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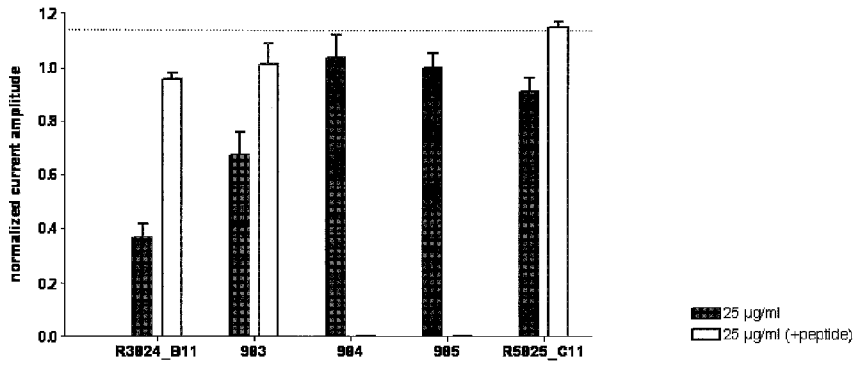
(54) **Title**: ANTIBODIES TO ION CHANNELS

Figure 1 Functional Effects of Selected Antibodies on Human Nav 1.7 current in HEK Cells



(57) **Abstract**: An anti- E1 ion channel antibody or binding fragment thereof, pharmaceutical compositions comprising said antibodies, use of the antibodies and compositions comprising the same, in treatment, for example in the treatment/modulation of pain and processes for generating and preparing said antibodies.

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ANTIBODIES TO ION CHANNELS

The present disclosure relates to anti-ion channel antibodies directed to an E1 portion thereof and fragments of said antibodies, with functionally modifying properties, pharmaceutical compositions comprising said antibodies, use of the antibodies and compositions comprising the same, in treatment, for example in the treatment/modulation of pain and processes for generating and preparing said antibodies.

Ion channels are pore-forming proteins that help establish and control cell membrane potential of all living cells by allowing the flow of ions down their electrochemical gradient. They are present in the membranes that surround all biological cells. The human genome contains more than 400 ion channel genes presenting a large diversity and play critical roles in many cellular processes such as secretion, muscular contraction and the generation and propagation of action potentials in cardiac and neuronal tissues.

Ion channels are integral membrane proteins that may adopt large molecular structures based on the assembly of several proteins. Such "multi-subunit" assemblies usually involve an arrangement of identical or homologous proteins closely packed around a water-filled pore through the plane of the membrane or lipid bilayer. The pore-forming subunit(s), usually called the α-subunit, may be associated with auxiliary subunits, either membrane bound or cytosolic, that help to control activity and cell surface expression of the ion channel protein. The X-ray structure of various ion channels was recently resolved (Doyle et al. Science 280:69 (1998); Jiang et al., Nature 423:33 (2003); Long et al., Science 309:897 (2005)) and indicate that the organization of the pore structure is largely conserved among ion channel family members. The opening and closing of the ion channel pore, referred as the gating process, may be triggered by various cellular or biochemical processes.

The largest family of ion channel proteins is composed of voltage-gated channels including e.g. sodium, calcium and potassium ion channels, transient receptor potential ion channels hyperpolarization activated ion channels, inward rectifier ion

channels, two-pore domain potassium channels and voltage gated proton channels. The latter depolarize in a pH-sensitive manner.

Inward rectifier ion channels are composed of 15 official and 1 unofficial members. The family can be further subdivided into 7 subfamilies based on homology.

At the present time there are about 10 voltage-gated calcium channels that have been identified.

Transient receptor potential ion channels are subdivided into 6 subfamilies based on homology: classical (TRPC), vanilloid receptors (TRPV), melastatin (TRPM), polycystins (TRPP), mucolipins (TRPML), and ankyrin transmembrane protein 1 (TRPA).

Hyperpolarization activated ion channels are sensitive to the cyclic nucleotides cAMP and cGMP, which alter the voltage sensitivity of the channel's opening. These channels are permeable to the monovalent cations K^+ and Na^+. There are 4 members of this family, all of which form tetramers of six-transmembrane α-subunits. As these channels open under hyperpolarizing conditions, they function as pacemaking channels in the heart, particularly the SA node.

The voltage-gated and ligand-gated ion channels are the most prominent members of the ion channel protein family. The activity of voltage-gated ion channels (*e.g.* calcium, sodium and potassium channels) is controlled by changes in cell membrane potentials whereas the ligand-gated ion channels (e.g. GABA-A receptors, Acethylcholine receptors) are controlled by the binding of specific intracellular or extracellular ligands. The gating mechanism is very complex, involving various membrane, pore and cytosolic structures, and differs between classes of ion channels.

Voltage-gated ion channels, sometimes referred to voltage-sensitive ion channels, are a class of transmembrane proteins that provide a basis for cellular excitability in cardiac and neuronal tissues. These channels are activated either by cell hyper- or depolarizations and generate ion fluxes that lead to control of cell membrane potential. Voltage-gated sodium channels are generally responsible for the initiation

of action potentials whereas voltage gated potassium channels mediate cell membrane repolarization. The fine tuned interplay between various voltage-gated ion channels is critical for the shaping of cardiac and neuronal action potentials.

