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(54) Title: MULTISPECIFIC ANTIBODIES FACILITATING SELECTIVE LIGHT CHAIN PAIRING

(57) Abstract: Generally, the present invention relates to the field of antibodies. In particular, it relates to multispecific antibodies, e.g. bispecific antibodies, which are modified such that the desired chain pairing takes place and/or can be selected for. Specifically, this is achieved by using different dimerization domains for light chain pairing. The invention further relates to nucleic acids encoding for these antibodies, expression vectors comprising these nucleic acids, cells expressing them, and further to pharmaceutical compositions comprising the antibodies. The invention also relates to methods of isolating the antibodies.

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Multispecific antibodies facilitating selective light chain pairing

Description:

Generally, the present invention relates to the field of antibodies. In particular, it relates to multispecific antibodies, e.g. bispecific antibodies, which are modified such that the desired chain pairing takes place and/or can be selected for. Specifically, this is achieved by using different dimerization domains for light chain pairing. The invention further relates to nucleic acids encoding for these antibodies, expression vectors comprising these nucleic acids, cells expressing them, and further to pharmaceutical compositions comprising the antibodies. The invention also relates to methods of isolating the antibodies.

Naturally occurring IgG antibodies are bivalent and monospecific. Multispecific, e.g. bispecific antibodies having binding specificities for multiple different antigens can be produced using recombinant technologies and are projected to have broad clinical applications. It is well known that complete IgG antibody molecules are Y-shaped molecules comprising four polypeptide chains: two heavy chains and two light chains. Each light chain consists of two domains, the N-terminal domain being known as the variable or V_L domain (or region) and the C-terminal domain being known as the constant (or C_L) domain (constant kappa (C_K) or constant lambda (C_λ) domain). Each heavy chain consists of four or five domains, depending on the class of the antibody. The N-terminal domain is known as the variable (or V_H) domain (or region), which is followed by the first constant (or C_H1) domain, the hinge region, and then the second and third constant (or C_H2 and C_H3) domains. In an assembled antibody, the VL and V_H domains associate together to form an antigen binding site. Also, the C_L and C_H1 domains associate together to keep one heavy chain associated with one light chain. The two heavy-light chain heterodimers associate together by interaction of the C_H2 and C_H3 domains and interaction between the hinge regions on the two heavy chains.

It has been of interest to produce multispecific antibodies, e.g. bispecific antibodies (BsAbs) that combine the antigen binding sites of several antibodies within a single molecule, and therefore, would be able to bind several different antigens simultaneously. Besides applications for diagnostic purposes, such molecules pave the way for new therapeutic applications, e.g., by redirecting potent effector systems to diseased areas (where cancerous cells often develop mechanisms to suppress normal immune responses triggered by monoclonal antibodies, like antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC)), or by increasing neutralizing or stimulating activities of antibodies. This potential was recognized early on, leading to a number of approaches for

obtaining such multispecific antibodies. Initial attempts to couple the binding specificities of two whole antibodies against different target antigens for therapeutic purposes utilized chemically fused heteroconjugate molecules (Staerz et al., 1985, Nature 314(6012): 628-31).

Production of e.g. bispecific IgG (BsIgG) by co-expression of the two light and two heavy chains in a single host cell can be highly challenging because of the low yield of desired BsIgG and the difficulty in removing closely related mispaired IgG contaminants (Suresh et al., Proc. Natl. Acad. Sci. U.S.A.83, 7989–7993, 1986). This reflects that heavy chains form homodimers as well as the desired heterodimers – the heavy chain-pairing problem. Additionally, light chains can mispair with non-cognate heavy chains – the light chain pairing problem. Consequently, coexpression of two antibodies can give rise to up to nine unwanted IgG species in addition to the desired BsIgG.

Engineering antibody heavy chains for heterodimerization has emerged as a successful strategy to overcome the BsIgG heavy chain-pairing problem. The homodimerization of the two heavy chains in an IgG is mediated by the interaction between the C_H3 domains alone. Heavy chains were first engineered for heterodimerization in the 1990s using a “knobs-into-holes” strategy. Starting from a “knob” mutation (T366W) (Ridgway et al., ‘Protein Eng. 9, 617–621, 1996) that disfavours C_H3 homodimerization, compensating “hole” mutations (T366S, L368A, and Y407V) (Atwell et al., J. Mol.Biol. 270, 26–35, 1997) were identified by phage display providing efficient pairing with the “knob” while disfavoring homodimerization. The promiscuity in the IgG domain interface led in recent years to several other successful strategies for heavy chain heterodimerization.

Further, several strategies have been developed to overcome the light chain pairing problem. Light chain mispairing can be obviated by using antibodies with a common light chain identified from phage display libraries with limited light chain diversity (Merchant et al., Nat. Biotechnol. 16, 677–681, 1998). More recently antibodies with a common light chain have been identified from other technologies including transgenic mice with a single light chain (McWhirter et al., WO2011/097603). Currently the most widely used route to BsIgG is by separate expression of the antibody components in two different host cells followed by purification and assembly into BsIgG *in vitro* (Jackman et al., J. Biol. Chem. 285, 20850–20859, 2010). The major advantage of the two host cell strategies over the common light chain approach is that they are much more broadly applicable to pre-existing antibodies. Moreover, the two different light chains typically contribute to antigen-binding affinity and specificity. Disadvantages of the two-host cell strategies compared to the common light chain are that they are associated with significantly greater expense and complexity in manufacturing.

The present invention addresses the light chain pairing problem and achieves the desired paring of the light chains of multispecific antibodies in the same cell with minimal or even without any undesired by-products, i.e. light chain pairings other than the desired ones. This is achieved by using the C_H2 domain of an IgM (MC_H2) or of an IgE (EC_H2) for pairing one light chain variable domain with a heavy chain variable domain as desired and using a different dimerization domain for pairing the other light chain variable domain with a heavy chain variable domain as desired. The function of MC_H2 and EC_H2 is unknown, very likely they correspond to the hinge region of IgG molecules. The IgM C_H2 domain (MC_H2) is a 111 amino acid (12.2 kDa) polypeptide forming a homodimer. The dimer has an S-S-bond formed between Cys110 of two domains and an internal S-S-bond between Cys30 and 93 within one domain. MC_H2 has a glycosylation site at Asn105. The inventors found that MC_H2 domains can be used to replace the C_H1/C_K on one arm of the antibody and discovered the MC_H2 fold is as stable as the C_H1/C_K and allows for a correct pairing of the variable domains. This is particularly advantageous in association with modifications facilitating the desired heavy chain pairing. EC_H2 is a highly homologous domain with the same function in the IgE antibody and will therefore be just as suitable for this purpose.

Before the present invention is described in detail below, it is to be understood that this invention is not limited to the particular methodology, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art. Preferably, the terms used herein are defined as described in “A multilingual glossary of biotechnological terms: (IUPAC Recommendations)”, Leuenberger, H.G.W, Nagel, B. and Kölbl, H. eds. (1995), Helvetica Chimica Acta, CH-4010 Basel, Switzerland).

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions etc.), whether supra or infra, is hereby incorporated by reference in its entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

In the following, the elements of the present invention will be described. These elements are listed with specific embodiments, however, it should be understood that they may be combined

in any manner and in any number to create additional embodiments. The variously described examples and preferred/particular embodiments should not be construed to limit the present invention to only the explicitly described embodiments. This description should be understood to support and encompass embodiments which combine the explicitly described embodiments with any number of the disclosed and/or preferred elements. Furthermore, any permutations and combinations of all described elements in this application should be considered disclosed by the description of the present application unless the context indicates otherwise.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word “comprise”, and variations such as “comprises” and “comprising”, are to be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integer or step. As used in this specification and the appended claims, the singular forms “a”, “an”, and “the” include plural referents, unless the content clearly dictates otherwise.

In the description that follows, amino acid numbers are used with respect to antibodies, which do not refer to a SEQ ID NO. These numbers refer to amino acid positions in antibodies according to the UniProtKB database (www.uniprot.org/uniprot) in the version as disclosed on August 26, 2016. Unless specified otherwise, the number(s) correspond to a position in human IgG, in particular in human IgG1. The UniProtKB sequences (in the version as disclosed on August 26, 2016) of antibody domains referred to herein, including those of human IgG1 are incorporated by reference as particular embodiments of the domains described herein, as well as variants thereof as defined below.

In a first aspect, the present invention relates to an antibody heavy chain A, light chain A or dimer thereof, wherein the heavy chain comprises a variable domain V_HA linked to a dimerization domain $CH2_H$ and the light chain comprises a variable domain V_LA linked to a dimerization domain $CH2_L$, and wherein both $CH2_H$ and $CH2_L$ are an IgM constant domain MC_H2 or an IgE constant domain EC_H2. MC_H2 and EC_H2 are homologs and have a highly similar amino acid sequence. If $CH2_H$ and $CH2_L$ are an IgM constant domain MC_H2, it is preferred that MC_H2 comprises a mutation of asparagine 209 to glutamine (position according to the numbering of UniProtKB - P01871 IgHM_HUMAN, as modified on July 1, 2008 - v3). If $CH2_H$ and $CH2_L$ are an IgE constant domain EC_H2, it is preferred that EC_H2 comprises a mutation of cysteine 105 to alanine (avoiding unwanted cysteine reactivity) and/or a mutation of asparagine 146 to glutamine (avoiding glycosylation) (positions according to the numbering of UniProtKB - P01854 IgHE_HUMAN, as modified on July 21, 1986 - v1).

In other words, the first aspect of the invention can be described as a fragment of an antibody or derivative thereof comprising an antibody heavy chain A, an antibody light chain A or a dimer thereof, as defined above. The term "dimer" refers to a dimer of the heavy chain A and the light chain A.

The term "antibody" as used herein refers to a molecule having the overall structure of an antibody, for example an IgG antibody, which has the light chain - heavy chain dimerization domains replaced by MC_H2 or EC_H2 domains. It also includes molecules which may have further chimeric domain replacements (i.e. at least one domain replaced by a domain from a different antibody), such as an IgG1 antibody comprising an IgG3 domain (e.g. the C_H3 domain of IgG3). Further, the term generally refers to multispecific, e.g. bispecific or trispecific antibodies. The antibody of the invention may alternatively be termed "antibody-like protein" or "chimeric antibody". An example is the "IgG configuration" with constant domains as described herein.

Usually, when referring to IgG in general, IgG1, IgG2, IgG3 and IgG4 are included, unless defined otherwise. In particular, IgG is IgG1.

The term "antibody derivative" as used herein refers to a molecule comprising at least the domains it is specified to comprise, but not having the overall structure of an antibody such as IgA, IgD, IgE, IgG, IgM, IgY or IgW, although still being capable of binding a target molecule. Said derivatives may be, but are not limited to functional (i.e. target binding, particularly specific target binding) antibody fragments thereof such as Fab2, or combinations of such derivatives, for example bivalent Fabs (e.g. as described herein). It also relates to an antibody to which further antibody domains have been added, such as further variable domains. Examples are the "tetravalent bispecific tandem immunoglobulin (TBTI) configuration (also termed and the same as the "dual variable domain configuration"), the "CODV configuration", the "CODV chimera configuration", in particular the "trivalent configuration", and the "tetravalent spider configuration" as described herein.

The term "heavy chain" as used herein includes also a fragment of a heavy chain that comprises at least a variable domain (usually of an IgG antibody) linked to a domain for dimerization with a light chain, wherein the dimerization domain is usually defined more specifically herein when using this term. It may but does not necessarily further comprise a constant domain C_H2 and optionally also a constant domain C_H3 (usually both of an IgG antibody). Accordingly, it may also be referred to as "fragment of a heavy chain comprising at least a variable domain linked to a domain for dimerization with a light chain".

Usually and unless one is modified further than the other, $CH2_H$ and $CH2_L$ are identical (i.e. they are homodimerization domains) and in one embodiment each have a sequence according to SEQ ID NO: 1 or according to SEQ ID NO: 2 (these sequences correspond to the CH2 domains used in the examples) or a variant of either with at least 80% sequence identity to SEQ ID NO: 1 or 2, respectively. Compared to human IgM, SEQ ID NO: 1 has a mutation of asparagine to glutamine at position 105 of SEQ ID NO: 1. This mutation is desired but not mandatory for the invention. Thus, a variant of SEQ ID NO: 1 may retain this mutation. Compared to human IgE, SEQ ID NO: 2 has a mutation of cysteine to alanine at position 105 of SEQ ID NO: 2 and a mutation of asparagine to glutamine at position 146 of SEQ ID NO: 2. One or both of these mutations are desired but not mandatory for the invention. Thus, a variant of SEQ ID NO: 2 may retain one or both of these mutations. Optionally, only one of $CH2_H$ and $CH2_L$ is a variant or the variants of $CH2_H$ and $CH2_L$ differ from each other. In any case, the variants are still capable of dimerization with each other and/or with the domain according to SEQ ID NO: 1 or 2, respectively. A variant has an amino acid sequence that it at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence it is derived from, for example SEQ ID NO: 1 or 2. The determination of percent identity between two sequences is accomplished using the mathematical algorithm of Karlin and Altschul, Proc. Natl. Acad. Sci. USA 90, 5873-5877, 1993. Such an algorithm is incorporated into the BLASTN and BLASTP programs of Altschul et al. (1990) J. Mol. Biol. 215, 403-410. To obtain gapped alignments for comparative purposes, Gapped BLAST is utilized as described in Altschul et al. (1997) Nucleic Acids Res. 25, 3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs are used. Alternatively, a variant can also be defined as having up to 20, 15, 10, 5, 4, 3, 2, or 1 amino acid substitutions, in particular conservative amino acid substitutions. Conservative substitutions are well known in the art (see for example Creighton (1984) Proteins. W.H. Freeman and Company). An overview of physical and chemical properties of amino acids is given in Table 1 below. In a particular embodiment, conservative substitutions are substitutions made with amino acids having at least one property according to Table 1 in common (i.e. of column 1 and/or 2). The term “variant” also includes fragments. A fragment has an N-terminal and/or C-terminal deletion of up to 20, 15, 10, 5, 4, 3, 2, or 1 amino acids in total. In addition or alternatively, the variant may be modified, for example by N-terminal and/or C-terminal amino acid additions of up to 50, 40, 30, 20, 10, 5, 4, 3, 2, or 1 amino acids in total.

Table 1: Properties of naturally occurring proteins.

Charge properties / hydrophobicity	Side group	Amino Acid
nonpolar hydrophobic	aliphatic	Ala, Ile, Leu, Val
	aliphatic, S-containing	Met
	aromatic	Phe, Trp
	imino	Pro
polar uncharged	aliphatic	Gly
	amide	Asn, Gln
	aromatic	Tyr
	hydroxyl	Ser, Thr
	sulfhydryl	Cys
positively charged	basic	Arg, His, Lys
negatively charged	acidic	Asp, Gly

The term "linked" generally and unless specified otherwise refers to a coupling of two domains by a peptide bond. Therein the two domains may be linked with or without a linker. Herein, it is referred to linking via a linker having a length of 0-X amino acids ("aa"). 0 aa means that the linking is via a peptide bond directly linking the two domains. For this also the term "fused" instead of linked may be used. An X of 1 or larger means that the linking is via a peptide linker. A peptide linker is in particular a flexible peptide linker, i.e. provides flexibility among the domains that are linked together. Such flexibility is generally increased if the amino acids are small and do not have bulky side chains that impede rotation or bending of the amino acid chain. Thus, preferably the peptide linker of the present invention has an increased content of small amino acids, in particular of glycines, alanines, serines, threonines, leucines and isoleucines. Preferably, at least 20%, 30%, 40%, 50%, 60% 70%, 80%, 90% or more of the amino acids of the peptide linker are such small amino acids. In a particular embodiment, the amino acids of the linker are selected from glycines and serines, i.e. said linker is a poly-glycine or a poly-glycine/serine linker, wherein "poly" means a proportion of at least 50%, 60%, 70%, 80%, 90% or even 100% glycine and/or serine residues in the linker. Linkers of CODV configurations as described herein provide sufficient mobility for the domains of the light and heavy chains to fold into cross over dual variable region immunoglobulins. Generally (not limited to CODV configurations), exemplary linkers comprise a sequence of the amino acid formula $(G_mS_n)_o$, wherein m is an integer from 0 to 4, n is 1 or 2, and o is an integer from 0 to 5. Particular examples are m=1, n=1, o=0-5; m=2, n=1, o=0-5; m=3, n=1, o=0-5; m=4, n=1, o=0-5; m=1, n=2, o=0-5; m=2, n=2, o=0-5; m=3, n=2, o=0-5; and m=4, n=2, o=0-5. If o>1, m and n can each also differ from the m and n, respectively, of one or more further (G_mS_n)

reiterations. In other words, for $(G_mS_n)_1$, m and n can differ from m and n, respectively, of any of $(G_mS_n)_{2-5}$. The same is possible for any of $(G_mS_n)_{2-5}$ with respect to the remaining $(G_mS_n)_o$.

Regarding the first aspect, V_HA is linked to $CH2_H$ via a 0-30 aa linker and V_LA is linked to $CH2_L$ via a 0-30 aa linker. Independently or in both cases, the length of each linker may be 1-30, 5-25 or 10-20, e.g. about 15 aa. In particular, the two linkers have the same length and may even be identical, i.e. have the same amino acid sequence. Generally and unless specified otherwise, each linkage referred to herein is by such linker.

In a particular embodiment, the C-terminus of V_HA is linked, in particular fused to the N-terminus of $CH2_H$ and the C-terminus of V_LA is linked, in particular fused to the N-terminus of $CH2_L$. Further, $CH2_H$ may be linked via a hinge region to a constant domain C_H2, wherein the constant domain C_H2 may be fused to a constant domain C_H3.

The term "hinge region" as used herein refers to the flexible amino acid stretch in the central part of the heavy chains of the IgG and IgA, in particular the IgG (i.e. Ig1, IgG2, IgG3 or IgG4, especially IgG1) immunoglobulin classes, which links these two chains by disulfide bonds.

