protein digestion; *A-24*
Protein disulfide isomerase; *A-32*
Protein dynamics; *D-12, E-48*
Protein E; *D-56*
Protein engineering; *SP.10.03, D-27, F-10*
Protein expression; *F-31*
Protein folding; *D-53, E-65, E-88*
Protein kinases; *Q-03*
Protein phosphatases; *U-03*
Protein scaffold; *SP.07.01*

Protein secretion; *R-12*
Protein serine/threonine phosphatase; *U-17*
Protein structure; *E-15, E-06, E-68*
Protein unfolding; *D-61*
Protein-ligand interaction; *E-29*
Protein-peptide docking; *D-55*
Protein-peptide interaction; *E-05*
Protein-protein docking; *D-62*
Protein-protein interactions; *K-34, E-69*
Proteins; *SP.05.01, SP.22.03*
Proteins interaction; *D-55*
Proteoglycans; *SP.06.04*
Proteoliposomes; *X-15*
Proteolytic activity; *P-15*
Proteolytic processing; *K-46*
Proteome-scale modelling; *D-49*
Proteomics; *A-09, R-12, M-03, O-19, O-04, R-08, R-10, O-18, F-22, O-22, S-24, K-26, K-54*
Prothrombotic; *SP.01.02*
Proximal tubule; *C-01*
Pseudomonas aeruginosa; *M-33, M-21, M-16*
PTA; *B-15*
PTEN; *K-25*
PTERINS; *SP.12.04*
PTMS; *B-08*
Ptychography; *SP.23.02*
Publishing practice; *SP.21.02, SP.21.03*
PVT1; *U-20*
Pyranoflavilyum cations; *I-09*
Pyrophosphate; *SM.01.04*

Q

QM/mm; *D-50*
Qsox1; *U-07*
Quantitative proteomics; *O-10*
Quantum dots; *J-01, I-11, I-06*
Quiescence; *M-05*

R

RAB 11; *E-75*
Radiation effects; *C-07*
Radiomodifier; *I-05*
Radiopharmaceutical; *C-06*
Radioresistance; *C-17*
Radiosensitization; *F-44*
Radiotherapy; *K-14*
Random-coil; *E-70*
Ranolazine; *C-10*
Ras; *K-45*
Rational design; *SP.07.01*
RBD; *D-64*
RBD-based elisa; *K-07*
Rbmti-A; *K-58*
R-bodies; *E-60*
RCC; *Q-01*
RDRP inhibitors; *E-19*
Reactivation; *E-58*
Reactive aldehydes; *S-16*
Reactive oxygen species; *K-64, F-51*
Reactive sulfur species; *D-63*
Reb genes; *E-60*
Recombinant; *F-39*
Recombinant enzyme; *F-27*
Red light; *I-01*
Redox; *S-24*
Redox balance; *B-16*
Redox imbalance; *K-21*
Redox signaling; *S-13*
Redox vulnerabilities; *S-30*
Refolding yield; *F-66*
Regulated cell death; *C-16*
Relapses; *K-62*
Remdesivir; *G-29*
Remote teaching; *SP.20.01*
Renal cell carcinoma; *Q-05*
Renal tubular injury; *K-18*
Renin-angiotensin system; *O-23, K-32*
Replication stress; *U-21*
Reproduction; *E-94*
Resilience; *K-03*
Resistance; *A-04*
Resistance nodulation division; *Q-04*
Respiratory control ratio; *C-07*
Respiratory distress syndrome; *SP.01.03*
Respiratory supercomplexes; *B-05*
Respiratory syncytial virus; *M-17*
Rest; *N-06*
Resveratrol-trans; *K-02*
Resveratrone; *F-18*
Rhipicephalus; *A-11*
Rhipicephalus microplus; *A-19, F-54*
RHO GTPASES; *K-14, SP.04.05*
Rhodnius prolixus; *SP.08.03, A-03, A-13, A-31, A-12, A-20, A-26, M-11*
Rhythms; *SP.16.01*
Ribose-5-phosphate isomerase; *E-38*
Ribose-5-phosphate-isomerase-b; *E-37*
Right atrium; *C-14*
Ring-shaped neutrophil; *K-70*
Rmeplox; *F-34*
RMN; *E-95*
RMPDI-1; *A-32*
RNA; *SP.09.02, O-02, D-20*
RNA binding protein; *M-10*
RNA interference; *A-03*
RNA; *SP.11.03*
RNA/DNA binding; *E-42*
Root; *SP.17.03*
Ros; *T-07, R-02*
Routes of folding; *D-19*
Rpl10; *CF.04*
Rt-lamp; *F-87, F-02*
Ru(ii) complexes; *K-04, D-36*
Ruthenium; *X-13*
Ruthenium complexes; *I-10, X-10*