One class of voltage-gated sodium channels comprises nine different isoforms (Nav1.1-1.9) and four different sodium channel specific accessory proteins have been described (SCN1b-SCN4b). The distinct functional activities of those isoforms have been described in a variety of neuronal cell types (Llinas et al., J. Physiol. 305:197-213 (1980); Kostyuk et al., Neuroscience 6:2423-2430 (1981); Bossu et al., Neurosci. Lett. 51:241-246 (1984) 1981; Gilly et al., Nature 309:448-450 (1984); French et al., Neurosci. Lett. 56:289-294 (1985); Ikeda et al., J. Neurophysiol. 55:527-539 (1986); Jones et al., J. Physiol. 389:605-627 (1987); Alonso & Llinas, 1989; Gilly et al., J. Neurosci. 9:1362-1374 (1989)) and in skeletal muscle (Gonoi et al., J. Neurosci. 5:2559-2564 (1985); Weiss et al., Science 233:361-364 (1986)). The $Na_v1.5$ and $Na_v1.4$ channels are the major sodium channel isoforms expressed in the cardiac and muscular tissue, respectively whereas $Na_v1.1$, 1.2, 1.3, 1.6, 1.7, 1.8 and 1.9 are specifically expressed in the central and peripheral nervous system. The use of the natural occurring toxin, tetrodotoxin (TTX), allowed to establish a pharmacological classification of the sodium channel isoforms based on their affinity to the toxin. The voltage-gated sodium channels were thus classified as TTX resistant ($Na_v1.5$, 1.8, 1.9) and TTX sensitive.

Certain ion channels have been associated with modulation of pain (see for example PNAS Nov 6, 2001. vol 98 no. 23 13373-13378 and The Journal of Neuroscience 22, 2004 24(38) 832-836). The ion channel $Na_v1.7$ is believed to have the ability to modulate pain, such as neuropathic pain and thus is a particularly interesting target for therapeutic intervention. $Na_v1.8$ and $Na_v1.9$ are also thought to have a role in the modulation of pain.

$Na_v1.7$ is a voltage-activated, tetrodotoxin-sensitive sodium channel encoded by the gene SCN9A. Both gain-of-function and loss-of-function mutations of $Na_v1.7$ result in clear pain-related abnormalities in humans.

Originally, gain-of-function mutations in SCN9A were identified by linkage analysis as the cause of erythromelalgia (or primary erythermalgia) and paroxysmal extreme pain disorder (formerly familiar rectal pain). Erythromelalgia is a rare autosomal dominant disorder associated with bouts of burning pain together with heat and redness in the extremities. The complete inability to sense pain by an otherwise healthy individual, devoid of neuropathy, is a very rare phenotype. Very recently, two studies, reported by Cox et al (2006) and by Goldberg et al (2007)., describe such a phenotype mapped, as an autosomal-recessive trait, to chromosome 2q24.3, a region containing the gene SCN9A. In both studies, detailed neurological tests revealed that these people are able to distinguish sharp/dull and hot/cold stimuli but have a global absence of pain sensation. All had injuries to lips and/or tongue caused by biting themselves. All had frequent bruises and cuts, and most suffered fractures or osteomyelitis.

This data constitutes strong evidence that SCN9A channelopathy, leading to loss of function of ion channel $Na_v1.7$, is associated with insensitivity to pain, in the absence of neuropathy or of cognitive, emotional or neurological disorders, and clinically validate $Na_v1.7$ as a pain-relevant target. Furthermore, from KO studies and animal pain models, it would appear that $Na_v1.7$ plays a major role in inflammatory pain.

Figure 2a is a diagrammatic representation of an ion channel, such as $Na_v1.7$, which comprises four domains A, B, C and D (also referred to as domain I, II, III and IV). Each domain comprises 6 transmembrane protein helixes S1, S2, S3, S4, S5 and S6. The exact amino acid number of each transmembrane protein varies depending on the database entry employed but UniProtKB/Swiss-Prot provides the following information for $Na_v1.7$:

in domain A transmembrane protein S1, S2, S3, S4, S5 and S6 are assigned amino acids 122-145, 154-173, 187-205, 212-231, 248-271 and 379-404, respectively;

in domain B transmembrane protein S1, S2, S3, S4, S5 and S6 are assigned amino acids 739-763, 775-798, 807-826, 833-852, 869-889 and 943-968 respectively;

in domain C transmembrane protein S1, S2, S3, S4, S5 and S6 are assigned amino acids 1188-1211, 1225-1250, 1257-1278, 1283-1304, 1324-1351 and 1431-1457 respectively; and

in domain D transmembrane protein S1, S2, S3, S4, S5 and S6 are assigned amino acids 1511-1534, 1546-1569, 1576-1599, 1610-1631, 1647-1669 and 1736-1760, respectively.

There are a number of natural variations of the sequence that are available in public databases, for example see UniProtKB/Swiss-Prot Q15858.

In the present disclosure S1, S2, S3, S4, S5 and S6 refers to the entities described above or a entity corresponding to same in an alternative ion channel, including wherein a different amino acid assignment is given to the same and including the corresponding entity in natural or non-natural variants and different isotypes of the same.

Each domain also contains extra-cellular hydrophilic loops E1, E2 and E3. The amino acid sequence of E1 in each domain starts after the transmembrane region S1 and ends at S2. E1 in each domain is distinct from E1 in other domains. The amino acid sequence of E2 in each domain starts after the transmembrane region S3 and ends at S4. E2 in each domain is distinct from E2 in other domains. The amino acid sequence of E3 in each domain starts after the transmembrane region S5 and ends at S6. E3 in each domain is also distinct from E3 in other domains.

Whilst the Na_v and Ca_v ion channels comprise four domains, A, B, C and D, each containing six transmembrane protein helixes, other ion channels, such as K_v ion channels, HCN ion channels and TRP ion channels comprise one domain. As for each domain in the Na_v and Ca_v ion channels, the K_v ion channels, HCN ion channels and TRP ion channels comprise 6 transmembrane protein helixes S1, S2, S3, S4, S5 and S6 and three extra-cellular hydrophilic loops E1, E2 and E3 as described above.