In a further embodiment, the heavy chain A and the light chain A are derived from IgG, e.g. IgG1, IgG2, IgG3 or IgG4. In particular they are derived from IgG1, wherein domains therein may be mutated and/or substituted by IgG2, IgG3 or IgG4, in particular by IgG3 domains, for example as described herein.

In some embodiments, heavy chain A has modifications as described in the second aspect, in particular one or more modifications facilitating the isolation of the antibody as described.

In a second aspect, the present invention relates to an antibody or derivative thereof, comprising a heavy chain A and a light chain A according to the first aspect.

Usually, the antibody or derivative thereof is isolated and/or recombinant. Further, the antibody or derivative thereof may be a mouse antibody, in particular a humanized mouse antibody, or derivative thereof. Alternatively, the antibody may be derived from any other species, particularly from a vertebrate species, e.g. a mammalian species such as a primate species including human, rabbit or rat.

It is envisaged that in the antibody or derivative thereof, the dimerization domain $CH2_H$ and the dimerization domain $CH2_L$ form a dimer. Furthermore, in one embodiment it is envisaged that

V_HA and V_LA form a variable domain F_VAA (i.e. FvA_HA_L) and thereby in particular a paratope AA (i.e. A_HA_L). In an alternative embodiment, V_HA and V_LA do not form a variable domain F_VAA (or a paratope AA). Particularly, instead V_HA forms a variable domain FvAX (i.e. $F_VA_HX_L$), thereby in particular a paratope AX (i.e. A_HX_L) with a further light chain variable domain V_LX comprised in light chain A and V_LA forms a variable domain XA (i.e. $F_VX_HA_L$), in particular a paratope XA (i.e. X_HA_L)) with a further heavy chain variable domain V_HX comprised in heavy chain A. This alternative is exemplified by the "CODV configuration" as described below (see also Fig. 1 D).

As indicated by these embodiments, the antibody or derivative thereof of the second aspect is usually multispecific, e.g. bispecific or trispecific. Accordingly, the antibody or derivative thereof of the second aspect further comprises a heavy chain B and a light chain B, wherein the heavy chain comprises a variable domain V_HB and the light chain comprises a variable domain V_LB. These may be arranged with heavy and light chains A in an IgG configuration, i.e. heavy chains A and B dimerize and light chain A dimerizes with heavy chain A and light chain B dimerizes with heavy chain B. Further, the multispecific, e.g. bispecific antibody or derivative thereof may be symmetric or asymmetric. Asymmetric means that the variable domains described above, e.g. F_VAA and FvBB and in particular the paratopes on the two antibody arms (i.e. of the two heavy chains) are different from each other. Symmetric as used herein means that the antibody or derivative thereof comprises identical variable domains on each arm of an IgG antibody or derivative thereof. A multispecificity of a higher degree than bispecificity is usually achieved in this antibody by further variable domains on the same arms.

A multispecific antibody or derivative is capable of binding multiple different antigens and a bispecific antibody or derivative is capable of binding two different antigens. For all antibodies or derivatives described herein, the antigen is for example independently selected for each specificity from the group consisting of B7.1, B7.2, BAFF, BlyS, C3, *C5,* CCL11 (eotaxin), CCL15 (MIP-1d), CCL17 (TARC), CCL19 (MIP-3b), CCL2 (MCP-1), CCL20 (MIP-3a), CCL21 (MIP-2), SLC, CCL24 (MPIF-2/eotaxin-2), CCL25 (TECK), CCL26 (eotaxin-3), CCL3 (MIP-1a), CCL4 (MIP-1b), CCL5 (RANTES), CCL7 (MCP-3), CCL8 (mcp-2), CD3, CD19, CD20, CD24, CD40, CD40L, CD80, CD86, CDH1 (E-cadherin), Chitinase, CSF 1 (M-CSF), CSF2 (GM-CSF), CSF3 (GCSF), CTLA4, CX3CL1 (SCYD1), CXCL12 (SDFI), CXCL13, EGFR, FCER1A, FCER2, HER2, IGF1R, IL-l, IL-12, IL13, IL15, IL17, IL18, IL1A, IL1B, IL1F1O, IL1β, IL2, IL4, IL6, IL7, IL8, IL9, IL12/23, IL22, IL23, IL25, IL27, IL35, ITGB4 (b 4 integrin), LEP (leptin), MHC class II, TLR2, TLR4, TLR5, TNF, TNF-α, TNFSF4 (OX40 ligand), TNFSF5 (CD40 ligand), Toll-like receptors, TREM1, TSLP, TWEAK, XCRI (GPR5/CCXCR1), DNGR-1 (CLEC91), and HMGB1. In particular it is from the group consisting of CD3, CD4, CD8, CD28, CD16, NKp46.

For all bispecific antibodies or derivatives described herein, the antigen pair is for example selected from the group consisting of the antigen pairs IL4 and IL13, IGF1R and HER2, IGF1R and EGFR, EGFR and HER2, BK and IL13, PDL-1 and CTLA-4, CTLA4 and MHC class II, IL-12 and IL-18, IL-Iα and IL-β, TNFα and IL12/23, TNFα and IL-12p40, TNFα and IL-β, TNFα and IL-23, and IL17 and IL23. For all trispecific antibodies or derivatives described herein, two antigens are for example selected from the group consisting of the antigen pairs IL4 and IL13, IGF1R and HER2, IGF1R and EGFR, EGFR and HER2, BK and IL13, PDL-1 and CTLA-4, CTLA4 and MHC class II, IL-12 and IL-18, IL-Iα and IL-β, TNFα and IL12/23, TNFα and IL-12p40, TNFα and IL-β, TNFα and IL-23, and IL17 and IL23. The third antigen can be another antigen selected from the list above. Further antigens are listed with respect to the sixth aspect below and can also be used for the multispecific antibody or derivative.

An example for a symmetric multispecific antibody of the invention is a "tetravalent spider configuration" (see Figure 1 E), which is defined by the antibody or derivative thereof of the second aspect comprising a first and a second heavy chain A and a first and a second light chain A each according to the first aspect, wherein (i) the $CH2_L$ domain of the first light chain A is linked to the V_H1 or V_L1 domain (e.g. V_HB or V_LB) of an IgG antibody or derivative thereof and the $CH2_L$ domain of the second light chain A is linked to the other V_H1 or V_L1 domain (e.g. V_HB or V_LB) of the IgG antibody or derivative thereof (for example in both cases to the V_H1 domain or to the V_L1 domain), or (ii) the $CH2_H$ domain of the first light chain A is linked to the V_H1 or V_L1 domain (e.g. V_HB or V_LB) of an IgG antibody or derivative thereof and the $CH2_H$ domain of the second light chain A is linked to the other V_H1 or V_L1 domain (e.g. V_HB or V_LB) of the IgG antibody or derivative thereof (for example in both cases to the V_H1 domain or to the V_L1 domain). In this respect, the linkage is usually via a 0-10 aa linker. Independently, the length of each linker may be 1-10 or 2-6 aa. In particular, the two linkers may have the same length and may even be identical, i.e. have the same amino acid sequence. A "derivative" of an IgG antibody in this respect is in particular an antigen-binding IgG fragment such as a Fab2 fragment.

In the "tetravalent spider configuration", usually the $CH2_L$ domain of the first light chain A forms a dimer with the $CH2_H$ domain of the first heavy chain A and the $CH2_L$ domain of the second light chain A forms a dimer with the $CH2_H$ domain of the second heavy chain A. Furthermore, usually V_HA and V_LA of the first heavy and light chains A form a variable domain F_VAA (i.e. FvA_HA_L), in particular a paratope AA (í.e. A_HA_L), and V_HA and V_LA of the second heavy and light chains A form a variable domain F_VAA (i.e. FvA_HA_L), in particular a paratope AA (i.e. A_HA_L). Further, the V_H1 and V_L1 domains of the IgG part are also symmetric, i.e. they form one variable domain F_V each which is the same for both (e.g. F_VBB).

An asymmetric multispecific antibody or derivative thereof of the invention, in particular an asymmetric bispecific antibody or derivative thereof of the invention, generally comprises a heavy chain B and a light chain B, wherein the heavy chain comprises a variable domain V_HB and the light chain comprises a variable domain V_LB.

Examples of this asymmetric antibody or derivative thereof are the "bivalent Fab configuration" (see Figure 1 B) and the "IgG configuration" (see Figure 1 A). Therein, V_HB is linked to a dimerization domain DIM_H and V_LB is linked to a dimerization domain DIM_L. In the "bivalent Fab configuration", the two Fabs may be linked to each other via the $CH2_H$ and the DIM_H domains as shown in Figure 1B, or one of the DIM domains may be linked to one of the VA domains (e.g. DIM_H to V_HA), or one of the CH2 domains may be linked to one of the VB domains (e.g. $VH2_H$ to V_HB). In this respect, the linkage is usually via a 0-30 or 1-30 aa linker. Independently, the length of each linker may be 5-25 or 10-20, e.g. about 15 aa. In particular, the two linkers have the same length and may even be identical, i.e. have the same amino acid sequence.

The term "DIM" is not used herein as an art known term for an antibody domain, but as a general designation for a dimerization domain, in particular a homodimerization domain. DIM_H and DIM_L usually form a dimer. Also, DIM_H and DIM_L are not constant domains C_H2, i.e. they are different from the pair $CH2_H$ and $CH2_L$. In particular, they are not an IgM constant domain MC_H2 or an IgE constant domain EC_H2. In one embodiment, a DIM domain is derived from an antibody. In a specific embodiment, DIM_H and DIM_L are C_{H1} and C_L, respectively, of an IgG antibody. Furthermore, it is envisaged that both the heavy chain B and the light chain B are derived from IgG, unless specified otherwise. In particular, the antibody or derivative thereof is bispecific. It is also envisaged that V_HA and V_LA may form a variable domain F_VAA (i.e. FvA_HA_L), in particular a paratope AA (i.e. A_HA_L), and V_HB and V_LB form a variable domain F_VBB (i.e. FvB_HB_L), in particular a paratope BB (i.e. B_HB_L)).

In the "bivalent Fab configuration", usually DIM_H is linked to $CH2_H$ or $CH2_L$, or DIM_L is linked to $CH2_H$ or $CH2_L$. Alternatively, one of the DIM domains may be linked to one of the VA domains (e.g. DIM_H to V_HA), or one of the CH2 domains may be linked to one of the VB domains (e.g. $VH2_H$ to V_HB). In this respect, the linkage is usually via a 0-30 or 1-30 aa linker. The length of the linker may be 5-25 or 10-20, e.g. about 15 aa.

In the "IgG configuration", usually $CH2_H$ is linked via a hinge region to a constant domain C_H2A and DIM_H is linked via a hinge region to a constant domain C_H2B. C_H2A may be linked, in

particular fused, to a constant domain C_H3A and C_H2B may be linked, in particular fused, to a constant domain C_H3B. Further, it is contemplated that heavy chain A is bound to heavy chain B via disulfide bonds, in particular two thereof, between the hinge region of heavy chain A and the hinge region of heavy chain B. Alternatively, in the IgG configuration, $CH2_H$ is not linked via a hinge region to a constant domain C_H2A and DIM_H is not linked via a hinge region to a constant domain C_H2B, but $CH2_H$ is linked to a different $CH2_H$ domain (e.g. a $ECH2_H$ domain if the $CH2_H$ of the Fab domain is an $MCH2_H$ domain, or vice versa), and DIM_H is linked to a $CH2_H$ domain capable of pairing with that different CH2H domain, in particular to a $CH2_H$ domain that is the same (in as of the same type) as that different $CH2_H$ domain (e.g. an $ECH2_H$ domain if the other $CH2_H$ domain is an $ECH2_H$ domain; or an $MCH2_H$ domain if the other $CH2_H$ domain is an $MCH2_H$ domain).

Another example of the asymmetric antibody or derivative thereof described above is the "TBTI configuration" (see Figure 1 C). This configuration is defined as the "IgG configuration", but

- heavy chain A further comprises a further heavy chain variable domain V_HX linked to V_HA,
- light chain A further comprises a further light chain variable domain V_LX linked to V_LA,
- heavy chain B further comprises a further heavy chain variable domain V_HY linked to V_HB, and
- light chain B further comprises a further light chain variable domain V_LY linked to V_LB.

These linkages are via a 0-15 or usually a 1-15 aa linker each. Independently, the length of each linker may be 5-10 or about 7 or 8 aa. In particular, the linkers have the same length and may even be identical, i.e. have the same amino acid sequence. In one embodiment, the C-termini of V_HX, V_LX, V_HY and V_LY are fused to N-termini of V_HA, V_LA, V_HB and V_LB, respectively. For this configuration it is contemplated that

- V_HA and V_LA form a variable domain F_VAA (i.e. FvA_HA_L), in particular a paratope AA (i.e. A_HA_L),
- V_HB and V_LB form a variable domain F_VBB (i.e. FvB_HB_L), in particular a paratope BB (i.e. B_HB_L),
- V_HX and V_LX form a variable domain F_VXX (i.e. FvX_HX_L), in particular a paratope XX (i.e. X_HX_L), and
- V_{HY} and V_LY form a variable domain F_VYY (i.e. FvY_HY_L), in particular a paratope YY (or Y_HY_L).

Yet another example of the asymmetric antibody or derivative thereof described above is the "cross-over dual variable configuration" or "CODV configuration". A general CODV configuration is described in WO 2012/2135345 A1 and comprises like the "TBTI configuration" a

- heavy chain A comprising a further heavy chain variable domain V_HX linked to V_HA,

- light chain A comprising a further light chain variable domain V_LX linked to V_LA,
- heavy chain B comprising a further heavy chain variable domain V_HY linked to V_HB, and
- light chain B comprising a further light chain variable domain V_LY linked to V_LB.

In one embodiment, the C-termini of V_HX, V_LX, V_HY and V_LY are fused to N-termini of V_HA, V_LA, V_HB and V_LB, respectively

However, in the "CODV configuration",

- V_HA forms a variable domain FvAX (i.e. $F_VA_HX_L$), in particular a paratope AX (i.e. A_HX_L)) with V_LX,
- V_LA forms a variable domain FvXA (i.e. $F_VX_HA_L$), in particular a paratope XA (i.e. X_HA_L)) with V_HX,
- V_HB forms a variable domain FvBY (i.e. $F_VB_HY_L$), in particular a paratope (i.e. B_HY_L)) with V_LY,
- V_LB forms a variable domain YB (i.e. $F_VY_HB_L$), in particular a paratope YB (i.e. Y_HB_L)) with V_HY.

Thus, unlike in the "TBTI configuration", V_HA and V_LA, V_HB and V_LB, V_HX and V_LX, V_HY and V_LY do not form paratopes AA, BB, XX, YY, respectively.

This is achieved by the following linkages (L1: linker 1, L2: linker 2, L3: linker 3, L4: linker 4):

(i)

- $CH2_H$ to V_HA by a 1-3 aa, in particular 1-2 aa, or about 2 aa linker, alternatively 0 aa linker (L4),
- $CH2_L$ to V_LA by a 3-14 aa, in particular 5-8 aa, or about 5 aa linker, alternatively 10 aa linker (L2),
- DIM_H to V_HB by a 1-3 aa, in particular 1-2 aa, or about 2 aa linker, alternatively 0 aa linker (L4),
- DIM_L to V_LB by a 3-14 aa, in particular 5-8 aa, or about 5 aa linker, alternatively 10 aa linker (L2),
- V_HA to V_HX by a 1-8 aa, in particular 1-5 aa, or about 1 aa linker, alternatively 0 aa linker (L3),
- V_LA to V_LX by a 3-12 aa, in particular 5-10 aa, or about 7 aa linker, alternatively 10 aa linker (L1),
- V_HB to V_HY by a 1-8 aa, in particular 1-5 aa, or about 1 aa linker, alternatively 0 aa linker (L3), and
- V_LB to V_LY by a 3-12 aa, in particular 5-10 aa, or about 7 aa linker, alternatively 10 aa linker (L1); or

(ii)

- $CH2_H$ to V_HA by a 2-15 aa, in particular 2-12 aa, or about 5 aa linker, alternatively 10 aa linker (L4),
- $CH2_L$ to V_LA by a 1-4 aa, in particular 1-2 aa, or about 2 aa linker, alternatively 0 aa linker (L2),

- DIM_H to V_HB by a 2-15 aa, in particular 2-12 aa, or about 5 aa linker, alternatively 10 aa linker (L4),
- DIM_L to V_LB by a 1-4 aa, in particular 1-2 aa, or about 2 aa linker, alternatively 0 aa linker (L2),
- V_HA to V_HX by a 2-15 aa, in particular 4-12 aa, or about 7 aa linker, alternatively 10 aa linker (L3),
- V_LA to V_LX by a 1-3 aa, in particular 1-2 aa, or about 1 aa linker, alternatively 0 aa linker (L1),
- V_HB to V_HY by a 2-15 aa, in particular 4-12 aa, or about 7 aa linker, alternatively 10 aa linker (L3), and
- V_LB to V_LY by a 1-3 aa, in particular 1-2 aa, or about 1 aa linker, alternatively 0 aa linker (L1).