S

Saccharomyces; *E-66*
Saccharomyces cerevisiae; *B-01, S-03, B-05, S-05, S-19, K-19, B-47, S-02, S-14, F-26, E-52*
Saliva; *SP.08.04*
Sample orientation; *M-30*
Sample preparation protocols; *O-22*
Sars-cov-1; *E-07*
Sars-cov-2; *CF.02, SP.10.02, SP.01.02, SP.01.03, SP.07.02, SP.07.04, SP.10.03, SP.18.03, F-79, E-03, K-07, D-07, E-19, E-23, G-35, F-02, G-06, D-06, D-22, G-24, D-38, E-42, E-84*
Sars-cov-2 nucleoprotein; *D-42*
Saxs; *SP.02.01, E-65, D-18*
Scaffold protein; *V-04*
Scanning electron microscopy; *F-70*
Schistosomiasis; *T-03, G-18, T-20*
Sciaridae; *O-26*
Scientific education; *H-01*
Scorpaena plumieri fish; *G-07*
Scorpion; *A-35, A-34*
Scorpion peptides; *G-16*

Scorpion venom; *A-02*
SCRNA-SEQ; *SP.09.01*
SDS; *E-88*
Second generation etanol; *F-62*
Secondary metabolites; *F-19*
Secretin; *E-22*
Secretory phospholipase A2; *T-04*
Selenium; *B-56*
Selenocompounds; *F-86*
Self-assembly; *G-21*
Semiconductor nanoparticles; *F-57*
Senna occidentalis; *T-05*
Separação de fase; *C-05*
Sepse; *A-32*
Septin; *E-69, E-50, E-66*
Serine protease; *T-13, T-02*
Serine protease cdtsp-2; *T-19*
Serine/threonine protein phosphatase; *U-13*
Sexual variation; *O-03*
SH2; *E-05*
SH3-like domain; *E-87*
Sialic acids; *D-17, J-06*
Sickle cell ulcers; *U-27*
Signal peptide; *R-12*
Signaling translation; *P-17*
Silk sericin; *F-59*
Silver nanoparticles; *F-80*
Simulation study; *SP.22.04*
Single nucleotide polymorphism; *K-09*
Single-cell transcriptomics; *O-24*
Singlet oxygen; *S-09, S-29, S-10*
Sirah force field; *E-90*
Sirna; *M-11*
Skeletal muscle; *B-55, B-39*
Small extracellular vesicles; *SP.04.04, K-24*
Small heat-shock protein; *A-27*
Small molecule drug discovery; *D-57*
Small-angle neutron scattering; *SM.04.02*
SMD; *E-67*
SMTGR; *G-18*
Snake envenomation; *F-56*
Snake venom; *O-13, O-17, O-21, O-02, O-08, T-10*
Snake venom serine protease; *O-14*
Socs3; *M-29*
Software; *D-01*
Soil; *M-07*
Soil bioindicator; *B-23*
Solar energy; *F-20*
Solid pharmaceutical formulations; *G-08*
Sonic hedgehog; *U-24*
Sorbitol; *B-13*
Sox9; *D-51*
Soybean; *SP.17.01*
Specific ion effect; *D-10*
Spectroscopy; *SP.23.01, F-18*
Sperm motility; *E-12*
Spider venom; *F-34*
Spike; *D-64*
Spike protein; *E-07, J-02*
Spinal cord; *N-14*
Splenectomy; *O-15*
Spliceosome; *U-06*
Splicing; *U-06*
Spodoptera frugiperda; *F-14*
Srpk2; *K-33, K-55*
Stability; *F-63*
Stacking proteins; *SM.04.04*
Staphylococcus aureus; *E-20*
Staphylococcus saprophyticus; *M-33*
Starch; *F-81, F-12*
Starch/collagen; *SM.03.02*
Starvation; *A-19*
Stem cell; *CF.06, D-51*
Stem cells proliferation; *A-08*
Sticholysin ii; *C-16*
Stomach cancer; *K-36*
Streptozotocin; *N-09*
Stress oxidative; *N-02, S-04*
Stress response; *U-18*
Strontium-substituted nanoparticles; *F-17*
Structural bioinformatics; *D-60*
Structural biology; *SP.10.01, SP.23.04*
Structural characterization; *E-49, E-71*
Structural modification; *P-08*
Structure; *E-31, E-81, E-14, E-54*
Structure activity relationship; *P-14*
Subclinical acute kidney injury; *K-56*
Sucrose gradient; *E-32*
Sugar interaction; *E-07*
Sugarcane; *SP.16.01*
Sulfasalazine; *D-40*
Sulfated glycosaminoglycans; *J-08*
Sulforaphane; *G-09, S-12*
Sunflower; *R-08*
Superoxide dismutase; *F-30*
Superoxide dismutase 1; *D-24*
Supramolecular structures; *G-15*
Surfactant-induced unfolding; *D-61*
Symbiont; *A-29*
Symbiosis; *SP.17.01, B-50*
Synaptosomes; *N-01*
Synchrotron; *SP.23.03*
Synchrotron radiation; *SP.23.01*
Synthesis; *SP.06.01*
Synthetic biology; *R-01*
Synthetic community (syncom); *SP.17.02*
Synthetic peptides; *P-19, D-11*
Synuclein; *E-10*