In a $Na_v1.7$ ion channel, the extracellular loops (E loops) are the following amino acid residues of SEQ ID NO:105 in Figure 2c:

$Na_v1.7$ Domain	E1 amino acids	E2 amino acids	E3 amino acids
A	146-153	206-211	272-378
B	764-774	827-832	890-942
C	1212-1224	1279-1282	1352-1430
D	1535-1545	1600-1609	1670-1735

The extracellular loops in some domains of $Na_v1.7$ share similarities with extracellular loops found in other ion channels.

$Na_v1.7$ is expressed in the peripheral nervous system i.e. in nociceptive dorsal root ganglions (DRG), most notably in nociceptive small-diameter DRG neurons, with little representation in the brain. $Na_v1.7$ distribution (e.g. sensory ending) and physiology predispose it to a major role in transmitting painful stimuli.

The expression of $Na_v1.7$ in the peripheral nervous system makes it a very attractive target for the generation of function blocking antibodies which represent an innovative approach for valuable treatment for pain with no side-effects or minimizing side effects to a tolerable level.

Neuropathic pain is a highly prevalent condition. In the United States, it is estimated to affect between 0.6 and 1.5 % of the population, or 1.8 to 4.5 million people. (Pullar and Palmer, 2003). At least 1.4 million people each year are diagnosed with painful diabetic neuropathy (PDN), post-herpetic neuropathy (PHN) or trigeminal neuralgia (TN) – three major causes of neuropathic pain. Other causes of neuropathic pain include spinal cord injuries, multiple sclerosis, phantom limb pain, post-stroke pain and HIV-associated pain. If patients with neuropathic-related chronic back pain, osteoarthritis and cancer were included, the total number would at least double. Nonsteroidal anti-inflammatory drugs (NSAIDs) although frequently used, are not very effective in the treatment of neuropathic pain. Moreover, their chronic use may lead to serious gastric damage. On the other hand, the use of opioids (morphine and derivatives) is restricted to the most severe form of neuropathic pain, i. e., cancer-related neuropathy, because serious side-effects are associated with chronic treatment, such as nausea, emesis, respiratory depression, constipation and tolerance, and the

potential for addiction and abuse. The latter have prevented the use of opioids in other neuropathies (Dellemijn, 1999; Namaka et al., 2004). Anti-epileptic drugs (AEDs) are known to attenuate abnormal neural hyperexcitability in the brain. In view of neural hyperexcitability playing a crucial role in neuropathic pain, it is understandable that AEDs were aimed at the treatment of chronic neuropathic pain (Renfrey, Downton and Featherstone, 2003). The most recent and important examples are gabapentin (Neurontin) and pregabalin (Lyrica, Frampton and Scott, 2004). However, even gabapentin, the gold standard for the treatment of neuropathic pain, reduces pain at best by 50% in about 40% of patients (Dworkin, 2002). Further, in contrast to opioids, gabapentin is not used in the treatment of cancer-related neuropathic pain.

As stated above, $Na_v1.7$ 'loss of function' mutation in human leads to insensitivity to pain (Cox et al., 2006). Moreover, $Na_v1.7$ 'gain of function' mutation in human leads to the pain phenotypes erythromelalgia and paroxysmal extreme pain disorder (Dib-Hajj, Yang, Waxman, 2008). Additionally, a peripherally acting small molecule blocking $Na_v1.7$ reverses hyperalgesia and allodynia in rat models of inflammatory and neuropathic pain (McGowan et al., 2009). Therefore a peripherally acting $Na_v1.7$ blocking antibody should be beneficial for pain therapy.

To date potent chemical inhibitors of ion channels have been identified but generally these are characterised by a poor selectivity against other ion channel isoforms. Given the ubiquitous distribution of ion channels in living organisms these non-selective inhibitors have been of limited utility.

Whilst antibodies are clearly desirable, due to their exquisite specificity, it has not been wholly straightforward to generate functionally modifying antibodies, in part, because clonal antibodies are ultimately required for therapeutic applications and some researchers in the field have indicated that polyclonal antibodies are required for effecting modification of the function of ion channels. Klionsky *et al* (The Journal of Pharmacology and Experimental Therapeutics Vol 319 No. 1 page 192-198) states on page 198:

"Since no rabbit, mouse or fully human monoclonal antibodies generated against the prepore region of human TRPV1 ..were effective in blocking channel activation we hypothesise that it may not be possible to lock the channel conformation through high-affinity binders to small epitopes in this region".

Sodium channels, particularly $Na_v1.7$, $Na_v1.8$. $Na_v1.9$ seems to have been a particularly challenging target in respect of generating functionally modifying antibodies. However, the present inventors have now found that the activity of said ion channels can be altered employing functionally modifying antibodies, for example a clonal population of antibodies. To date whilst antibodies to ion channels have been generated it is believed that no E1 binding functionally modifying antibodies to ion channels involved in the modulation of pain have been disclosed.

The present inventors have now established that functionally modifying antibodies can be raised against an E1 loop of ion channels involved in the modulation of pain. This is surprising because the E1 loop in each of the domains is a relatively short amino acid sequence.

Summary of the Invention

Thus the invention provides an anti-E1 ion channel antibody or binding fragment thereof, wherein said ion channel has a function in the modulation of pain, and said antibody or fragment is functionally modifying to said ion channel after binding thereto.

Brief Description of the Drawings.

Figure 1 shows the functional effects of certain monoclonal antibodies on human $Na_v1.7$ current in HEK cells.

Figure 2a shows a diagrammatic representation of $Na_v1.7$.

Figure 2b shows the amino acid sequence for domain A (SEQ ID NO:101), B (SEQ ID NO:102), C (SEQ ID NO:103) and D (SEQ ID NO:104) of $Na_v1.7$.