In (i) above, the light chain linkers are longer than the heavy chain linkers on both arms, and in (ii) above, the heavy chain linkers are longer than the light chain linkers on both arms. In other words, with respect to the linkers, the arms are symmetric. However, they can also be asymmetric as indicated in Fig. 1D, i.e. on one arm, the light chain linkers are longer than the heavy chain linkers and on the other arm the heavy chain linkers are longer than the light chain linkers. This results directly in the following alternative embodiments (iii) and (iv), respectively:

(iii)

- $CH2_H$ to V_HA by a 1-3 aa, in particular 1-2 aa, or about 2 aa linker, alternatively 0 aa linker (L4),
- $CH2_L$ to V_LA by a 3-14 aa, in particular 5-8 aa, or about 5 aa linker, alternatively 10 aa linker (L2),
- DIM_H to V_HB by a 3-14 aa, in particular 5-8 aa, or about 5 aa linker, alternatively 10 aa linker (L4),
- DIM_L to V_LB by a 1-3 aa, in particular 1-2 aa, or about 2 aa linker, alternatively 0 aa linker (L2),
- V_HA to V_HX by a 1-8 aa, in particular 1-5 aa, or about 1 aa linker, alternatively 0 aa linker (L3),
- V_LA to V_LX by a 3-12 aa, in particular 5-10 aa, or about 7 aa linker, alternatively 10 aa linker (L1),
- V_HB to V_HY by a 3-12 aa, in particular 5-10 aa, or about 7 aa linker, alternatively 10 aa linker (L3), and
- V_LB to V_LY by a 1-8 aa, in particular 1-5 aa, or about 1 aa linker, alternatively 0 aa linker (L1);

or

(iv)

- $CH2_H$ to V_HA by a 2-15 aa, in particular 2-12 aa, or about 5 aa linker, alternatively 10 aa linker (L4),
- $CH2_L$ to V_LA by a 1-4 aa, in particular 1-2 aa, or about 2 aa linker, alternatively 0 aa linker (L2),

- DIM_H to V_HB by a 1-4 aa, in particular 1-2 aa, or about 2 aa linker, alternatively 0 aa linker (L4),
- DIM_L to V_LB by a 2-15 aa, in particular 2-12 aa, or about 5 aa linker, alternatively 10 aa linker (L2),
- V_HA to V_HX by a 2-15 aa, in particular 4-12 aa, or about 7 aa linker, alternatively 10 aa linker (L3),
- V_LA to V_LX by a 1-3 aa, in particular 1-2 aa, or about 1 aa linker, alternatively 0 aa linker (L1),
- V_HB to V_HY by a 1-3 aa, in particular 1-2 aa, or about 1 aa linker, alternatively 0 aa linker (L3), and
- V_LB to V_LY by a 2-15 aa, in particular 4-12 aa, or about 7 aa linker, alternatively 10 aa linker (L1).

In the above, the most general ranges are usually combined with each other, or the particular ranges with each other, or the single values mentioned last with each other (first alternatives which each other and second alternative with each other). With respect to (i), usually the $CH2_L$ to V_LA and DIM_L to V_LB linkers are longer than the $CH2_H$ to V_HA and DIM_H to V_HB linkers, respectively, and the V_LX to V_LA and V_LY to V_LB linkers are longer than the V_HA to V_HX and V_HB to V_HY linkers, respectively. With respect to (ii), usually the $CH2_H$ to V_HA and DIM_H to V_HB linkers are longer than the $CH2_L$ to V_LA and DIM_L to V_LB linkers, respectively, and the V_HA to V_HX and V_HB to V_HY linkers are longer than the V_LX to V_LA and V_LY to V_LB linkers, respectively. With respect to (iii), usually the $CH2_L$ to V_LA and DIM_H to V_HB linkers are longer than the $CH2_H$ to V_HA and DIM_L to V_LB linkers, respectively, and the V_LX to V_LA and V_HY to V_HB linkers are longer than the V_HA to V_HX and V_LB to V_LY linkers, respectively. With respect to (iv), usually the $CH2_H$ to V_HA and DIM_L to V_LB linkers are longer than the $CH2_L$ to V_LA and DIM_H to V_HB linkers, respectively, and the V_HA to V_HX and V_LB to V_LY linkers are longer than the V_LX to V_LA and V_HY to V_HB linkers, respectively. Longer herein may mean at least 1.5 times as long, at least 1.75 times as long or at least 2 times as long (in as far as possible given the specified ranges).

Further, the above can also be described with heavy (I) and light chain (II) domain formulas for each arm, which are

(I) $CH2_H/DIM_H – L4 – V_H – L3 – V_H$ and (II) $CH2_L/DIM_L – L2 – V_L – L1 – V_L$,

wherein one arm has $CH2_H$ and $CH2_L$ for (I) and (II), respectively, and the other arm has DIM_H and DIM_L for (I) and (II), respectively,

and wherein L4 is (a) a 1-3 aa, in particular 1-2 aa, or about 2 aa linker, alternatively 0 aa linker, or (b) a 2-15 aa, in particular 2-12 aa, or about 5 aa linker, alternatively 10 aa linker, L3 is (a) a 1-8 aa, in particular 1-5 aa, or about 1 aa linker, alternatively 0 aa linker, or (b) a 2-15

aa, in particular 4-12 aa, or about 7 aa linker, alternatively 10 aa linker, L2 is (a) a 3-14 aa, in particular 5-8 aa, or about 5 aa linker, alternatively 10 aa linker, or (b) a 1-4 aa, in particular 1-2 aa, or about 2 aa linker, alternatively 0 aa linker. Therein, embodiments (a) combine with each other, and embodiments (b) combine with each other. Also, the most general ranges are usually combined with each other, or the particular ranges with each other, or the single values mentioned last with each other (first alternatives which each other and second alternative with each other).

One specific embodiment of the "CODV configuration" is the "CODV bivalent Fab configuration", which comprises the left "A" arm of the "CODV configuration" shown in Fig. 1D without the constant domains C_H2A and C_H3A, i.e. it comprises the domains V_HA, V_LA, V_HX and V_LX configured as described for the "CODV configuration".

A further example of an asymmetric antibody or derivative thereof described above is the "CODV chimera configuration" with a particular example being the "trivalent configuration" (see Figure 1 F). In the "CODV chimera configuration", the antibody comprises one arm A not in the "CODV configuration" and one arm B in the "CODV configuration". Either the arm A comprises CH2 domains as described herein and the arm B comprises DIM domains as described herein, or the arm A comprises the DIM domains and arm B the CH2 domains.

The arm A not in the "CODV configuration" can have any antibody arm configuration, including an arm of configurations described herein, in particular of asymmetric configurations described herein, such as the "IgG configuration" or the "TBTI configuration". In the arm A in the "IgG configuration", the heavy chain and the light chain are configured as described above for that configuration (with CH2 domains if arm B comprises DIM domains or with DIM domains instead if arm B comprises CH2 domains). In the arm A in the "TBTI configuration", the heavy chain and the light chain are configured as described above for that configuration (with CH2 domains if arm B comprises DIM domains or with DIM domains instead if arm B comprises DH2 domains).

The arm B in the "CODV configuration" is configured as defined as described above for that configuration (with DIM domains if arm A comprises CH2 domains or with CH2 domains instead if arm A comprises DIM domains). The linkage of the domains can be either as in (i) or (ii) defined above.

The embodiment with arm A having the "IgG configuration" is a particular configuration also termed "trivalent configuration" herein (see Fig. 1 F) as it forms three paratopes. This

configuration can be monospecific (all paratopes are the same), but is usually bispecific (two of the three paratopes are same) or trispecific (all paratopes are different). The configuration is particularly useful in the trispecific form.

In further embodiments of the multispecific, in particular bispecific antibody or derivative thereof of the second aspect, the antibody or derivative thereof comprises one or more modifications facilitating the isolation of the antibody, especially modifications facilitating the pairing of heavy chain A with heavy chain B or allowing for the selection of this pairing. This applies in particular to an asymmetric antibody or derivative of the second aspect.

For example, the modifications facilitating the isolation of the antibody may be one or more of the following: a knobs into holes technology modification, a DuoBody technology modification, an Azymmetric technology modification, a Charge Pair technology modification, a HA-TF technology modification, a SEEDbody technology modification or a Differential protein A affinity modification. Specifically, they may be one or more of the following:

- first heavy chain (e.g. heavy chain A): T366Y mutation and optionally further S354 (S354C) and/or T166W mutation, second heavy chain (e.g. heavy chain B): Y407T mutation and optionally further Y349C, T366S, L368A and/or Y407V mutation (knobs into holes technology),
- first heavy chain (e.g. heavy chain A): T366W mutation, second heavy chain (e.g. heavy chain B): T366S, L368A and/or Y407V mutation (knobs into holes technology),
- first heavy chain (e.g. heavy chain A): F405L mutation, second heavy chain (e.g. heavy chain B): K409R mutation (DuoBody technology),
- first heavy chain (e.g. heavy chain A): T350V, L351Y, F405A and Y407V mutation, second heavy chain (e.g. heavy chain B): T350V, T366L, K392L and T394W mutation (Azymmetric technology),
- first heavy chain (e.g. heavy chain A): K409D and K392D mutation, second heavy chain (e.g. heavy chain B): D399K and E356K mutation (Charge Pair technology),
- first heavy chain (e.g. heavy chain A): D221E, P228E and L368E mutation, second heavy chain (e.g. heavy chain B): D221R, P228R and K409R mutation (Charge Pair technology),
- first heavy chain (e.g. heavy chain A): S364H and F405A mutation, second heavy chain (e.g. heavy chain B): Y349T and T394F mutation (HA-TF technology),
- first heavy chain (e.g. heavy chain A): IgG/A chimera, second heavy chain (e.g. heavy chain B): IgA/G chimera (SEEDbody technology),
- first heavy chain (e.g. heavy chain A): Fc region or part thereof (e.g. C_H3 domain) is from IgG3, second heavy chain (e.g. heavy chain B) Fc region or part thereof (e.g. C_H3 domain) is from IgG1, 2 or 4, in particular IgG1 (Differential protein A affinity),

- first heavy chain (e.g. heavy chain A): H435R and Y436F mutation, second heavy chain (e.g. heavy chain B): T407T mutation (Differential protein A affinity), and/or
- first heavy chain (e.g. heavy chain A): H435R mutation, second heavy chain (e.g. heavy chain B): no mutation (Differential protein A affinity).

Therein, the first heavy chain may alternatively be heavy chain B and the second heavy chain may be heavy chain A.

In a particular embodiment, the antibody has a Y-shaped IgG like form (including the "IgG configuration", the "TBTI configuration" and the "CODV configuration"), the constant domains C_H2A, C_H2B, C_H3A and/or C_H3B, preferably C_H3A and/or C_H3B, facilitate heavy chain heterodimerization or a selection of heavy chain heterodimers. By heavy chain heterodimerization a linkage of heavy chain A to heavy chain B is meant, so a linkage of heavy chain A to a further heavy chain A and/or a linkage of chain B to a further heavy chain B is impeded or selected against.

For example, the Fc regions of heavy chain A and heavy chain B (e.g. constant domains C_H2A and C_H2B, and/or C_H3A and C_H3B), in particular C_H3A and C_H3B comprise knob-into-hole mutations. Such knob-into-hole mutations are in particular T366Y in one of C_H3A or C_H3B and Y407T in the other, wherein C_H3A and C_H3B are IgG1 constant domains, and optionally wherein the Fc region comprising the T366Y mutation ("knob" chain) further comprises the mutations S354 and T166W and the Fc region comprising the Y407T mutation ("hole" chain) further comprises the mutations Y349C, T366S, L368A and Y407V.

Alternatively or in addition, (i) either the Fc region of heavy chain A or a part thereof (e.g. C_H2A or C_H3A), in particular C_H3A, or the Fc region of heavy chain B or a part thereof (e.g. C_H2B or C_H3B), in particular C_H3B, is derived from IgG3, or (ii) either the Fc region of heavy chain A (i.e. C_H2A and C_H3A), in particular C_H3A, or the Fc region of heavy chain B (i.e. C_H2B and C_H3B), in particular C_H3B, comprises one or more mutations decreasing and in particular abolishing the binding to Protein A. Such mutations may be H435R and Y436F in either C_H3A or C_H3B, wherein C_H3A and C_H3B are IgG1 constant domains.

It is particularly envisaged that the Fc regions of heavy chain A and heavy chain B (e.g. constant domains C_H2A and C_H2B, and/or C_H3A and C_H3B), in particular C_H3A and C_H3B comprise knob-into-hole mutations and either the Fc region of heavy chain A (i.e. C_H2A and C_H3A), in particular C_H3A, or the Fc region of heavy chain B (i.e. C_H2B and C_H3B), in particular C_H3B, comprises one or more mutations abolishing the binding to Protein A. Even more particularly, it is envisaged that the heavy chain comprising the hole mutation(s) of the knob-

into-hole mutations comprises the one or more mutations decreasing or in particular abolishing the binding to Protein A. This has the advantage that the isolation of this antibody can be performed without a Kappa Select chromatography as described below, since the knob-into-hole approach allows for dimerization also of two "hole" heavy chains, but not for dimerization of two "knob" heavy chains. Therefore, if the "hole" heavy chain has the one or more mutations decreasing and in particular abolishing the binding to Protein A and it is selected with Protein A chromatography, one will select against the "hole" dimers and isolate only the multispecific, in particular bispecific antibody without any further steps.

Further, the antibody or derivative thereof of the second aspect may have reduced or no Fc effector functions. An Fc effector function is the interaction with complement protein C1q and/or the binding to Fc receptors. A reduced or a lack of effector functions can be achieved for example by a double mutation L234A and L235A (so-called "LALA mutation") in the C_H2A and/or the C_H2B domain.

All terms used with respect to the second aspect have the meanings as defined with respect to the first aspect of the invention, unless specifically defined otherwise. Further, all embodiments specified for the first aspect that are applicable to the second aspect are also envisaged for the second aspect.

In a third aspect, the present invention relates to one or more polynucleotides encoding for the heavy chain A and/or the light chain A of the first aspect. In one embodiment, the one or more polynucleotides also encode for the heavy chain B and/or for the light chain B of the antibody or derivative of the second aspect. Particularly, the one or more polynucleotides encode for the antibody or derivative of the second aspect. This refers to all embodiments described above, in particular to any of the antibody configurations described herein. In one embodiment, the one or more polynucleotides are isolated.

All terms used with respect to the third aspect have the meanings as defined with respect to the first and second aspect of the invention, unless specifically defined otherwise. Further, all embodiments specified for the first and second aspect that are applicable to the third aspect are also envisaged for the third aspect.

In a fourth aspect, the present invention relates to one or more expression vectors comprising the one or more polynucleotides of the third aspect.

The term "vector" as used herein refers to any molecule (e.g., nucleic acid, plasmid, or virus) that is used to transfer coding information to a host cell. The term "vector" includes a nucleic acid molecule that is capable of transporting another nucleic acid to which it has been fused. One type of vector is a "plasmid," which refers to a circular double-stranded DNA molecule into which additional DNA segments may be inserted. Another type of vector is a viral vector, wherein additional DNA segments may be inserted into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell and thereby are replicated along with the host genome. In addition, certain vectors are capable of directing the expression of genes they comprise. Such vectors are referred to herein as "expression vectors".

All embodiments specified for the first, second and third aspect that are applicable to the fourth aspect are also envisaged for the fourth aspect.

In a fifth aspect, the present invention relates to a cell comprising the one or more polynucleotides of the third aspect or the one or more expression vectors of the fourth aspect. In one embodiment, the cell is isolated.

A wide variety of cell expression systems can be used to express said polynucleotides including the use of prokaryotic and eukaryotic cells, such as bacterial cells (e.g. E. coli), yeast cells, insect cells or mammalian cells (e.g. mouse cells, rat cells, human cells etc.). For this purpose, a cell is transformed or transfected with said polynucleotide(s) or expression vector(s) such that the polynucleotide(s) of the invention are expressed in the cell and, in one embodiment, secreted into the medium in which the cells are cultured, from where the expression product can be recovered.

All embodiments specified for the first, second, third and fourth aspect that are applicable to the fifth aspect are also envisaged for the fifth aspect.

In a sixth aspect, the present invention relates to a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a dimer of the heavy chain A and the light chain A of the first aspect or the antibody or derivative of the second aspect, wherein in a particular embodiment the dimer or the antibody or derivative specifically binds to a pathogen, a diseased cell, a cell receptor or a cell signalling molecule.

The pharmaceutical compositions of the invention can be selected for parenteral delivery. Alternatively, the compositions can be selected for inhalation or for delivery through the digestive tract, such as orally. The preparation of such pharmaceutically acceptable compositions is within the skill of the art.

The term "pharmaceutically acceptable carrier" or "physiologically acceptable carrier" as used herein refers to one or more formulation materials suitable for accomplishing or enhancing the delivery of an antibody-like binding protein. The primary carrier in a pharmaceutical composition can be either aqueous or non-aqueous in nature. For example, a suitable vehicle or carrier for injection can be water, physiological saline solution, or artificial cerebrospinal fluid, possibly supplemented with other materials common in compositions for parenteral administration. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles. Other exemplary pharmaceutical compositions comprise Tris buffer of about pH 7.0-8.5, or acetate buffer of about pH 4.0-5.5, which can further include sorbitol or a suitable substitute. In one embodiment of the invention, antibody-like binding protein compositions can be prepared for storage by mixing the selected composition having the desired degree of purity with optional formulation agents in the form of a lyophilized cake or an aqueous solution. Further, the antibody-like binding protein can be formulated as a lyophilizate using appropriate excipients such as sucrose.

The pharmaceutical composition can contain formulation materials for modifying, maintaining, or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption, or penetration of the composition. Suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine, or lysine), antimicrobials, antioxidants (such as ascorbic acid, sodium sulfite, or sodium hydrogen-sulfite), buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates, or other organic acids), bulking agents (such as mannitol or glycine), chelating agents (such as ethylenediaminetetraacetic acid (EDTA)), complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin, or hydroxypropylbeta-cyclodextrin), fillers, monosaccharides, disaccharides, and other carbohydrates (such as glucose, mannose, or dextrins), proteins (such as serum albumin, gelatin, or immunoglobulins), coloring, flavoring and diluting agents, emulsifying agents, hydrophilic polymers (such as polyvinylpyrrolidone), low molecular weight polypeptides, salt-forming counterions (such as sodium), preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid, or hydrogen peroxide), solvents (such as glycerin, propylene glycol, or polyethylene glycol), sugar alcohols (such as mannitol or sorbitol), suspending agents, surfactants or wetting agents (such as pluronics; PEG;

sorbitan esters; polysorbates such as polysorbate 20 or polysorbate 80; triton; tromethamine; lecithin; cholesterol or tyloxapal), stability enhancing agents (such as sucrose or sorbitol), tonicity enhancing agents (such as alkali metal halides, preferably sodium or potassium chloride - or mannitol sorbitol), delivery vehicles, diluents, excipients and/or pharmaceutical adjuvants (see, e.g., REMINGTON's PHARMACEUTICAL SCIENCES (18th Ed., A.R. Gennaro, ed., Mack Publishing Company 1990), and subsequent editions of the same).