T

T lymphocytes; *K-62*
T6SS; *E-91, E-96*
Tails; *K-46*
Targeted alpha therapy; *C-06*
TCDHODH; *G-14*
T. cruzi; *A-35*
Teaching and learning; *H-02*
Tebuconazole; *Q-07*
Telomeres; *M-13, E-39, M-26*
Temsirolimus; *Q-01*
Terpenes; *F-55*
Theragnostic; *G-31*
Therapeutic potential; *J-02*
Therapeutic; *F-09*
Thermal shock; *B-42*
Thermodynamic; *SP.22.02*
Thermophilic protein; *E-63*
Thermostability; *Q-08*
Thimerosal; *B-32*
Thiobase; *D-26*
Thioredoxin; *S-07*
Thioredoxin reductase-1; *S-30*
Thiosemicarbazones; *G-37*
Three finger toxins; *E-45*
Thrombocytopenia; *M-23*
Thyroid hormones; *B-30*
Tiba; *F-83*
Ticks; *A-11, A-01, A-21*
Tissue engeneering; *F-06*
Tissue metabolism; *B-04*
Tityus serrulatus; *A-02*
Tityus sp; *A-34*
TMED; *C-11*
TMEM176B; *D-44*
TNAP; *SP.03.01*
TNAP and ANXAS; *SM.03.01*
Tolerance; *K-66*
Toxicity; *M-33, B-40*