Figure 2c shows the full amino acid sequence of Nav1.7 (SEQ ID NO:105).

Figure 3a shows that the clonal 983 anti- $Na_v1.7$ antibody reduces electrically induced DRG spike frequency *in vitro*.

Figure 3b shows that anti- $Na_v1.7$ monoclonal antibody 983 reduces electrically induced DRG spike frequency *in vitro*

Figure 3c shows that anti- $Na_v1.7$ monoclonal antibody 1080 reduces electrically induced DRG spike frequency *in vitro*

Figure 3d(a) shows automated Patch Clamp analysis of recombinant human Nav1.7 channels expressed in HEK cells. 983 monoclonal antibody produces a dose-dependent inhibition of Nav1.7 currents.

Figure 3d(b) shows automated Patch Clamp analysis of recombinant human Nav1.7 channels expressed in HEK cells. 1080 monoclonal antibody produces a dose-dependent inhibition of Nav1.7 currents.

Figure 3e shows automated Patch Clamp analysis of recombinant rat Nav1.7 channels expressed in HEK cells. 983 monoclonal antibody produces a dose-dependent inhibition of Nav1.7 currents. 1080 monoclonal antibody produces a ~26% inhibition of Nav1.7 currents at 25μg/ml.

Figure 3f Kinetics of human Nav1.7 inhibition by 983 monoclonal antibody.

Figure 3g shows ELISA data for antibody 983 specific binding to Nav 1.7 peptide

Figure 3h shows ELISA data for antibody 1080 specific binding to Nav 1.7 peptide.

Figure 4-7 shows the amino acid sequence of certain anti- $Na_v1.7$ antibodies.

Detailed Description of the invention

In one aspect the present disclosure provides an ion channel E1 loop binding entity that functionally modifies the activity of an ion channel associated with the modulation of pain, including an antibody, an antibody fragment, a protein or proteinaceous scaffold, a nucleic acid or nucleotide, a small molecule such a synthetic molecule or the like, in particular an antibody, an antibody fragment, a protein or proteinaceous scaffold or a nucleic acid or nucleotide.

Ion channels thought to be involved and/or associated with modulation of pain include but are not limited to Na_v 1.3, Na_v 1.6, Na_v 1.7, Na_v 1.8, Na_v 1.9, Ca_v 3.1, Ca_v 3.2, Ca_v 3.3, Ca_v 2.1, Ca_v 2.2, Ca_v 2.3, K_v 2.1, K_v 2.2, K_v 7.x, HCN1, HCN2, TRPV1, TRPA1, ASIC1, TRPM8, TRPV3 and TRP4.

In one embodiment the ion channel is a sodium channel, for $Na_v1.7$, $Na_v1.8$ or $Na_v1.9$, such as a $Na_v1.7$. The peptide employed for immunization may comprise at least part of an extracellular sequence of the ion channel wherein the extracellular sequence is the E1 loop and may be derived from the A domain, B domain, C domain or D domain of the ion channel. In a preferred embodiment the peptide comprises at least a part of an E1 extracellular region derived from the A domain, B domain, C domain or D domain of the ion channel. In a further preferred embodiment the peptide comprises at least a part of an E1 extracellular region derived from the A domain or the B domain of the ion channel. Preferably the peptide comprises at least a part of the BE1 extracellular region.

In one embodiment of the present invention the ion channel is not Nav1.7.
In one embodiment the present invention provides an anti-E1 ion channel antibody or binding fragment thereof, which binds to an E1 extracellular loop of the ion channel, wherein said ion channel has a function in the modulation of pain, and said antibody or fragment is functionally modifying to said ion channel after binding thereto and wherein said ion channel is not Nav1.7.

In one embodiment the ion channel is a potassium ion channel $K_v2.1$, $K_v2.2$ or $K_v7.x$.

In one embodiment the ion channel is a calcium ion channel, for example $Ca_v3.1$, $Ca_v3.2$, $Ca_v3.3$, $Ca_v2.1$, $Ca_v2.2$ or $Ca_v2.3$.

In one embodiment the ion channel is a hyperpolarisable channel HCN1 or HCN2.

In one embodiment the ion channel is a non-gated ion channel, for example TRPV1, TRPA1, ASIC1, TRPM8, TRPV3 or TRP4.

In one embodiment there is provided an E1 binding anti-ion channel antibody for use in treatment, for example for use in the modulation of pain, in particular the amelioration of pain.

In one embodiment there is provided an E1 binding anti-ion channel antibody for use in the modulation, for example amelioration of pain.

In one embodiment there is provided an E1 binding anti-Na_v 1.7 antibody for use in the modulation, for example amelioration of pain, in particular a sub-group of pain described herein.

In one embodiment there is provided an E1 binding anti-Na_v 1.8 antibody for use in the modulation, for example amelioration of pain, in particular a sub-group of pain described herein.

In one embodiment there is provided an E1 binding anti-Na_v 1.9 antibody for use in the modulation, for example amelioration of pain, in particular a sub-group of pain described herein.

In one embodiment there is provided an E1 binding anti-HCN1 antibody for use in the modulation, for example amelioration of pain, in particular a sub-group of pain described herein.

In one embodiment there is provided an E1 binding anti-HCN2 antibody for use in the modulation, for example amelioration of pain, in particular a sub-group of pain described herein.

In one embodiment there is provided an E1 binding anti-TRPA1 antibody for use in the modulation, for example amelioration of pain, in particular a sub-group of pain described herein.

In one embodiment there is provided an E1 binding anti-TRPV1 antibody for use in the modulation, for example amelioration of pain, in particular a sub-group of pain described herein.

In one embodiment there is provided an E1 binding anti-TRPV3 antibody for use in the modulation, for example amelioration of pain, in particular a sub-group of pain described herein.