The term "specifically binds" as used herein refers to a binding reaction which is determinative of the presence of the target molecule *in vitro* or *in vivo*, in particular in an organism such as the human body. As such, the specified ligand binds to its particular target molecule and does not bind in a substantial amount to other molecules present. Generally, an antibody or derivative thereof that “specifically binds” a target molecule has an equilibrium affinity constant greater than about 10^5 (e.g., 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, and 10^{12} or more) mole/liter for that target molecule.

The term "pathogen" refers to any organism which may cause disease in a subject. It includes but is not limited to bacteria, protozoa, fungi, nematodes, viroids and viruses, or any combination thereof, wherein each pathogen is capable, either by itself or in concert with another pathogen, of eliciting disease in vertebrates including but not limited to mammals, and including but not limited to humans. As used herein, the term “pathogen” also encompasses microorganisms which may not ordinarily be pathogenic in a non-immunocompromised host, but are in an immunocompromised host.

The diseased cell may be a tumor cell, a chronically infected cell, a senescent cell, a cell showing an inflammatory phenotype, a cell accumulating amyloid proteins or a cell accumulating misfolded proteins.

In case of a tumor cell, the underlying disease is a tumor, in particular selected from the group consisting of Adrenal Cancer, Anal Cancer, Bile Duct Cancer, Bladder Cancer, Bone Cancer, Brain/CNS, Tumors, Breast Cancer, Cancer of Unknown Primary, Castleman Disease, Cervical Cancer, Colon/Rectum Cancer, Endometrial Cancer, Esophagus Cancer, Ewing Family Of Tumors, Eye Cancer, Gallbladder Cancer, Gastrointestinal Carcinoid Tumors, Gastrointestinal Stromal Tumor (GIST), Gestational Trophoblastic Disease, Hodgkin Disease, Kaposi Sarcoma, Kidney Cancer, Laryngeal and Hypopharyngeal Cancer, Leukemia, Liver Cancer, Lung Cancer, Lymphoma, Lymphoma of the Skin, Malignant Mesothelioma, Multiple Myeloma, Myelodysplastic Syndrome, Nasal Cavity and Paranasal Sinus Cancer, Nasopharyngeal Cancer, Neuroblastoma, Non-Hodgkin Lymphoma, Oral Cavity and

Oropharyngeal Cancer, Osteosarcoma, Ovarian Cancer, Pancreatic Cancer, Penile Cancer, Pituitary Tumors, Prostate Cancer, Retinoblastoma, Rhabdomyosarcoma, Salivary Gland Cancer, Sarcoma - Adult Soft Tissue Cancer, Skin Cancer, Small Intestine Cancer, Stomach Cancer, Testicular Cancer, Thymus Cancer, Thyroid Cancer, Uterine Sarcoma, Vaginal Cancer, Vulvar Cancer, Waldnstrom Macroglobulinemia, and Wilms Tumor. In particular, the antigen is a cancer associated antigen, such as HER2, EGFR, EGFRvIII, EGFR3 (ERBB3), MET, FAP, PSMA, CXCR4, ITGB3, CEA, CAIX, Mucins, Folate-binding protein, GD2, VEGFR1, VEGFR2, CD20, CD30, CD33, CD52, CTLA4, CD55, integrin αVβ3, integrin α5β1, IGF1R, EPHA3, RANKL, TRAILR1, TRAILR2, IL13Ralpha, UPAR, Tenascin, PD-1, PD-L1, Tumor-associated glycoprotein 72, Ganglioside GM2, A33, Lewis Y antigen or MUC1.

In case of a chronically infected cell, the underlying disease is a chronic infectious disease, such as tuberculosis, malaria, chronic viral hepatitis (HBV, Hepatitis D virus and HCV), Acquired immune deficiency syndrome (AIDS, caused by HIV, Human Immunodeficiency Virus), or EBV related disorders: Systemic Autoimmune Diseases (Systemic Lupus Erithematosus, Rheumatoid Arthritis, and Sjogren Syndrome) and Multiple Sclerosis (MS). In particular, the chronically infected cell comprises a pathogen or part thereof of the above-recited infectious diseases.

In case of a senescent cell, the underlying disease is a senescence associated disease, such as (i) Rare genetic diseases called Progeroid syndromes, characterized by pre-mature aging: Werner syndrome (WS), Bloom syndrome (BS), Rothmund–Thomson syndrome (RTS), Cockayne syndrome (CS), Xeroderma pigmentosum (XP), Trichothiodystrophy or Hutchinson–Gilford Progeria Syndrome (HGPS) or (ii) Common age related disorders: Obesity, type 2 diabetes, sarcopenia, osteoarthritis, idiopathic pulmonary fibrosis and chronic obstructive pulmonary disease, cataracts, neurodegenerative diseases, or cancer treatment related disorders. In particular, the senescent cell expresses, in particular in a misfolded form and/or presented on the cell surface, one or more protein such as prion protein (PrP), FasR, Fas ligand, CD44, EGF receptor, CD38, Notch-1, CD44, CD59, or TNF receptor. Notwithstanding, the cell may also be a non-diseased cell expressing one or more of these proteins.

In case of a cell showing an inflammatory phenotype, the underlying disease is an inflammatory disease, such as an Allergy, Asthma, Artherosclerosis, Autoimmune diseases, Autoinflammatory diseases, Celiac disease, Chronic prostatitis, Glomerulonephritis, Hypersensitivities, Inflammatory Bowel disease, Inflammatory myopathies, Obesity, Pelvic inflammatory disease, Reperfusion injury, Rheumatoid arthritis, Sarcoidosis, Transplant

rejection, Vasculitis, or Interstitial cystitis. In particular, a cell showing an inflammatory phenotype is a cell overexpressing one or more proinflammatory factors such as Bradykinin, C3, C5a, Factor XII, Membrance attack complex, Plasmin, Thrombin, Lysosome granules, Histamine, IFN-gamma, IL-8, IL-6, IL-8, IL-18, Leukotriene B4, Nitric oxide, Prostaglandins, TNF-alpha, or C-reactive Protein.

In case of a cell accumulating amyloid proteins, the underlying disease is a disease associated with the abnormal accumulation of amyloid fibrils such as Alzheimer's disease, Diabetes mellitus type 2, Parkinson's disease, Transmissible spongiform encephalopathy, Fatal familial insomnia, Huntington's disease, Medullary carcinoma of the thyroid, Cardiac arrythmias, Atherosclerosis, Rheumatoid arthritis, Aortic medial amyloid, Prolactinomas, Familial amyloid polyneuropathy, Hereditary non-neuropathic systemic amyloidosis, Dialysis related amyloidosis, Lattice corneal dystrophy, Cerebral amyloid angiopathy Cerebral amyloid angiopathy, Systemic AL amyloidosis, or Sporadic inclusion body myositis. In particular, a cell accumulating amyloid proteins is a cell overexpressing one or more amyloids such as Beta amyloid, IAPP, Alpha-synuclein, PrP^{Sc}, Huntingtin, Calcitonin, Atrial natriuretic factor, Apolipoprotein A1, Serum amyloid A, Medin, Prolactin, Transthyretin, Lysozyme, Beta-2 microglobulin, Gelsolin, Keratoepithelin, Cystatin, Immunoglobulin light chain AL, or S-IBM.

In case of a cell accumulating misfolded proteins, the underlying disease is a proteopathy such as Alzheimer's disease, Cerebral β-amyloid angiopathy, Retinal ganglion cell degeneration in glaucoma, Prion diseases, Parkinson's disease, Tauopathies, Frontotemporal lobar degeneration, FTLD–FUS, Amyotrophic lateral sclerosis, Huntington's disease, Familial British dementia, Familial Danish dementia, Hereditary cerebral hemorrhage with amyloidosis, CADASIL, Alexander disease, Seipinopathies, Familial amyloidotic neuropathy, Senile systemic amyloidosis, AL (light chain) amyloidosis, AH (heavy chain) amyloidosis, AA (secondary) amyloidosis, Type II diabetes, Aortic medial amyloidosis, ApoAI amyloidosis, ApoAII amyloidosis, ApoAIV amyloidosis, Familial amyloidosis of the Finnish type, Lysozyme amyloidosis, Fibrinogen amyloidosis, Dialysis amyloidosis, Inclusion body myositis/myopathy, Cataracts¸ Retinitis pigmentosa with rhodopsin mutations, Medullary thyroid carcinoma, Cardiac atrial amyloidosis, Pituitary prolactinoma, Hereditary lattice corneal dystrophy, Cutaneous lichen amyloidosis, Mallory bodies, Corneal lactoferrin amyloidosis, Pulmonary alveolar proteinosis, Odontogenic (Pindborg) tumor amyloid, Seminal vesicle amyloid, Cystic Fibrosis, Sickle cell disease, or Critical illness myopathy. Preferably, a cell accumulating misfolded proteins is a cell misfolding one or more proteins such as Amyloid β peptide (Aβ), Tau protein, Amyloid β peptide (Aβ), Amyloid β peptide (Aβ), Prion protein, α-Synuclein, Microtubule-associated protein tau (Tau protein), TDP-43, Fused in sarcoma (FUS) protein,

Superoxide dismutase, TDP-43, FUS, Proteins with tandem glutamine expansions, ABri, ADan, Cystatin C, Notch3, Glial fibrillary acidic protein (GFAP), Seipin, Transthyretin, Serpins, Monoclonal immunoglobulin light chains, Immunoglobulin heavy chains, Amyloid A protein, Islet amyloid polypeptide (IAPP; amylin), Medin (lactadherin), Apolipoprotein AI, Apolipoprotein AII, Apolipoprotein AIV, Gelsolin, Lysozyme, Fibrinogen, Beta-2 microglobulin, Amyloid β peptide (Aβ), Crystallins, Rhodopsin, Calcitonin, Atrial natriuretic factor, Prolactin, Keratoepithelin, Keratins, Keratin intermediate filament proteins, Lactoferrin, Surfactant protein C (SP-C), Odontogenic ameloblast-associated protein, Semenogelin I, cystic fibrosis transmembrane conductance regulator (CFTR) protein, Hemoglobin, or Hyperproteolytic state of myosin ubiquitination.

The term "cell receptor" is not limited to any particular receptor. For example it may be a G-protein coupled receptor, an ion channel or a cross-membrane transporter. Specific examples are CD3, CD4, CD8, CD28, CD16, and NKp46.

The cell signalling molecule may be a cytokine, such as a chemokine, an interferon, an interleukin, a lymphokine or a tumour necrosis factor, or a hormone or growth factor.

In a particular embodiment of the sixth aspect, the antibody or derivative thereof is multispecific, in particular bispecific, and further binds to an effector molecule, e.g. a cytotoxic substance or a receptor ligand.

Further targets the dimer or the antibody or derivative of the sixth aspect can bind to are the antigens described with respect to the second aspect above.

In another particular embodiment of the sixth aspect, the antibody or derivative thereof is multispecific, in particular bispecific, and binds to said pathogen, diseased cell or cell signalling molecule with a further variable region.

All terms used with respect to the sixth aspect have the meanings as defined with respect to the first, second, third, fourth and fifth aspect of the invention, unless specifically defined otherwise. Further, all embodiments specified for the first, second, third, fourth and fifth aspect that are applicable to the sixth aspect are also envisaged for the sixth aspect.

In a seventh aspect, the present invention relates to a method of isolating the antibody or derivative thereof of the second aspect, comprising the steps of

(i) providing a solution comprising a heavy chain A and a light chain A according to the first aspect and a heavy chain B and a light chain B as defined in the second aspect,

(ii) purifying the antibody or derivative without a means for selecting for the pairing of heavy chain A with light chain A and/or the pairing of heavy chain B with light chain B.

In particular, the solution may comprise

- the antibody or derivative of the second aspect,
- an antibody or derivative comprising two heavy chains A according to the first aspect, and
- an antibody or derivative comprising two heavy chains B as defined in the second aspect.

In particular, the solution does not comprise an antibody or derivative wherein light chain A is paired with heavy chain B and/or wherein light chain B is paired with heavy chain A.

In one embodiment, step (i) comprises expressing the one or more polynucleotides of the third aspect in a cell and optionally lysing the cell. The purifying of the antibody or derivative may include a means known in the art, for example physicochemical fractionation (e.g. differential precipitation, size-exclusion or solid-phase binding of immunoglobulins based on size, charge or other shared chemical characteristics of antibodies), class-specific affinity, or antigen-specific affinity.

In a particular embodiment, the antibody or derivative thereof is the antibody or derivative of the second aspect according to the "bivalent Fab configuration" or a symmetric antibody or derivative thereof of the second aspect (e.g. in the "tetravalent spider configuration") and the antibody or derivative is purified without a means for selecting for heavy chain pairing, e.g. a pairing of heavy chain A with heavy chain B.

In another particular embodiment, in which the heavy chains of the antibody or derivative thereof may comprise knob-into-hole mutations, the antibody or derivative thereof is the antibody or derivative of the second aspect has a Y-shaped IgG like form (including the "IgG configuration", the "TBTI configuration" and the "CODV configuration"), and the antibody or derivative is purified including a means for selecting for a pairing of heavy chain A with heavy chain B. In particular, the means for selecting for a pairing of heavy chain A with heavy chain B includes

- selecting for Protein A binding (e.g. by Protein A chromatography),
- selecting for the presence of a C_H1 domain or a C_L (or Cκ) domain (e.g. by Kappa Select chromatography), or for the absence of a CH2 domain, or

- selecting for Protein A binding (e.g. by Protein A chromatography), followed by selecting for the presence of a C_H1 domain or a C_L (or Cκ) domain (e.g. by Kappa Select chromatography), or for the absence of a CH2 domain.

In a particularly advantageous embodiment, wherein the Fc regions of heavy chain A and heavy chain B comprise knob-into-hole mutations and either the Fc region of heavy chain A or the Fc region of heavy chain B comprises one or more mutations abolishing the binding to Protein A, and wherein the heavy chain comprising the hole mutation of the knob-into-hole mutations comprises the one or more mutations abolishing the binding to Protein A, the method of the seventh aspect does not comprise selecting for the presence of a C_H1 domain or a C_L (or Cκ) domain (e.g. by Kappa Select chromatography), or for the absence of a CH2 domain. The omission of this selection is possible since the knob-into-hole approach allows for dimerization also of two "hole" heavy chains, but not for dimerization of two "knob" heavy chains. Therefore, if the "hole" heavy chain has the one or more mutations decreasing and in particular abolishing the binding to Protein A and it is selected with Protein A chromatography, one will select against the "hole" dimers and isolate only the bispecific antibody without any further steps.

All terms used with respect to the seventh aspect have the meanings as defined with respect to the first, second, third, fourth, fifth and sixth aspect of the invention, unless specifically defined otherwise. Further, all embodiments specified for the first, second, third, fourth, fifth and sixth aspect that are applicable to the seventh aspect are also envisaged for the seventh aspect.

Some exemplary antibodies are represented by amino acid sequences as follows:

A tetravalent spider antibody (see Fig. 1E) having
- light chains A according to SEQ ID NO: 3, wherein residues 1-113 are the variable domain A and may be substituted with a variable domain having a different specificity than the one exemplified,
- heavy chains according to SEQ ID NO: 4, wherein residues 1-120 are the variable domain A and may be substituted with a variable domain having a different specificity, and residues 239-358 are the variable domain B and may be substituted with a variable domain having a different specificity than the one exemplified, and
- light chains B according to SEQ ID NO: 5, wherein residues 1-107 are the variable domain B and may be substituted with a variable domain having a different specificity than the one exemplified.

A bispecific IgG1 antibody (see Fig. 1A) with MCH2 domains, having:
- a light chain A according to SEQ ID NO: 6, wherein residues 1-112 are the variable domain A and may be substituted with a variable domain having a different specificity than the one exemplified,
- a heavy chain A according to SEQ ID NO: 7, wherein residues 1-113 are the variable domain A and may be substituted with a variable domain having a different specificity than the one exemplified,
- a light chain B according to SEQ ID NO: 8, wherein residues 1-107 are the variable domain B and may be substituted with a variable domain having a different specificity than the one exemplified, and
- a heavy chain B according to SEQ ID NO: 9, wherein residues 1-118 are the variable domain B and may be substituted with a variable domain having a different specificity than the one exemplified.

A bispecific IgG1 antibody (see Fig. 1A) with MCH2 domains, having:
- a light chain A according to SEQ ID NO: 10, wherein residues 1-111 are the variable domain A and may be substituted with a variable domain having a different specificity than the one exemplified,
- a heavy chain A according to SEQ ID NO: 11, wherein residues 1-118 are the variable domain A and may be substituted with a variable domain having a different specificity than the one exemplified,
- a light chain B according to SEQ ID NO: 12, wherein residues 1-107 are the variable domain B and may be substituted with a variable domain having a different specificity than the one exemplified, and
- a heavy chain B according to SEQ ID NO: 13, wherein residues 1-123 are the variable domain B and may be substituted with a variable domain having a different specificity than the one exemplified.

A bivalent Fab (Fig. 1B) wherein $CH2_H$ is linked to V_HB instead of DIM_H, having
- a light chain A according to SEQ ID NO: 14, wherein residues 1-112 are the variable domain A and may be substituted with a variable domain having a different specificity than the one exemplified,
- a heavy chain according to SEQ ID NO: 15, wherein residues 1-121 are the variable domain A and may be substituted with a variable domain having a different specificity, and wherein residues 240-359 are the variable domain B and may be substituted with a variable domain having a different specificity than the one exemplified, and

- a light chain B according to SEQ ID NO: 16, wherein residues 1-113 are the variable domain A and may be substituted with a variable domain having a different specificity than the one exemplified.