Toxicity in aquatic ecosystems; *R-13*
Toximetabolomics; *B-25*
Toxin exfoliative e; *E-20*
Toxins; *M-25*
TPR-domain protein; *E-41, E-46*
Traditional medicine; *F-89*
Transcription factor; *D-51*
Transcriptome; *U-27*
Transcriptomic signatures; *U-14*
Transcriptomics; *P-18*
Transgenic rat model; *SP.18.02*
Transglycosylation; *A-07*
Transmembrane receptors; *P-17*
Transport; *SP.19.01*
Transthyretin; *D-27, E-53*
TRAP-1; *E-33, E-71*
Trastuzumab; *SP.06.04*
Traumatic brain injury; *SP.05.01*
Trbibolium castaneum; *J-03*
Treatment; *N-03*
Triacylglycerol; *A-10*
Triatomine insects; *SP.08.03*
Triatomines; *SP.08.01*
Triazole derivatives; *S-03*
Tribolium castaneum; *A-23, A-10*
Trichoderma; *F-90*
Trichoderma reesei; *F-27*
Triple-negative breast cancer; *R-11*
Triterpenoic acids; *C-02*
TRKA; *E-49, K-57*
TRNA; *M-02*
Trypanosoma; *M-02*
Trypanosoma cruzi; *SP.08.01, SP.15.02, M-10, M-14, E-38*
Trypsin; *T-02*
Trypsin inhibitor; *M-27*
Tuberculosis; *D-46*
Tubule-interstitial injury; *G-32*
Tumo microenviroment; *SP.09.01*
Tumor; *SP.24.01*
Tumor cell; *G-10*
Two photon absorption; *F-18*
Type 3 secretion system; *M-04*
Type IV pili; *E-22*
Type IV pilus; *E-28, M-16*
Tyrosine phosphatases; *S-06*
Tβ4; *K-55*

U

Ubiquitination; *K-59*
Uga1p; *E-52*
Ulcerative colitis; *K-11*
Ultrafast spectroscopy; *D-26*
Ultramicroscopy; *SP.23.03*
Uncaria tomentosa; *K-64*
Urea moyet; *B-35*
Usp2b; *K-06*

V

Vaccine; *SP.01.04, A-21, H-04*
Variability; *O-07*
Variation; *O-21*
Variations; *O-13*
Vascular calcification; *SM.03.01*
V-ATPase; *K-38*
Vector control; *F-48*
Vector diseases; *A-09*
Vector surveillance; *A-28*
Vegetable hormones; *R-01*
Vemurafenib; *B-10, K-28*
Venom; *G-07, E-16*
Venom variation; *O-03*
Vesicle properties; *X-04*
Vesicles; *SP.19.05, M-03, X-12*
VGRG; *E-91*
Viability; *O-11*
Violacein; *K-28*
Vipa; *E-95*
Viperidae; *F-05*
Viral assembling; *D-35*
Viral neutralization; *F-10*
Virtual screening; *D-11, D-33, D-47, D-38*
Virus; biophysics; *SP.13.01*
Visceral leishmaniasis; *K-62*
Vitamina C; *E-80*

W

Wasp; *A-22*
Water shortage tolerant plant; *S-15*
Water structure; *X-16*
Wheat; *F-87*
White mangrove; *F-85*
Whsmv; *F-87*
Wolbachia; *B-50*
Wolbachia pipientis; *B-19, B-33*
Wound healing; *F-59*
Xanthene; *F-53*
Xanthomonas citri; *M-09, M-04*

X

XIAP; *U-25*
X-ray crystallography; *E-75, G-34*
X-rays; *SM.04.02, SP.23.02*
X-STR; *O-27*

Y

Yeast; *S-20*
Yellow fever virus; *M-23*

Z

Zea mays; *SP.17.04*
Zebrafish; *F-64*
Zika; *SP.10.03*
Zika virus; *E-13, E-43, F-10, E-90, G-04*
Zika virus infection; *K-08*
Zikv; *D-05, F-16, G-28*
Zinc; *K-63*
Zinc transporters; *Q-05*
ZN channels; *Q-01*
Zwitterionic detergents; *X-01*

A-1 adrenergic receptor; *K-39, K-50*
α-glucosidase; *A-07*
α -helix; *P-12*
α -hemolysin; *D-43*
β-cyclodextrin; *F-72*
β -glucosidase; *E-04*
β -xylosidases; *F-62*
(Hemi-)cellulases ; *F-84*
(Na^+, k^+)-atpase regulation; *Q-03*
1,2-CCD; *F-47*
15-lipoxygenase-2 (15-lox-2); *D-47*
3D Bio-Printing; *SM.03.02*
3D Printing; *F-12*
3D Skin Model; *F-42*
4-PBA; *B-41*
4T1 Cells; *F-44*
6-phosphogluconate pathway; *H-05*
6-thioguanine; *S-10*