In one embodiment there is provided an E1 binding anti-TRPM8 antibody for use in the modulation, for example amelioration of pain, in particular a sub-group of pain described herein.

In one embodiment there is provided an E1 binding anti-TRP4 antibody for use in the modulation, for example amelioration of pain, in particular a sub-group of pain described herein.

In one embodiment there is provided an E1 binding anti-ASIC1 antibody for use in the modulation, for example amelioration of pain, in particular a sub-group of pain described herein.

In one embodiment there is provided an E1 binding anti- Ca_v 3.1 antibody for use in the modulation, for example amelioration of pain, in particular a sub-group of pain described herein.

In one embodiment there is provided an E1 binding anti- Ca_v 3.2 antibody for use in the modulation, for example amelioration of pain, in particular a sub-group of pain described herein.

In one embodiment there is provided an E1 binding anti- Ca_v 3.3 antibody for use in the modulation, for example amelioration of pain, in particular a sub-group of pain described herein.

In one embodiment there is provided an E1 binding anti- Ca_v 2.1 antibody for use in the modulation, for example amelioration of pain, in particular a sub-group of pain described herein.

In one embodiment there is provided an E1 binding anti- Ca_v 2.2 antibody for use in the modulation, for example amelioration of pain, in particular a sub-group of pain described herein.

In one embodiment there is provided an E1 binding anti- Ca_v 2.3 antibody for use in the modulation, for example amelioration of pain, in particular a sub-group of pain described herein.

Functionally modifying antibody as employed herein is intended to refer to an antibody or fragment (such as a binding fragment) that changes the activity of the ion channel, for example by reducing an activity by at least 5%, for example 10 or 15% such as 20% in at least one *in vitro* or *in vivo* assay. Suitable *in vitro* assays include a patch clamp assay or other assay as described herein. In one embodiment the functionally modifying antibody reduces the amplitude of current through a patch clamp assay by 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70% or more percent.

An antibody that provides a functional modification to the ion channel and a functionally modifying antibody are terms used interchangeably herein.

In one embodiment the functional modification is, for example sufficient to block, close or inhibit the pore of the ion channel. This functional modification may be effected by any mechanism including, physically blocking the pore, causing a conformational change in the ion channel which for example blocks the pore or eliciting the ion channel to adopt a non-functional state (resting or inactivated state) and/or maintaining the ion channel in a non-functional state (allosteric modulation).

In one embodiment the functional modification is sufficient to reduce the cell surface levels of the ion channel protein. This functional modification may be effected by any mechanism including but not limited to, antibody induced internalization or endocytosis or increased cycling of the ion channel, leading to a reduce number of functional ion channel proteins at the cell surface.

The mechanisms proposed *supra* for functional modification of the ion channel are examples and are not intended to be limiting in respect of ways the a functionally modifying antibody may generate a functionally modifying effect in the ion channel.

Examination of the differences in the sequence of $Na_v1.7$ extracellular domains vs the extracellular domains of other family members allows areas of particular interest to be identified which may be of use in the generation of antibodies, for example by generating peptides based on those sequences. In Na_v 1.7 domain A amino acids 146-153, domain B amino acids 764-774, domain C amino acids 1213-1224 and 1216-1224, and domain D amino acids 1535-1545 are regions of particular difference/distinction and thus may be particularly suitable for generating antibodies.

In one embodiment the antibody or fragment binds an extra-cellular (or extra-cellular accessible region) of domain A in the ion channel in the E1 loop.

In one embodiment the antibody or fragment binds an extra-cellular (or extra-cellular accessible region) of domain B in the ion channel in the E1 loop.

In one embodiment the antibody or fragment binds an extra-cellular (or extra-cellular accessible region) of domain C in the ion channel, in the E1 loop.

In one embodiment the antibody or fragment binds an extra-cellular (or extra-cellular accessible region) of domain D in the ion channel, in the E1 loop.

In one embodiment the antibody or fragment according to the present invention may be employed in combination with other entities which functionally modify the ion channel in question, for antibodies or fragment that bind an E3 region of the ion channel, for example part of the E3 region of domain A, B, C or D, such as domain A.

In one embodiment there is provided an E1 binding anti- $Na_v1.7$ antibody.

An anti- $Na_v1.7$ antibody is an antibody that binds specifically to $Na_v1.7$. An E1 binding anti- $Na_v1.7$ antibody is an antibody that binds specifically to $Na_v1.7$ in an E1 region.

Specific binding is intended to refer to the fact that the antibody is selective for the relevant ion channel, for example $Na_v1.7$ and can distinguish it from other ion channels and proteins, for example other ion channels in the same family. A selective antibody is one that, for example can be used to affinity purify the relevant ion channel, such as $Na_v1.7$ including from other ion channels.

In one embodiment the anti- $Na_v1.7$ antibody is specific to mammalian $Na_v1.7$, for example human $Na_v1.7$.

In one embodiment the E1 binding anti-ion channel antibody, for example anti-$Na_v1.7$ antibody cross-reacts with the relevant human ion channel and the corresponding rat ion channel.

In one embodiment the functionally modifying antibody or fragment is a clonal population of antibodies, for example a monoclonal population. It has been suggested in the literature that polyclonal antibodies are required to obtain functional modification of ion channels. Therefore, it is particularly surprising that the present disclosure provides monoclonal antibodies which are functionally modifying to a relevant ion channel.

Monoclonal as employed herein is intended to refer antibodies or fragments derived from a single cell, for example employing hybridoma technology.

A clonal population is intended to refer to a population of antibodies or fragments with the same properties, characteristics including the same amino acid sequence and specificity.

In one embodiment two or more, for example three or four clonal populations of anti-ion channel antibodies are employed in admixture, wherein the mixture comprises at least one antibody, for example two, three or four according to the present invention.