A bivalent bispecific antibody, wherein two variable domains B are each part of a heavy chain and these heavy chains HC1 and HC2 are paired with each other (thereby pairing the variable domains B), and wherein heavy chain HC1 is linked to an MCH2 domain linked to a variable domain A and the MCH2 domain pairs with a further MCH2 domain which is linked to a variable domain A to form a light chain LC1 (thereby pairing the variable domains A), having

- a light chain LC1 according to SEQ ID NO: 17, wherein residues 1-112 are the variable domain A and may be substituted with a variable domain having a different specificity than the one exemplified,

a heavy chain HC1 according to SEQ ID NO: 18, wherein residues 1-121 are the variable domain A and may be substituted with a variable domain having a different specificity, and wherein residues 240-359 are the variable domain B and may be substituted with a variable domain having a different specificity than the one exemplified, and

- a light chain LC2 according to SEQ ID NO: 19, wherein residues 1-113 are the variable domain B and may be substituted with a variable domain having a different specificity than the one exemplified.

A CH2E-pseudo-CODV antibody (see Fig. 7A) having

- a chain 1 according to SEQ ID NO: 20, wherein residues 1-118 are the variable domain 1 and residues 120-242 are the variable domain 2 (both may be substituted with a variable domain having a different specificity than the one exemplified), and
- a chain 2 according to SEQ ID NO: 21, wherein residues 1-107 are the variable domain 2 and residues 115-225 are the variable domain 1 (both may be substituted with a variable domain having a different specificity than the one exemplified).

A pseudo hinge-CH2E-hybrid-Fab2 (see Fig. 8A) having

- a heavy chain A according to SEQ ID NO: 22, wherein residues 1-123 are the variable domain A and may be substituted with a variable domain having a different specificity than the one exemplified,
- a light chain A according to SEQ ID NO: 23, wherein residues 1-106 are the variable domain A and may be substituted with a variable domain having a different specificity than the one exemplified,

- a heavy chain B according to SEQ ID NO: 24, wherein residues 1-118 are the variable domain B and may be substituted with a variable domain having a different specificity than the one exemplified,
- a light chain B according to SEQ ID NO: 25, wherein residues 1-111 are the variable domain B and may be substituted with a variable domain having a different specificity than the one exemplified,

A bispecific IgG1 antibody (see Fig. 1A) with ECH2 domains, having:
- a light chain A according to SEQ ID NO: 26, wherein residues 1-110 are the variable domain A and may be substituted with a variable domain having a different specificity than the one exemplified,
- a heavy chain A according to SEQ ID NO: 27, wherein residues 1-123 are the variable domain A and may be substituted with a variable domain having a different specificity than the one exemplified,
- a light chain B according to SEQ ID NO: 28, wherein residues 1-111 are the variable domain B and may be substituted with a variable domain having a different specificity than the one exemplified, and
- a heavy chain B according to SEQ ID NO: 29, wherein residues 1-118 are the variable domain B and may be substituted with a variable domain having a different specificity than the one exemplified.

A trivalent antibody (see Fig. 1F) with MCH2 domains, having:
- a light chain A according to SEQ ID NO: 30, wherein residues 1-111 are the variable domain A and may be substituted with a variable domain having a different specificity than the one exemplified (anti-IL13-(huzdBB13)-VL-huMCH2),
- a heavy chain A according to SEQ ID NO: 31, wherein residues 1-118 are the variable domain A and may be substituted with a variable domain having a different specificity than the one exemplified (anti-IL13-(huzdBB13)-VH-huMCH2-huIgG1-Fc (hole-RF)),
- a light chain B according to SEQ ID NO: 32, wherein residues 118-224 are the variable domain B and residues 1-107 are the variable domain Y, and both may be substituted with variable domains having a different specificity than the ones exemplified (CODV-anti-IL4-(huzd8D4-8)-VL--anti-TNFalpha-(Adalimumab)-VL--huIGLC2), and
- a heavy chain B according to SEQ ID NO: 33, wherein residues 1-121 are the variable domain B and residues 122-244 are the variable domain Y, and both may be substituted with variable domains having a different specificity than the ones exemplified (CODV-anti-TNFalpha-(Adalimumab)-VH-anti-IL4-(huzd8D4-8)-VH-huIGHG1(knob)). This antibody is trispecific, but

variable domains can be substituted also in a way making the antibody bispecific or monospecific.

Instead of the above chains, variants thereof may be used. A variant has an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence it is derived from, for example SEQ ID NO: 3 to 33. The determination of percent identity between two sequences is accomplished as described above. Also, in all the above examples, the MCH2/ECH2 domains can be swapped with the DIM/C_H1C_k domains, as applicable.

In the following figures and examples, some particular embodiments of the invention are described in more detail. Yet, no limitation of the invention is intended by the details of the particular embodiments. In contrast, the invention pertains to any embodiment which comprises details which are not explicitly mentioned in the embodiments herein, but which the skilled person finds without undue effort.

Description of the figures:

Figure 1: Examples of antibody or derivative configurations facilitated by the invention. Different dimerization domains ensure correct pairing of the variable domains. A: IgG configuration (linkers not shown except Fc-Fab linking by the hinge region), B: Bivalent Fab configuration (linkers not shown except Fab-Fab linker; this linker may alternatively link $CH2_H$ with DIM_L, $CH2_L$ with DIM_H or $CH2_L$ with DIM_L), C: TBTI configuration (linkers not shown except Fc-Fab linking by the hinge region), D: CODV configuration showing two possible linker configurations on the left and on the right arm (both arms can also have the same linker configuration, i.e. that of the left or of the right arm shown), E: Tetravalent spider configuration (linkers not shown except Fc-Fab linking by the hinge region and Fab-Fab linkers), F: trivalent configuration (example for the CODV chimera configuration) containing three binding sites allowing for mono-, bi- and tri-specificity. The right arm shown is a CODV arm having the linker configuration of the right arm of the CODV configuration of Fig. 1 D, but it may instead also have the linker configuration of this CODV configuration. The left arm is a conventional IgG arm. Left and right arms are exchangeable, i.e. the left arm can be the CODV arm and the right arm the IgG arm. Further, the MCH2 domains are shown to be in the IgG arm, but they can instead be in the CODV arm (independent of its linker configuration) with the DIM domains then being in the IgG arm.

Figure 2: Schematic representation of the architecture of the asymmetric antibody. A: On one half of the antibody like structure the CH1/kappa region is replaced by the MCH2 domains. The Fc of the Fab containing half is chimeric, harbouring the CH3 from IgG3 whereas all other Fc domains are of IgG1 origin. A two step purification including protein A and kappa select chromatography allows the isolation of the correct assembled heterodimeric molecule. B: SDS-PAGE showing the chain composition under reducing conditions. C: SEC profile of the purified heterodimeric antibody.

Figure 3: Schematic representation of the architecture of the asymmetric antibody. A: On one half of the antibody like structure the CH1/kappa region is replaced by the MCH2 domains. The heterodimerization is driven by knobs-into-holes mutations within the Fc parts. The Fc part with the hole mutations further contains the RF mutation. A two step purification including protein A and kappa select chromatography allows the isolation of the correct assembled heterodimeric molecule. B: SDS-PAGE showing the chain composition under reducing conditions. C: SEC profile of the purified heterodimeric antibody.

Figure 4: Tetravalent spider configuration. A: Schematic representation of the symmetric IgG like structure. A single step protein A chromatography allows the isolation of the correctly assembled homodimeric molecule. B: SDS-PAGE showing the chain composition under reducing conditions. C: SEC profile of the purified heterodimeric antibody. D and E: Biacore sensograms showing binding to the corresponding antigens.

Figure 5: Schematic representation of the architecture of the asymmetric antibody. A: On one half of the antibody like structure the CH1/kappa region is replaced by the ECH2 domains. The heterodimerization is driven by knobs-into-holes mutations within the Fc parts. The Fc part with the knob mutations further contains the RF mutation. B: SDS-PAGE showing the chain composition under reducing conditions. C: SEC profile of the purified protein. D: Biacore sensograms showing binding to IL4 and IL13.

Figure 6: Bivalent Fab configuration. A: schematic representation of the bivalent bispecific Fab fragment. B: SEC profile of the purified protein. C and D: Biacore sensograms showing binding to CD3 and CD123 respectively.

Figure 7: CODV bivalent Fab configuration (EC_H2). A: schematic representation of the protein architecture. B: SEC profile of the purified protein. C: Biacore sensograms showing binding to both IL4 and IL13.

Figure 8: F(ab')2 like configuration. A: schematic representation of the bivalent bispecific F(ab')2 like fragment. B: SEC profile of the purified heterodimeric antibody. C:SDS-PAGE showing the chain composition under reducing conditions. D: Biacore sensograms showing binding to IL4 and IL13.

Description of the examples:

Material & Methods

Generation of expression plasmids

All described constructs were built from gene synthesis fragments (GeneArt), cleaved with restriction enzymes and ligated into mammalian expression vector pXL. The vector was used to transform *E. coli* and clones were selected and amplified to isolate DNA for transient transfection using Qiagen Kits. The DNA sequence was verified by sequencing of the relevant ORF and sequence context. The resulting coding ORFs are in accordance with the before listed and described sequences.

Expression of recombinant proteins

All protein production campaigns were realized via transient expression. Freestyle HEK293 (Life) cells growing in F17 serum free suspension culture (Life) were transfected with the expression plasmid. Transfections were performed using Cellfectin transfection reagent (Life). The cells were cultured at 37°C for 7 days at 8% CO_2 in shaker flasks. The culture supernatant containing recombinant protein was harvested by centrifugation and was clarified by filtration (0.22µm). Recombinant IgG1 constructs were purified by affinity chromatography on Protein A (HiTrap Protein A HP Columns, GE Life Sciences). After elution from the column with 100 mM acetate buffer and 100 mM NaCl, pH 3.5, the CODV-IgG1 constructs were desalted using HiPrep 26/10 Desalting Columns, formulated in PBS at a concentration of 1 mg/mL and filtered using a 0.22 µm membrane. Fab-like constructs were purified by IMAC on HiTrap IMAC HP Columns (GE Life Sciences). After elution from the column with a linear gradient (Elution buffer: 20 mM sodium phosphate, 0.5 M NaCl, 50 - 500 mM imidazole, pH 7.4), the protein containing fractions were pooled and desalted using HiPrep 26/10 Desalting Columns, formulated in PBS at a concentration of 1 mg/mL and filtered using a 0.22 µm membrane. For purification of asymmetric heterodimeric constructs the protein samples were additionally applied on His-Trap column (GE) and/or captured by Kappa select (GE) after capture on protein A and desalting step. The protein was polished by SEC using a Superdex 200 (GE). After a final ultrafiltration concentration step the protein was used for different assays. This strategy was used to isolate heterodimers from homodimers. Protein concentration was determined by

measurement of absorbance at 280 nm. Each batch was analyzed by SDS-PAGE under reducing and non-reducing conditions to determine the purity and molecular weight of each subunit and of the monomer.

Characterization of MCH2 and ECH2 Variants

To determine whether the MCH2 and ECH2 antibody-like protein heavy and light chains were pairing and folding properly, the aggregation level was measured by analytical size-exclusion chromatography (SEC). Analytical SEC was performed on assembled pairs using an ÄKTA explorer 10 (GE Healthcare) equipped with a TSKgel G3000SWXL column (7.8mm x 30cm) and TSKgel SWXL guard column (Tosoh Bioscience). The analysis was run at 1ml/min. using 250 mM NaCl, 100 mM Na-phosphate, pH 6.7, with detection at 280 nm. 30 µl of protein sample (at 0.5-1 mg/ml) were applied onto the column. For estimation of the molecular size, the column was calibrated using a gel filtration standard mixture (MWGF-1000, SIGMA Aldrich). Data evaluation was performed using UNICORN software v5.11.

Binding analysis by SPR

Two pairs of heavy and light chains were selected for full kinetic analysis. Recombinant human IL13 and IL4 was purchased from Chemicon (USA). Kinetic characterization of purified antibodies was performed using surface plasmon resonance technology on a BIACORE 3000 (GE Healthcare). A capture assay using a species specific antibody (e.g., human-Fc specific MAB 1302, Chemicon) for capture and orientation of the investigated antibodies was used. The capture antibody was immobilized via primary amine groups (11000 RU) on a research grade CM5 chip (GE Life Sciences) using standard procedures. The analyzed antibody was captured at a flow rate of 10 µL/min with an adjusted RU value that would result in maximal analyte binding of 30 RU. Binding kinetics were measured against recombinant human IL4 and IL13 over a concentration range between 0 to 25 nM in HBS EP (10 mM HEPES, pH 7.4, 150 mM NaCl, 3 mM EDTA, and 0.005 % Surfactant P20) at a flow rate of 30 µl/min. Chip surfaces were regenerated with 10 mM glycine, pH 2.5. Kinetic parameters were analyzed and calculated in the BIAevaluation program package v4.1 using a flow cell without captured antibody as reference.

"Redirected" cell killing

Peripheral blood mononuclear cells (PBMCs) were isolated from 200 ml peripheral blood of healthy donors treated with EDTA by Ficoll density centrifugation. 15 ml Histopaque (Sigma-Aldrich) was preloaded on a 50 ml Leucosep-Tube (Greiner bio-one). Blood was diluted with autoMACS Rinsing Buffer + 1% BSA (Miltenyi Biotec) and loaded on the membrane of a total of ten prepared tubes. Tubes were centrifuged without brake for 10 min at 1000 xg. PBMCs

were collected and washed with autoMACS Rinsing Buffer + 1% BSA three times. Finally, PBMCs were resuspended in autoMACS Running Buffer (Miltenyi Biotec) for isolation of T lymphocytes by autoMACSpro technology using the Pan T Cell isolation Kit (Miltenyi Biotec) according to manufacturer's instructions. Purity of separated T cells was analyzed by MACSQuant flow cytometry using the human 7-Color Immunophenotyping Kit (Miltenyi Biotec). T-cell engaging effect of bispecific antibodies was analyzed by a flow cytometry based cytotoxic assay. Target cells (i.e. THP-1 cell line) were stained for 15 min at 37°C with 1 µM CFSE in 1 ml RPMI + GlutaMAX I (Gibco) per 1E7 cells. Afterwards, cells were washed twice and resuspended in RPMI + GlutaMAX I + 10% FCS (Invitrogen). 2.5E4 target cells were seeded in 96-well U-bottom suspension culture plates (Greiner bio-one) in 50 µl medium per well. Isolated primary human T lymphocytes were resuspended in RPMI + GlutaMAX I + 10% FCS and were added at indicated effector-to-target ratio in 50 µl per well to the target cells (in general E:T=10:1). Bispecific antibodies were diluted 1:3 in serial in PBS (Invitrogen) and 5 µl each were added to the cells at a final maximum concentration of 3000 ng/ml. The assay was incubated for 20 h at 37°C in 5% CO2. To detect dead target cells, all cells were stained with 7-AAD. Therefore, 5 µg/ml 7-AAD diluted in Stain Buffer with FBS (BD Pharmingen) were added to each well and were incubated for 15 min at 4°C in the dark. Cells were measured using the MACSQuant (Miltenyi Biotec) or LSRII (BD) flow cytometer, respectively. Further data analyses were performed using the FlowJo software (Tree Star, Inc.). Read out was percentage of CFSE and 7-AAD double positive cells. The results of these investigations demonstrate the ability of the CD123xCD3 Fabs to mediate redirected killing of tumor cells.

Example 1: IgG configuration (MC_H2), RF mutations

As described in the Material & Methods section, an antibody in the IgG configuration comprising an IgG3-CH3 domain in one Fc chain and an IgG1-CH3 domain in the other and variable domains being IL13 and IL4 was generated, wherein the $CH2_H$ and $CH2_L$ domains are an IgM constant domain MC_H2 (see Fig. 2A). It was purified with the following steps:

1. Protein A chromatography
2. Kappa select chromatograpy
3. Desalting on HiPrep 26/10

The yield was 4 mg/l. SDS-PAGE on NuPAGE® Novex® 4-12% Bis-Tris (Fig. 2B) showed the expected number and size of fragments under reducing conditions. SEC analysis of the purified protein showed a monomer level of 96% (Fig. 2C). Biacore analysis showed binding to the corresponding antigens and binding kinetics as expected from the parental antibodies (Table 2).

Table 2: Binding kinetics of the purified bispecific antibody against IL4 and IL13

Analyte	ka (1/Ms)	Kd (1/s)	KD (M)
IL13	1,49E+06	8,21E-05	5,50E-11
IL4	1,51E+08	2,53E-04	1,68E-12

Example 2: IgG configuration (MC_H2), RF and knob-into-hole mutations

As described in the Material & Methods section, an antibody in the IgG configuration comprising mutations H435R and Y436F (RF mutations) in one CH3 domain as well as know-into-hole mutations and variable domains binding IL13 and IL4 was generated (see Fig. 3A). It was purified with the following steps:

1. Protein A chromatography
2. Kappa select chromatograpy
3. Desalting on HiPrep 26/10

The yield was with 10 mg/l more than double as high as for the variant described in example 1, indicating a higher efficiency of correct chain association driven by the knob-into-hole modification of the Fc fragments. SDS-PAGE on NuPAGE® Novex® 4-12% Bis-Tris (Fig. 3B) showed under reducing conditions the expected number and size of fragments SEC analysis of the purified protein showed a monomer level of 96% (Fig. 3C). Biacore analysis showed binding to the corresponding antigens and binding kinetics as expected from the parental antibodies.