In one embodiment the antibody according to the disclosure is a whole antibody.

In one embodiment the antibody or fragment thereof is multivalent and/or bi-specific. Multivalent as employed herein is intended to refer multiple binding sites (for example at least two binding sites) in the antibody or fragment entity. Multivalent entities, in the context of the present specification, have at least two binding sites with the same specificity. Mutivalent antibodies with binding sites that bind the same epitope will not be considered bi-specific unless the entity comprises a third binding site with different specificity to a first and second binding site. Thus bi-specific as employed in the present disclosure requires two or more binding sites in the antibody or fragment to bind to different, i.e. distinct target antigens.

In an entity with multiple binding sites where each binding site binds a different epitope on the same or different target antigen, then the antibody or fragment entity will be considered bi-specific, within the meaning of the present disclosure.

Binding site as employed herein is intended to refer to an area of the antibody or fragment, which specifically binds a target antigen. A heavy chain variable domain and light chain variable domain pairing will be considered an example of a binding site herein.

In one embodiment an antibody fragment according to the disclosure is monovalent. That is to say, has only one binding site.

In one embodiment the antibody is a fragment, for example a single domain antibody, a single chain Fv, a Fab, a Fab' or a pairing of a full length heavy and light chain.

The antibody of the disclosure and fragments thereof, of use in the present invention, can be from any species but is preferably derived from a monoclonal antibody, a human antibody, or are humanised fragments. An antibody fragment for use in the present invention can be derived from any class (e.g. IgG, IgE, IgM, IgD or IgA) or subclass of immunoglobulin molecule and may be obtained from any species including for example mouse, rat, shark, rabbit, pig, hamster, camel, llama, goat or human.

The residues in antibody variable domains are conventionally numbered according to a system devised by Kabat *et al.* This system is set forth in Kabat *et al.*, 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA (hereafter "Kabat et al. (supra)"). This numbering system is used in the present specification except where otherwise indicated.

The Kabat residue designations do not always correspond directly with the linear numbering of the amino acid residues. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering corresponding to a shortening of, or insertion into, a structural component, whether framework or complementarity determining region (CDR), of the basic variable domain structure. The correct Kabat numbering of residues may be determined for a given antibody by alignment of residues of homology in the sequence of the antibody with a "standard" Kabat numbered sequence.

The CDRs of the heavy chain variable domain are located at residues 31-35 (CDR-H1), residues 50-65 (CDR-H2) and residues 95-102 (CDR-H3) according to the Kabat numbering system. Kabat numbering will be employed herein.

An alternative number system, not used herein, is Chothia (Chothia, C. and Lesk, A.M. J. Mol. Biol., 196, 901-917 (1987)), where the loop equivalent to CDR-H1 extends from residue 26 to residue 32. A combination of Chothia and Kabat numbering for 'CDR-H1', would for example comprises residues 26 to 35.

The CDRs of the light chain variable domain are located at residues 24-34 (CDR-L1), residues 50-56 (CDR-L2) and residues 89-97 (CDR-L3) according to the Kabat numbering system.

Below is provided the CDRs for various rabbit antibodies:

CA167 00983	CDR-L1	QSSQSVYKNNDLA	Seq Id No: 1
	CDR-L2	YASTLAS	Seq Id No: 2
	CDR-L3	LGSYDCSSADCNA	Seq Id No: 3
	CDR-H1	NYAMS	Seq Id No: 4
	CDR-H2	IIGKSGSTAYASWAKG	Seq Id No: 5

	CDR-H3	FVLL	Seq Id No: 6

CA167 00984	CDR-L1	QSSQSVNNNNFLS	Seq Id No: 7
	CDR-L2	RASTLAS	Seq Id No: 8
	CDR-L3	AGGYSGNIYA	Seq Id No: 9
	CDR-H1	DYIIN	Seq Id No: 10
	CDR-H2	IMGTSGTAYYASWAKG	Seq Id No: 11
	CDR-H3	GGVATSNF	Seq Id No: 12

CA167 00985	CDR-L1	QSSQSVYGNNWLG	Seq Id No: 13
	CDR-L2	SASTLAS	Seq Id No: 14
	CDR-L3	VGGYSGNIHV	Seq Id No: 15
	CDR-H1	DYDMS	Seq Id No: 16
	CDR-H2	TIYVSGNTYYATWAKG	Seq Id No: 17
	CDR-H3	AVPGSGKGL	Seq Id No: 18

CA167 01080	CDR-L1	QSSQSVWKNNDLS	Seq Id No: 19
	CDR-L2	YASTLAS	Seq Id No: 20
	CDR-L3	VGSYDCSSADCNA	Seq Id No: 21
	CDR-H1	KWPMT	Seq Id No: 22
	CDR-H2	IIGRSGSTNYASWAKG	Seq Id No: 23
	CDR-H3	GGSYYDL	Seq Id No: 24

CA167 01081	CDR-L1	QSSQSVDNNNYLS	Seq Id No: 25
	CDR-L2	DASDLAS	Seq Id No: 26
	CDR-L3	AGGYITSSDIFYD	Seq Id No: 27
	CDR-H1	TYAMS	Seq Id No: 28
	CDR-H2	IVGKSGIIKYASWAKG	Seq Id No: 29
	CDR-H3	LWSL	Seq Id No: 30
CA167 01082	CDR-L1	QASQSISNWLA	Seq Id No: 31
	CDR-L2	RASTLAS	Seq Id No: 32
	CDR-L3	QSDYGIDTYGSA	Seq Id No: 33
	CDR-H1	SYAMT	Seq Id No: 34
	CDR-H2	MVRRSGTTYYASWAKG	Seq Id No: 35
	CDR-H3	CDNSAGDWSYGMDL	Seq Id No: 36