Example 3: Tetravalent spider configuration

As described in the Material & Methods section, an antibody in the Tetravalent spider configuration (see Fig. 4A) was generated. It was purified with the following steps:

1. Protein A chromatography
2. Desalting on HiPrep 26/10

The yield was 5 mg/l. SDS-PAGE on NuPAGE® Novex® 4-12% Bis-Tris (Fig. 4B) showed all expected fragments with the expected size under non reducing conditions. SEC analysis showed a monomer level of 98% (Fig. 4C). Biacore analysis (Fig. 4D and E) showed binding against the corresponding antigens.

Example 4: IgG configuration (EC_H2), RF and knob-into-hole mutations

This bivalent bispecific antibody (see Fig. 5A) contains a CH2 domain of human IgE (Uniprot P01854) from amino acid position 104 to 210. The domain contains mutation of cysteine 105 to alanine to avoid unwanted cys reactivity and mutation of asparagine 146 to glutamine to

avoid glycosylation. In the following examples called “CH2E”.To fuse the CH2E part to the HC of the construct, a flexible linker (GSGSGS) was introduced.

Example using variable domains “*huBB13*” and “*hu8D4-8*” recognizing antigens: IL4 and IL13 described before (US 20100226923 A1) composed in an IgG like structure consisting of two different Fabs each representing a different target VD fused to a modified IgG1-Fc domain. To enhance the expression and formation of heterodimers KIH (knob into hole) mutations were introduced into the CH3 domains. L234A, L235A mutations (Hezareh et al. 2001, J. Virol., 75: 12161) were incorporated to prevent effector function of the Fc backbone. The ECH2 domain is replacing CH1/kappa.

The antibody comprises the chains

anti-IL4(hu8D4-8-VL1)-CL1gk

anti-IL4(hu8D4-8-VH1)-CH1g-Fc(IgG1 LALA knob RF)

anti-IL13-VL(huBB13-VL3)–CH2e

anti-IL13-(huBB13-VH2)–CH2e-Fc(IgG1 LALA hole)

A two-step affinity chromatography was performed to ensure enrichment of heterodimeric chain pairs. The protein was captured by *HiTrap Protein A 5ml (GE Healthcare)* and eluted by pH shift. Protein fractions were collected and buffer was instantly exchanged to PBS by a *HiPrep 26/10 Desalting 53ml desalting column (GE Healthcare)*. The protein solution was applied to *HiTrap KappaSelect 5ml (GE Healthcare)* and eluted via pH shift. Protein containing fractions were collected and buffer was instantly exchanged to PBS by a *HiPrep 26/10 Desalting 53ml desalting column (GE Healthcare).* Fractions were pooled and sample volume was reduced via ultrafiltration. The protein was polished by SEC using a *Superdex* 200 (*GE Healthcare*) column. After a final ultrafiltration and 0.22 µm filtration step the protein solution was used for further assays.

The yield after purification was 18 mg/l. SDS-PAGE on NuPAGE® Novex® 4-12% Bis-Tris (Fig. 5B) showed the expected number and size of fragments under reducing conditions. SEC analysis of the purified protein showed a monomer level of ~99% (Fig. 5C). Biacore analysis showed binding to the corresponding antigens IL4 and IL13 (Fig. 5D).

Example 5: Bivalent Fab configuration

As described in the Material & Methods section, an antibody in Bivalent Fab configuration was generated, wherein the two Fabs are linked via a linkage of a MCH2 domain of one Fab-like fragment (CD3) to a variable domain of the other Fab (CD123), see Figure 6A. The yield was 7 mg/l. SEC analysis showed a monomer level of 97% (Fig. 6B). Biacore analysis showed

binding to the corresponding antigens (Fig. 6C and D) and binding kinetics as expected from the parental antibodies (Table 3)

Table 3: Binding kinetics of the prurified bispecific bivalent Fab against CD3 and CD123

Analyte	ka (1/Ms)	Kd (1/s)	KD (M)
CD123	4.5E+05	3.77E-05	8.38E-11
CD3	1.581E+05	9.00E-04	5.71E-09

Example 6: CODV bivalent Fab configuration (EC_H2)

The bispecific F-like fragment (see Fig. 7A) contains a CH2 domain of human IgE (Uniprot P01854) from amino acid position 104 to 210. The domain contains mutation of cysteine 105 to alanine to avoid unwanted cys reactivity and mutation of asparagine 146 to glutamine to avoid glycosylation. In the example variable domains "*huBB13*" and "*hu8D4-8*" are used recognizing antigens IL4 and IL13 described before (US 20100226923 A1). The molecule is composed in the CODV format. In this example the ECH2 domain is replacing the CH1 and CL domains usually located at the C-terminal end of both chains to connect HC and LC via disulfide bridges. A 8x histidine tag was added for purification.

The antibody comprises 2 unique chains:

Chain 1: anti-IL13(huBB13-VH2)-anti-IL4(hu8D4-8-VH1)-ECH2-His

Chain 2: anti-IL4(hu8D4-8-VL1)-anti-IL13(huBB13-VL2)-ECH2

The His-tagged protein was captured on *HisTrap High Performance 5ml(GE Healthcare)* and eluted by an imidazole gradient. The protein was polished by SEC using a *Superdex* 200 (*GE Healthcare*) By a final ultrafiltration concentration step the protein was concentrated and used for further assays. Fig. 7B shows the SEC profile of the Fab-like fragment after purification. Biacore analysis showed binding of both IL4 and IL13 (Fig. 7C).

Example 7: F(ab')2 like configuration (EC_H2 replacing the hinge region)

This F(ab')2-like antibody (see Fig. 8A) contains a CH2 domain of human IgE (Uniprot P01854) from amino acid position 104 to 210. The domain contains mutation of cysteine 105 to alanine to avoid unwanted cys reactivity and mutation of asparagine 146 to glutamine to avoid glycosylation. Also in this example an inclusion of flexible linker connecting the CH2E domain on the heavy chain was introduced. Example using variable domains "*huBB13*" and "*hu8D4-8*" recognizing antigens IL4 and IL13 described before (US 20100226923 A1) composed in a Fab_2 like format. In this example the ECH2 domain is replacing the hinge region to connect two Fab modules via HC parts. Generation of a bispecific molecule is done via hetero-dimerization of two different Fabs representing two separate target specifities. One Fab arm

contains a MCH2 domain the other corresponds to a native Fab structure. In this example anti-IL4 VD is connected to a CH1/kappa domain and the anti-IL13 VD is fused to a modified MCH2 domain. An 8x histidine tag was added at the Cter of one ECH2 domain for purification.

The antibody comprises 4 unique chains:

anti-IL4 (hu8D4-8-VH1)-CH1g-CH2e

anti-IL4 (hu8D4-8-VL1)-Ck

anti-IL13 (huBB13-VH2)-MCH2-ECH2-8xHis

anti-IL13 (huBB13-VL3)-MCH2

A two-step affinity chromatography was performed to ensure enrichment of heterodimeric chain pairs. The protein was captured by *HiTrap KappaSelect 5ml (GE Healthcare)* and eluted following manufacturer's instructions by pH shift. By a desalting column the buffer was immediately exchanged to PBS. After the protein solution was applied to *HisTrap High Performance* 5ml (GE Healthcare) and eluted by an imidazole gradient. Afterwards the protein containing fractions were pooled and further polished by SEC using a Superdex 200 (*GE Healthcare*). After a final ultrafiltration concentration step the protein was used for further assays. The SEC profile after purification revealed a monomeric fraction of ~99% (Fig. 8B). A reducing SDS PAGE (Novex 4-12% Bis-Tris) showed the expected 4 fragment sizes (Fig. 8C). The construct is able to bind both IL4 and IL13 as demonstrated in Biacore analysis (Fig. 8D).

Claims:

1. An antibody heavy chain A, light chain A or dimer thereof, wherein the heavy chain comprises a variable domain V_HA linked to a dimerization domain $CH2_H$ and the light chain comprises a variable domain V_LA linked to a dimerization domain $CH2_L$, and wherein both $CH2_H$ and $CH2_L$ are an IgM constant domain MC_H2 or an IgE constant domain EC_H2.

2. An antibody or derivative thereof, comprising a heavy chain A and a light chain A according to claim 1.

3. The antibody or derivative of claim 2, further comprising a heavy chain B and a light chain B, wherein the heavy chain B comprises a variable domain V_HB and the light chain comprises a variable domain V_LB, and wherein the antibody or derivative preferably is multispecific.

4. The antibody or derivative of claim 3, comprising a first and a second heavy chain A and a first and a second light chain A each as defined in claim 1, wherein
(i) the $CH2_L$ domain of the first light chain A is linked to the V_H1 or V_L1 domain of an IgG antibody or derivative thereof and the $CH2_L$ domain of the second light chain A is linked to the other V_H1 or V_L1 domain of the IgG antibody or derivative thereof, or
(ii) the $CH2_H$ domain of the first light chain A is linked to the V_H1 or V_L1 domain of an IgG antibody or derivative thereof and the $CH2_H$ domain of the second light chain A is linked to the other V_H1 or V_L1 domain of the IgG antibody or derivative thereof.

5. The antibody or derivative of any one of claims 2 to 3, wherein the antibody or derivative is asymmetric, and wherein preferably V_HB is linked to a dimerization domain DIM_H and V_LB is linked to a dimerization domain DIM_L, wherein DIM_H and DIM_L are dimerization domains different from $CH2_H$ and $CH2_L$, respectively.

6. The antibody or derivative of claim 5, wherein V_HB is linked to a dimerization domain DIM_H and V_LB is linked to a dimerization domain DIM_L, wherein DIM_H and DIM_L are dimerization domains different from $CH2_H$ and $CH2_L$, respectively, and wherein
(i) DIM_H is linked to $CH2_H$ or $CH2_L$, or DIM_L is linked to $CH2_H$ or $CH2_L$,
(ii) $CH2_H$ is linked via a hinge region to a constant domain C_H2A, DIM_H is linked via a hinge region to a constant domain C_H2B, and optionally C_H2A is linked to a constant domain C_H3A and C_H2B is linked to a constant domain C_H3B, or

(iii) further to (ii), heavy chain A further comprises a further heavy chain variable domain V_HX linked to V_HA and light chain A further comprises a further light chain variable domain V_LX linked to V_LA; and/or heavy chain B further comprises a further heavy chain variable domain V_HY linked to V_HB and light chain B further comprises a further light chain variable domain V_LY linked to V_LB.

7. The antibody or derivative of any one of claims 5 to 6, wherein the antibody or derivative thereof comprises one or more modifications facilitating the isolation of the antibody, wherein the one or more modifications preferably facilitate the pairing of heavy chain A with heavy chain B or allow for selecting for this pairing, and wherein the modifications are preferably one or more of:

- a T366Y mutation and optionally further an S354C and T166W mutation in one heavy chain, and a Y407T mutation and optionally further a Y349C, T366S, L368A and Y407V mutation in the other heavy chain,
- a T366W mutation in one heavy chain, and a T366S, L368A and Y407V mutation in the other heavy chain,
- a F405L mutation in one heavy chain, and a K409R mutation in the other heavy chain,
- a T350V, L351Y, F405A and Y407V mutation in one heavy chain, and a T350V, T366L, K392L and T394W mutation in the other heavy chain,
- a K409D and K392D mutation in one heavy chain, and a D399K and E356K mutation in the other heavy chain,
- a D221E, P228E and L368E mutation in one heavy chain, and a D221R, P228R and K409R mutation in the other heavy chain,
- a S364H and F405A mutation in one heavy chain, and a Y349T and T394F mutation,
- an IgG/A chimera for one heavy chain, and an IgA/G chimera for the other heavy chain,
- an Fc region or part thereof of one heavy chain from IgG3, and an Fc region or part thereof of the other heavy chain from IgG1, IgG2 or IgG4,
- a H435R and Y436F mutation in one heavy chain, and a T407T mutation in the other heavy chain, and/or
- a H435R mutation in one heavy chain, and no mutation in the other heavy chain.

8. The antibody or derivative of claim 7, wherein the heavy chains comprise knob-into-hole mutations and one or more mutations decreasing the binding to Protein A, and wherein preferably the heavy chain comprising the hole mutation(s) of the knob-into-hole mutations comprises the one or more mutations decreasing the binding to Protein A.

9. One or more polynucleotides encoding for the heavy chain A and/or the light chain A of claim 1 or for the antibody or derivative of any one of claims 2 to 8.

10. One or more expression vectors comprising the one or more polynucleotides of claim 9.

11. A cell comprising the one or more polynucleotides of claim 9 or the one or more expression vectors of claim 10.

12. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a dimer of the heavy chain A and the light chain A of claim 1 or the antibody or derivative of any one of claims 2 to 8.

13. A method of isolating the antibody or derivative thereof of the second aspect, comprising the steps of

(i) providing a solution comprising a heavy chain A and a light chain A as defined in claim 1 and a heavy chain B and a light chain B as defined in claim 2,

(ii) purifying the antibody or derivative without a means for selecting for the pairing of heavy chain A with light chain A and/or the pairing of heavy chain B with light chain B.

14. The method of claim 13, wherein the antibody or derivative is purified with a means for selecting for a pairing of heavy chain A with heavy chain B, and wherein the means preferably is

a) selecting for Protein A binding,

b) selecting for the presence of a C_H1 domain or a Cκ domain, or for the absence of a CH2 domain, or

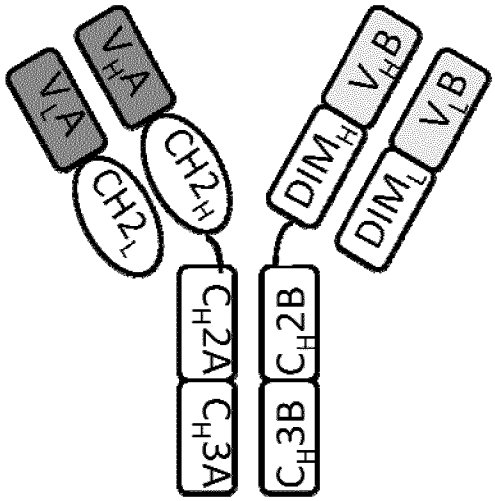
c) a combination of a) and b), preferably in that order.

15. The method of claim 13, wherein the antibody or derivative thereof is that of claim 7 wherein the heavy chain comprising the hole mutation(s) of the knob-into-hole mutations comprises the one or more mutations decreasing the binding to Protein A, and wherein the antibody or derivative is purified by selecting for Protein A binding, characterized in that the method does not comprise selecting for the presence of a C_H1 domain or a Cκ domain, or for the absence of a CH2 domain.

Figures:

Figure 1

A



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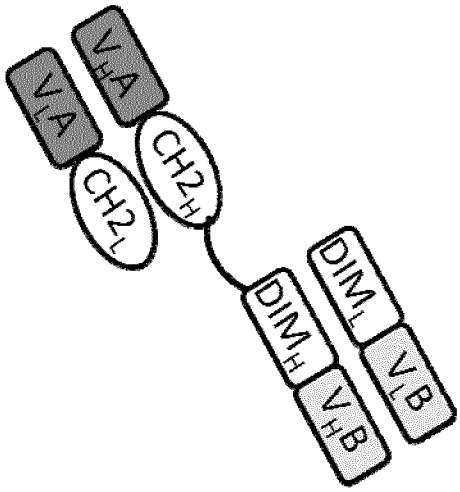

Figure 1 (continued)

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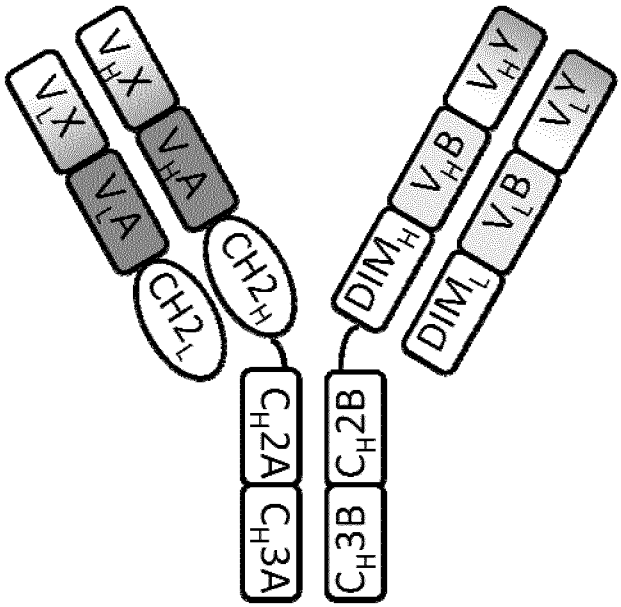

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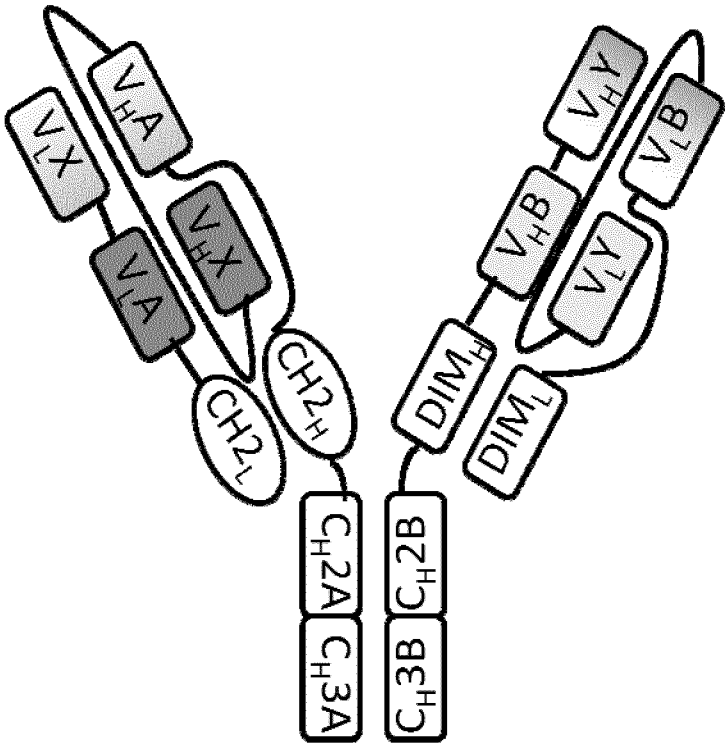