CA167 01083	CDR-L1	QASQSVYQNNYLA	Seq Id No: 37
	CDR-L2	SASTLAS	Seq Id No: 38
	CDR-L3	LGAYDCSGVDCSA	Seq Id No: 39
	CDR-H1	TNAMI	Seq Id No: 40
	CDR-H2	VIAGSGSTSYASWAKG	Seq Id No: 41
	CDR-H3	GGWVSGPESL	Seq Id No: 42

CA167 01084	CDR-L1	QSSPSVYGNNWLG	Seq Id No: 43
	CDR-L2	SASTLAS	Seq Id No: 44
	CDR-L3	AGGYSGNIHV	Seq Id No: 45
	CDR-H1	NYDMT	Seq Id No: 46
	CDR-H2	SIFVSGNIYYASWAKG	Seq Id No: 47

	CDR-H3	AILGSSKGL	Seq Id No: 48

CA167 01085	CDR-L1	QASQSIYSYLA	Seq Id No: 49
	CDR-L2	SASYLAS	Seq Id No: 50
	CDR-L3	QHGYISGNVDNA	Seq Id No: 51
	CDR-H1	IYDMS	Seq Id No: 52
	CDR-H2	SIYVSGNIYYASWAKG	Seq Id No: 53
	CDR-H3	AVPGSSKGL	Seq Id No: 54

CA167 01086	CDR-L1	QSSQSIYTNYLS	Seq Id No: 55
	CDR-L2	SASTLAS	Seq Id No: 56
	CDR-L3	QAYFTGEIFP	Seq Id No: 57
	CDR-H1	NYHMG	Seq Id No: 58
	CDR-H2	FITRGGTTYYASWAKG	Seq Id No: 59
	CDR-H3	GSGASGFYL	Seq Id No: 60

In one embodiment the disclosure herein extends to an antibody comprising 1, 2, 3, 4, 5, or 6 CDR sequences disclosed in this specification.

In one embodiment the disclosure extends to an antibody comprising a single variable domain or a pair of variable domain from a sequence or sequences herein.

In one embodiment the variable domain of the heavy chain comprises at least one of a CDR having the sequence given in the table listed above, for example where the CDR is in its "natural position". The natural position of a CDR such as H1, H2, H3, L1, L2 or L3 is given above in the tables, for example the natural position for the CDR of Seq ID No: 4 is H1, the natural position for the CDR of Seq ID No: 5 is H2, the natural position for the CDR of Seq ID No: 6 is H3, and so on. Analogous interpretations also apply to the light chain sequences.

In one example an antibody of the present invention comprises a heavy chain wherein at least two of CDR-H1, CDR-H2 and CDR-H3 of the variable domain of the heavy chain are selected from sequences given in the tables above, for example the CDRs are in their natural position and optionally in their natural pairing. Natural pairing as employed herein is intended to refer to pairing of CDRs from the same antibody (i.e from one table above). An example of natural pairing for CA167 00983 is Seq ID No: 1 and 2, 1 and 3, and 2 and 3.

In one embodiment an antibody according to the present invention comprises a heavy chain, wherein the variable domain comprises the sequence given in:

SEQ ID NO:4 for CDR-H1,
SEQ ID NO:5 for CDR-H2 and
SEQ ID NO: 6 for CDR-H3,
or
SEQ ID NO:10 for CDR-H1,
SEQ ID NO:11 for CDR-H2 and
SEQ ID NO:12 for CDR-H3,
or
SEQ ID NO:16 for CDR-H1,
SEQ ID NO:17 for CDR-H2 and
SEQ ID NO:18 for CDR-H3,
or,
SEQ ID NO:22 for CDR-H1,
SEQ ID NO:23 for CDR-H2 and
SEQ ID NO:24 for CDR-H3,
or
SEQ ID NO:28 for CDR-H1,
SEQ ID NO:29 for CDR-H2 and
SEQ ID NO:30 for CDR-H3,
or
SEQ ID NO:34 for CDR-H1,
SEQ ID NO:35 for CDR-H2 and
SEQ ID NO:36 for CDR-H3,
or
SEQ ID NO:40 for CDR-H1,
SEQ ID NO:41 for CDR-H2 and
SEQ ID NO:42 for CDR-H3,
or
SEQ ID NO:46 for CDR-H1,
SEQ ID NO:47 for CDR-H2 and
SEQ ID NO:48 for CDR-H3,
or
SEQ ID NO:52 for CDR-H1,
SEQ ID NO:53 for CDR-H2 and
SEQ ID NO:54 for CDR-H3,
or
SEQ ID NO:58 for CDR-H1,
SEQ ID NO:59 for CDR-H2 and

SEQ ID NO:60 for CDR-H3,
or a sequence having at least 60%, 70%, 80% such as at least 90%, 95% or 98% identity or similarity thereto.

In one embodiment an antibody according to the present invention comprises a heavy chain wherein the variable domain of the heavy chain comprises a variable domain or variable domain components as disclosed herein, for example listed herein.

Variable domain components as employed herein is intended to refer to CDRs and combinations thereof, particularly as explicitly disclosed herein.

In another embodiment, the antibody of the present invention comprises a heavy chain, wherein the variable domain of the heavy chain comprises a sequence having at least 60%, 70%, 80% identity or similarity, such as at least 90%, 95% or 98% identity or similarity to a heavy chain variable region disclosed herein.