Figure 1 (continued)

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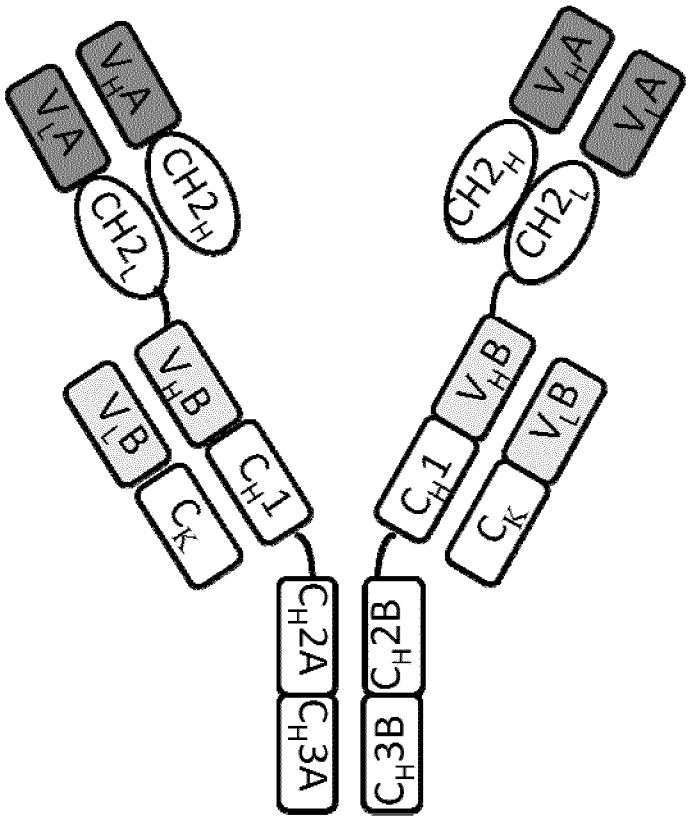


Figure 1 (continued)

F

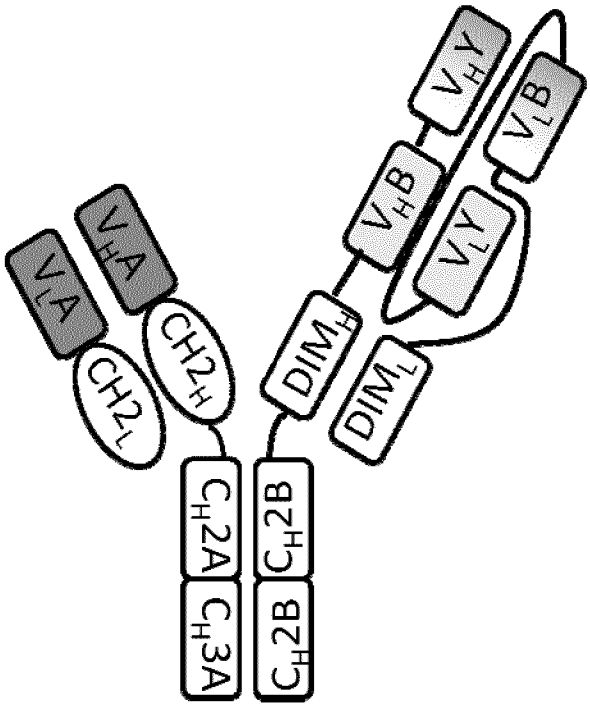
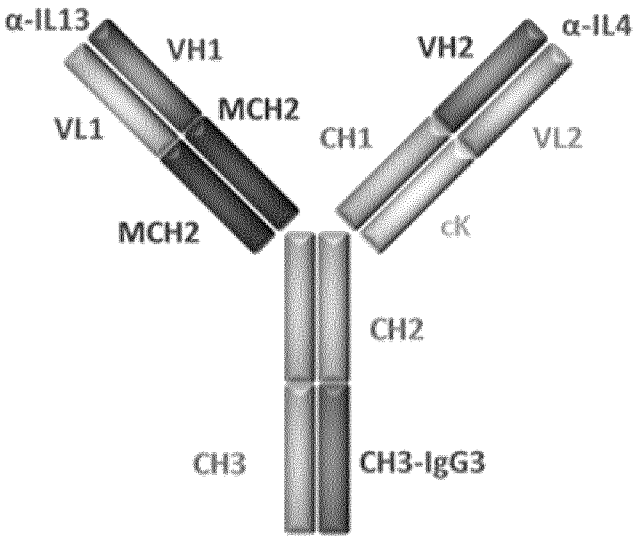
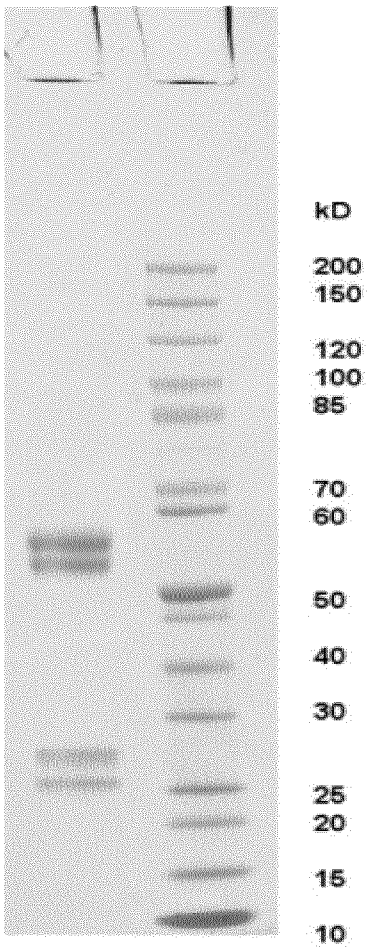


Figure 2

A


α-IL13
VH1
MCH2
VL1
MCH2
VH2
α-IL4
CH1
VL2
cK
CH2
CH3
CH3-IgG3

B


kD
200
150
120
100
85
70
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20
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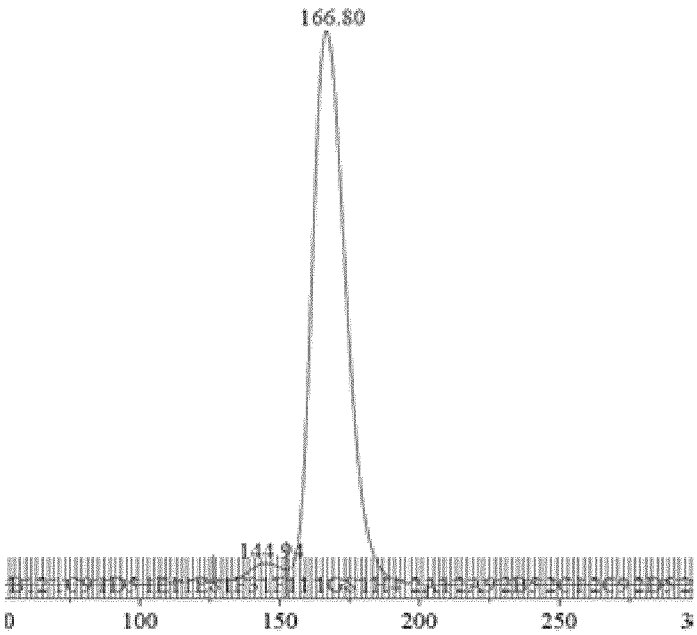
10

C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200
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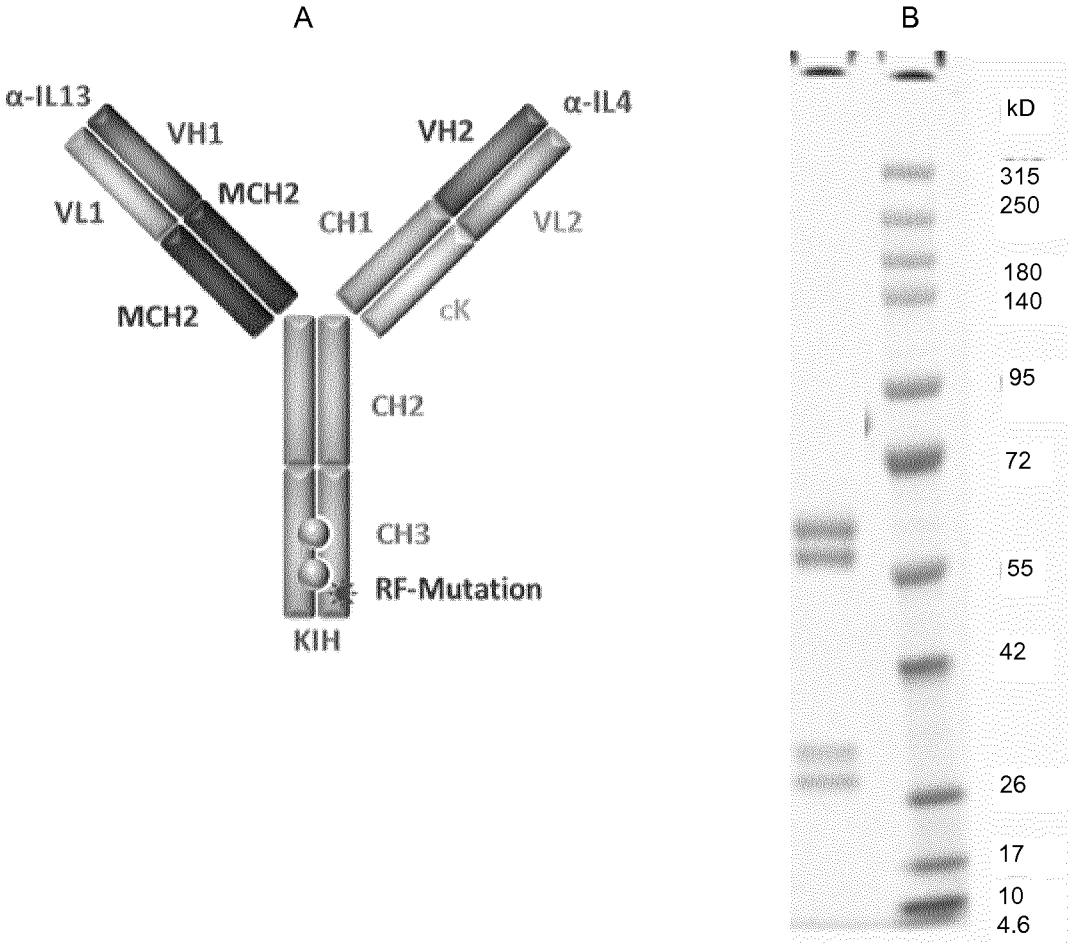
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VL1
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VH2
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VL2
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CH2
CH3
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KIH
B
kD
315
250
180
140
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17
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C
166.80
141.94
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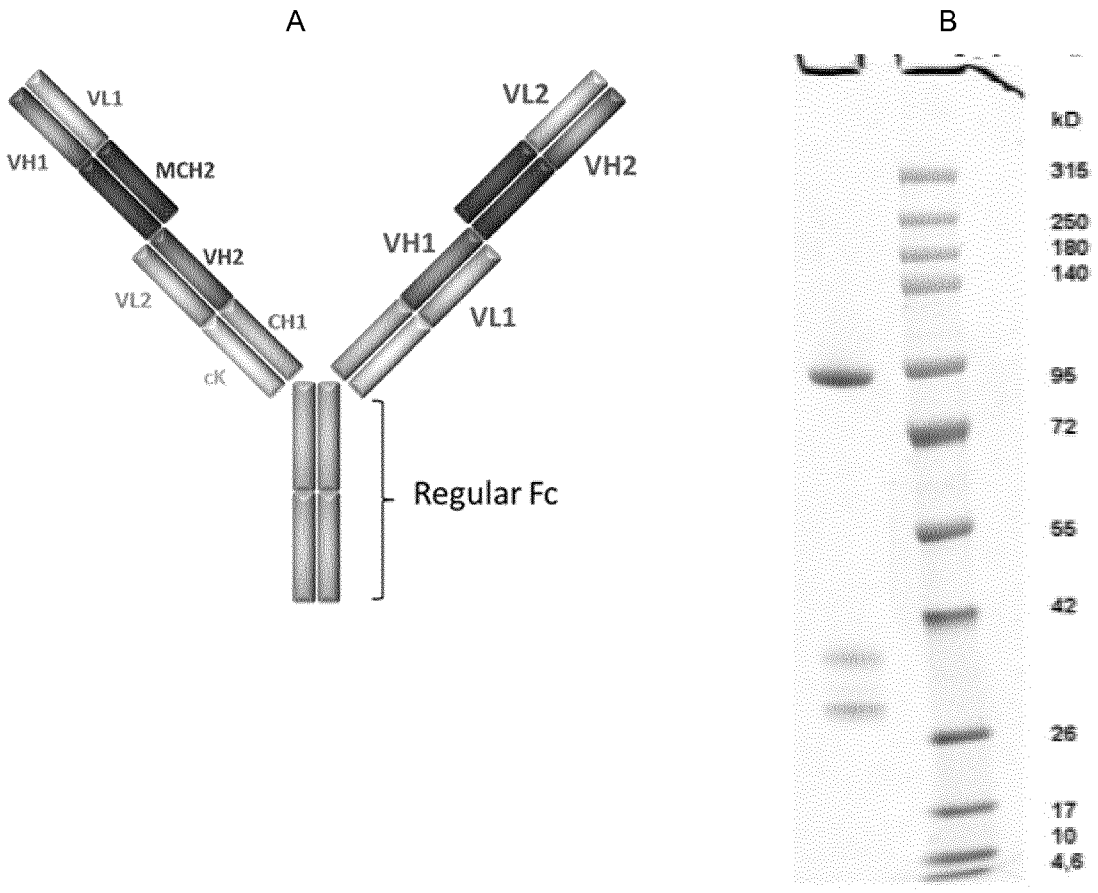
100
150
200

Figure 4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VH1
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VL2
CH1
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VL2
VH2
VH1
VL1
Regular Fc
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kD
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180
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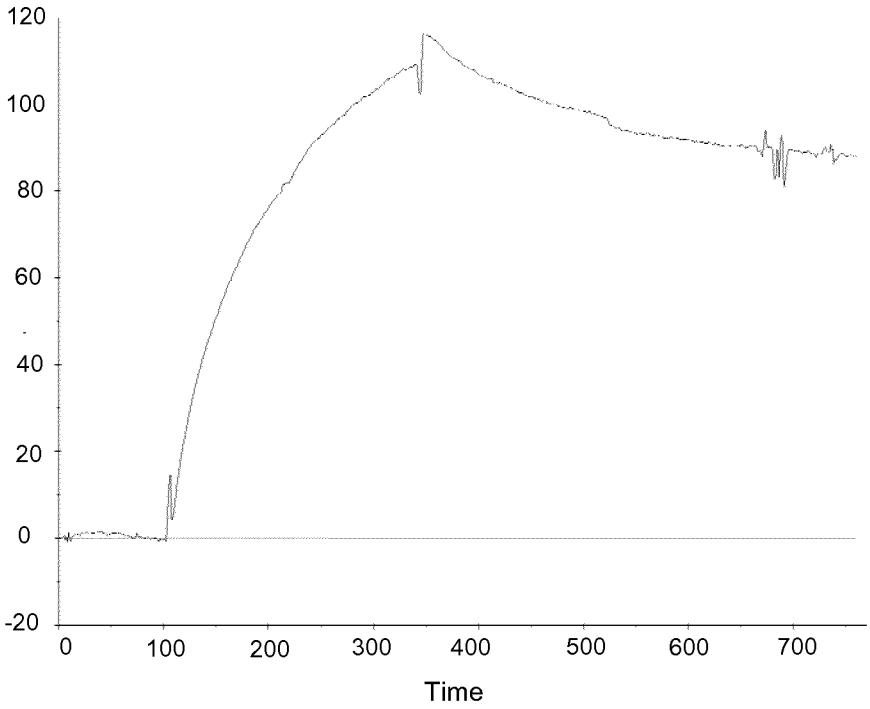
72
55
42
26
17
10
4,6
C
154.50
100
150
200
250

D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80
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40
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0
-20
0
100
200
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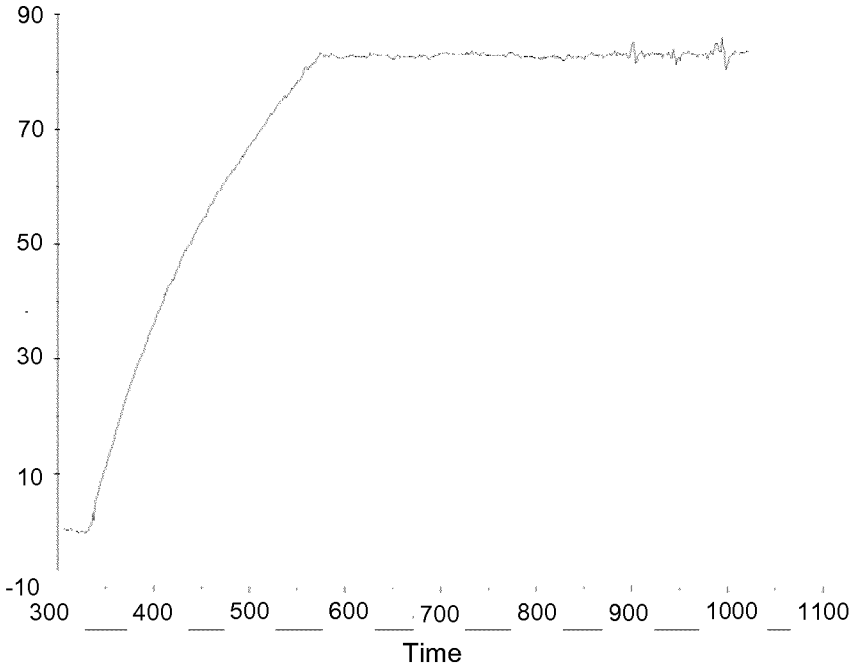
400
500
600
700
Time

E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50
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-10
300
400
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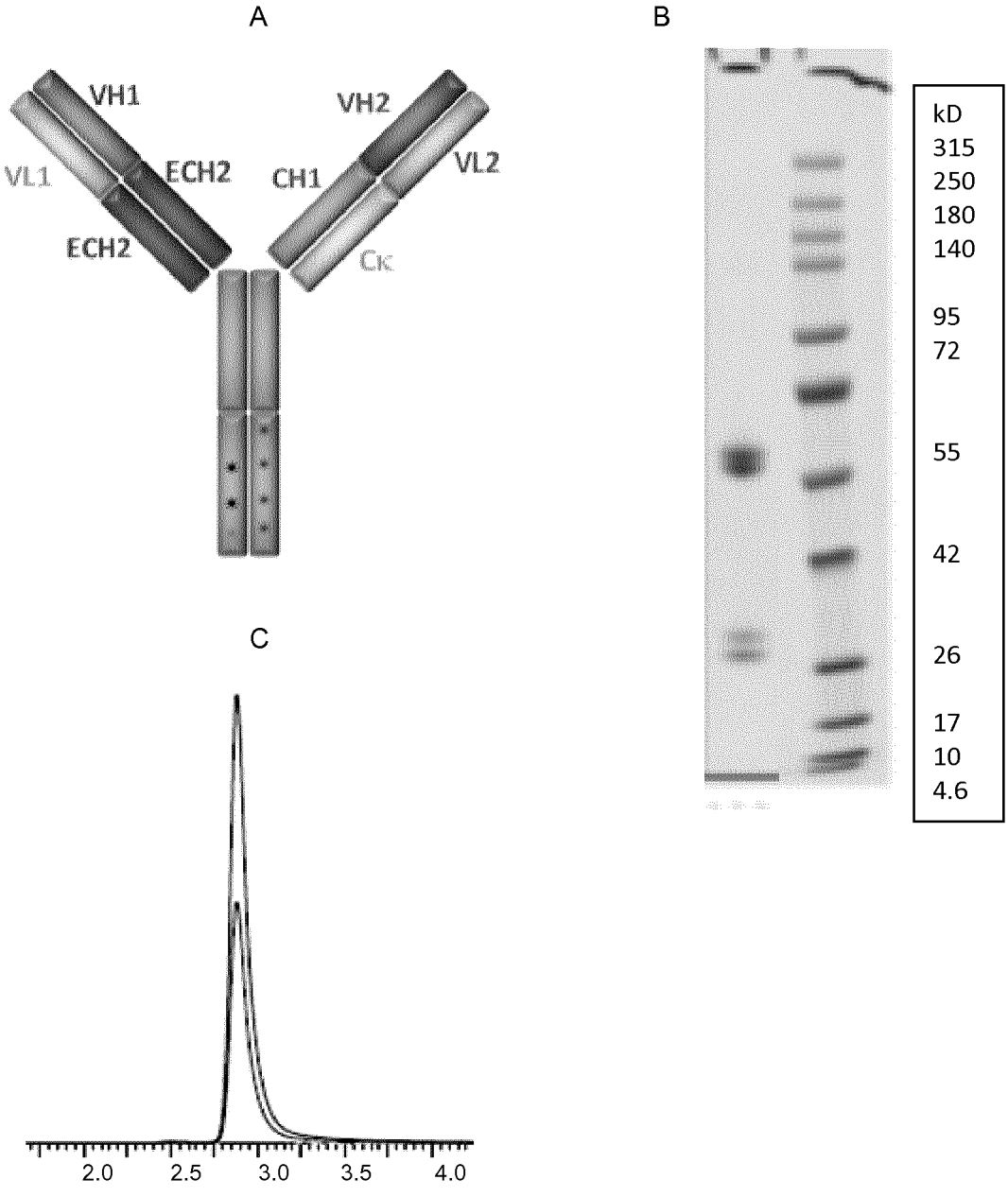
600
700
800
900
1000
1100
Time

Figure 5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VL2
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Ck
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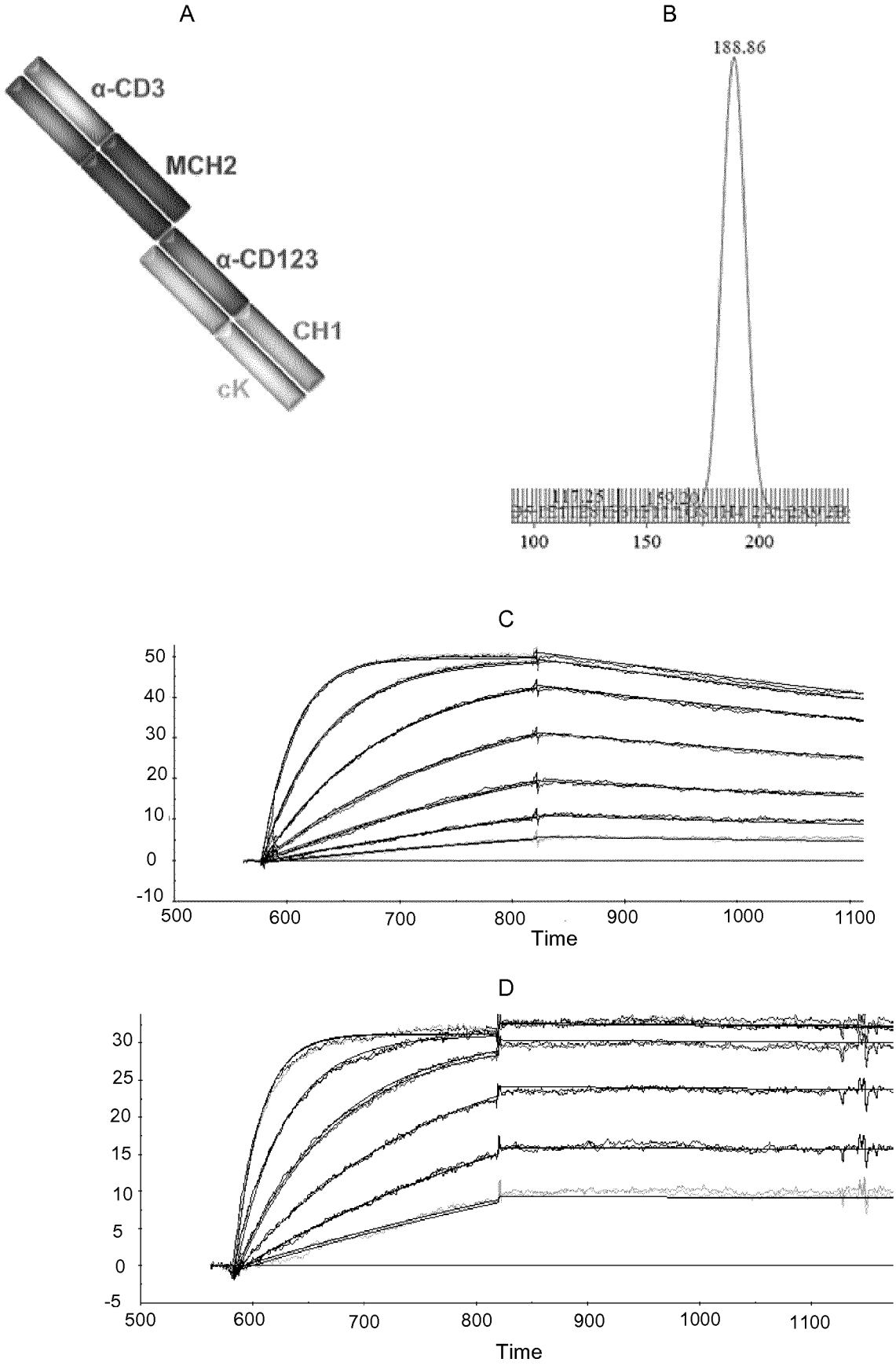
42
26
17
10
4.6
C
2.0
2.5
3.0
3.5
4.0
D
20
15
10
5
0
-5
-10
Time
500
600
700
800
900
1000
1100

Figure 6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CH1
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500
600
700
800
900
1000
1100
Time
D
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25
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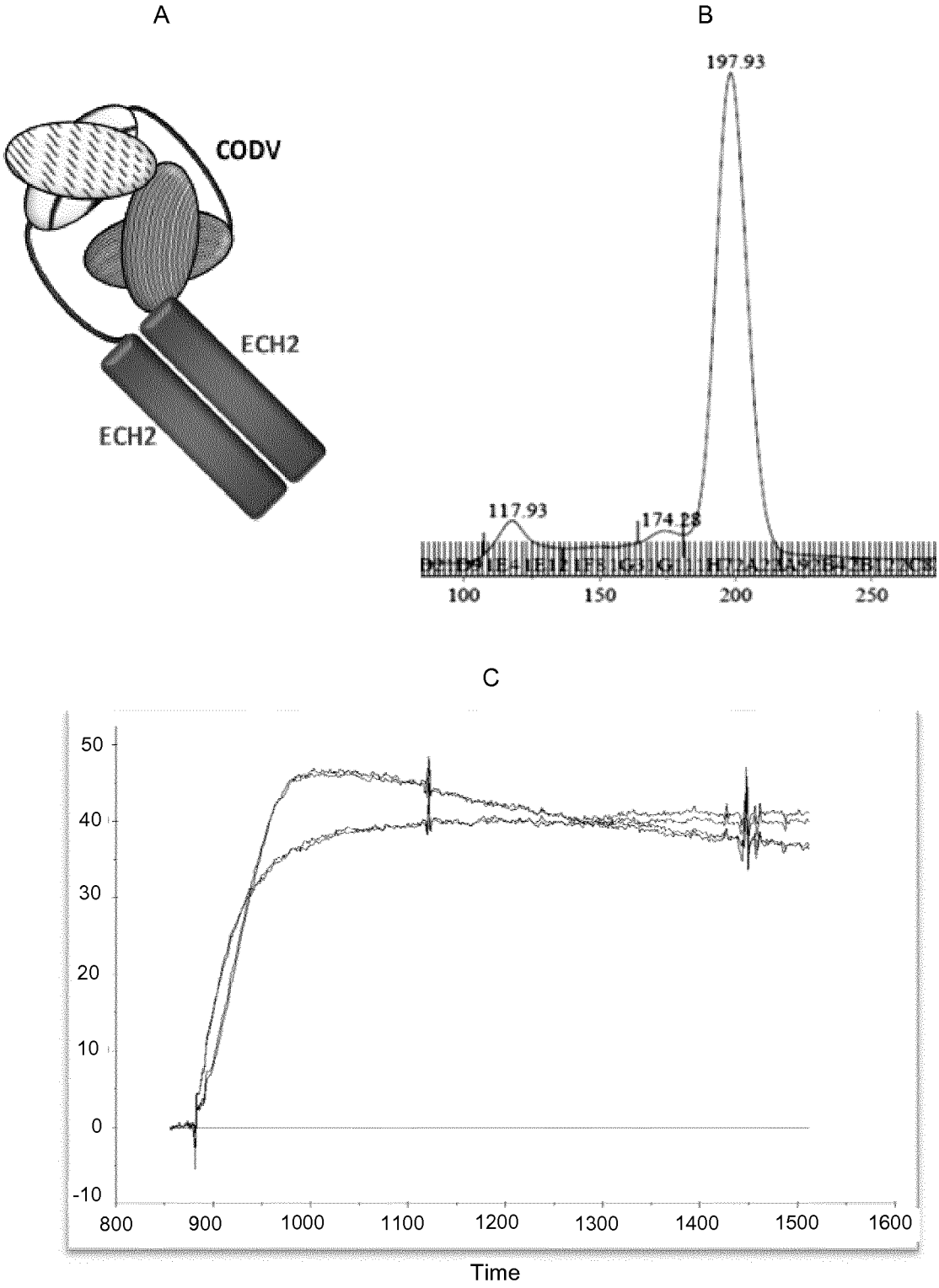
15
10
5
0
-5
500
600
700
800
900
1000
1100
Time

Figure 7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0
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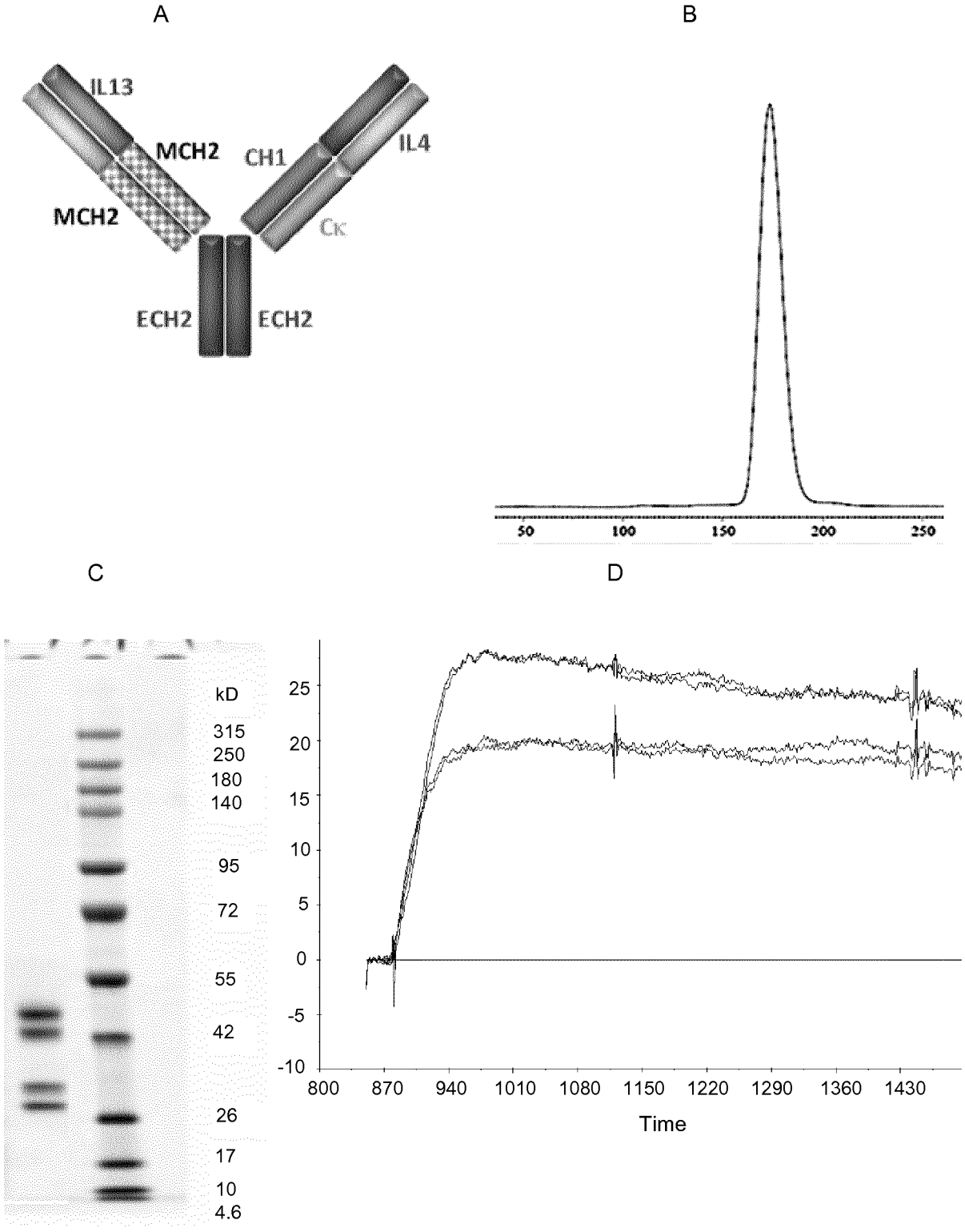
800
900
1000
1100
1200
1300
1400
1500
1600
Time

Figure 8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CH1
IL4
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ECH2
ECH2
B
50
100
150
200
250
C
kD
315
250
180
140
95
72
55
42
26
17
10
4.6
D
25
20
15
10
5
0
-5
-10
800
870
940
1010
1080
1150
1220
1290
1360
1430
Time

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/071375

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/00 C07K16/24
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Kenneth Murphy ET AL: "Structural variation in immunoglobulin constant regions" In: "Janeway's Immunobiology (7th Ed.)", 1 January 2008 (2008-01-01), Garland, XP055320566, ISBN: 978-0-8153-4123-9 pages 160-167, The whole document, in particularthe structires of the antibody isotypes -----	1
X	EP 2 210 902 A1 (TCL PHARMA [FR]; INST NAT SANTE RECH MED [FR]) 28 July 2010 (2010-07-28) The whole document, in particular Fig.1c ----- -/--	1

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 November 2017	20/11/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Chapman, Rob

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHRISTIAN KLEIN ET AL: "Progress in overcoming the chain association issue in bispecific heterodimeric IgG antibodies", MABS, vol. 4, no. 6, 1 November 2012 (2012-11-01), pages 653-663, XP055106060, ISSN: 1942-0862, DOI: 10.4161/mabs.21379 The whole document, in particular Fig. 4 and 5	1-15
X	SPIESS CHRISTOPH ET AL: "Alternative molecular formats and therapeutic applications for bispecific antibodies", MOLECULAR IMMUNOLOGY, PERGAMON, GB, vol. 67, no. 2, 27 January 2015 (2015-01-27), pages 95-106, XP029246892, ISSN: 0161-5890, DOI: 10.1016/J.MOLIMM.2015.01.003 The whole document, in particular Fig.1	1-15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2017/071375

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2017/071375

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 2210902 A1	28-07-2010	CA 2749627 A1	22-07-2010
		EP 2210902 A1	28-07-2010
		EP 2387587 A1	23-11-2011
		JP 5755148 B2	29-07-2015
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		US 2011313135 A1	22-12-2011
		US 2017166643 A1	15-06-2017
		WO 2010082136 A1	22-07-2010