"Identity", as used herein, indicates that at any particular position in the aligned sequences, the amino acid residue is identical between the sequences. "Similarity", as used herein, indicates that, at any particular position in the aligned sequences, the amino acid residue is of a similar type between the sequences. For example, leucine may be substituted for isoleucine or valine. Other amino acids which can often be substituted for one another include but are not limited to:

- phenylalanine, tyrosine and tryptophan (amino acids having aromatic side chains);
- lysine, arginine and histidine (amino acids having basic side chains);
- aspartate and glutamate (amino acids having acidic side chains);
- asparagine and glutamine (amino acids having amide side chains); and
- cysteine and methionine (amino acids having sulphur-containing side chains).

Degrees of identity and similarity can be readily calculated (Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing. Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991).

The present invention also provides an E1 binding anti-ion channel antibody or fragment, wherein the ion channel has a role/function in the modulation of pain *in vivo*, in particular an antibody directed to an ion channel described herein, for example an anti- $Na_v1.7$ antibody, which selectively inhibits the function of said ion channel, said antibody or fragment having a light chain which comprises at least one CDR having the sequence given herein for light chain CDRs (see tables and lists provides), for example where the CDR is in its "natural position".

In one embodiment the antibody of the present invention comprises a light chain, wherein at least two of CDR-L1, CDR-L2 and CDR-L3 in the variable domain of the light chain are selected from sequences given herein for light chain CDRs, for example the CDRs are in their natural position and optionally in their natural pairing. Natural pairing as employed herein is intended to refer to the fact that a CDR from the one antibody is paired/co-located in a variable domain with a CDR derived from the same antibody, in particular the same chain of the same antibody (i.e. from one table above).

For the avoidance of doubt, it is understood that all permutations are included.

In one example the antibody of the present invention comprises a light chain, wherein the variable domain comprises the sequence given in:

SEQ ID NO:1 for CDR-L1,
SEQ ID NO:2 for CDR-L2 and
SEQ ID NO:3 for CDR-L3,
or
SEQ ID NO:7 for CDR-L1,
SEQ ID NO:8 for CDR-L2 and
SEQ ID NO:9 for CDR-L3,
or
SEQ ID NO:13 for CDR-L1,
SEQ ID NO:14 for CDR-L2 and
SEQ ID NO:15 for CDR-L3,
or,
SEQ ID NO:19 for CDR-L1,
SEQ ID NO:20 for CDR-L2 and

SEQ ID NO:21 for CDR-L3,
or
SEQ ID NO:25 for CDR-L1,
SEQ ID NO:26 for CDR-L2 and
SEQ ID NO:27 for CDR-L3,
or
SEQ ID NO:31 for CDR-L1,
SEQ ID NO:32 for CDR-L2 and
SEQ ID NO:33 for CDR-L3,
or
SEQ ID NO:37 for CDR-L1,
SEQ ID NO:38 for CDR-L2 and
SEQ ID NO:39 for CDR-L3,
or
SEQ ID NO:43 for CDR-L1,
SEQ ID NO:44 for CDR-L2 and
SEQ ID NO:45 for CDR-L3,
or
SEQ ID NO:49 for CDR-L1,
SEQ ID NO:50 for CDR-L2 and
SEQ ID NO:51 for CDR-L3,
or
SEQ ID NO:55 for CDR-L1,
SEQ ID NO:56 for CDR-L2 and
SEQ ID NO:57 for CDR-L3,
a sequence having at least 60%, 70%, 80% such as at least 90%, 95% or 98% identity or similarity thereto.

In one embodiment, the present invention comprises a light chain, wherein the variable domain of the light chain comprises a variable domain or variable domain components as disclosed herein, for example from any heavy chain described.

In another embodiment, the antibody of the present invention comprises a light chain, wherein the variable domain of the light chain comprises a sequence having at least 60%, 70%, 80% identity or similarity, such as at least 90%, 95% or 98% identity or similarity to a heavy chain variable region disclosed herein.

In one embodiment there is provided pair of variable domains, for example a heavy chain variable domain and light chain variable domain. In one aspect there is provided a heavy and light chain variable domain pair which is a cognate pair.

The antibody molecules of the present invention comprise a complementary light chain or a complementary heavy chain, respectively.

In one embodiment the heavy and light chain are a natural pairing, that is to say are derived from the same antibody, for example as shown in a single table herein.

In one embodiment the heavy and the light chain have a non-natural pairing.

One antibody provided by the present invention is referred to herein as antibody 983 shown in Figure 4.

In a further aspect the invention also provides a nucleotide sequence encoding an antibody or fragment thereof according to the present disclosure.

Also provided by the present invention is a CDR-grafted (or humanised) anti-ion channels antibody (as per the current invention) for example directed to an ion channel described herein, in particular an anti-$Na_v1.7$ antibody characterised in that the antibody is functionally modifying to said ion channel. In one embodiment one or more of the CDRs in the CDR-grafted antibody molecule have been obtained from the rat antibody 983. As used herein, the term 'CDR-grafted antibody molecule' refers to an antibody molecule wherein the heavy and/or light chain contains one or more CDRs (including, if desired, one or more modified CDRs) from a donor antibody (e.g. a rat or rabbit antibody as described herein) grafted into a heavy and/or light chain variable region framework of an acceptor antibody (e.g. a human antibody). For a review, see Vaughan et al, Nature Biotechnology, 16, 535-539, 1998.

When the CDRs are grafted, any appropriate acceptor variable region framework sequence may be used having regard to the class/type of the donor antibody from which the CDRs are derived, including rat, rabbit, mouse, primate and human framework regions. Preferably, the CDR-grafted antibody of the present invention

has a variable domain comprising human acceptor framework regions as well as one or more of the CDRs derived from the donor antibody as referred to herein. Thus, provided is a CDR-grafted antibody wherein the variable domain comprises human acceptor framework regions and non-human, preferably rat, mouse or rabbit donor CDRs.

Examples of human frameworks which can be used in the present invention are KOL, NEWM, REI, EU, TUR, TEI, LAY and POM (Kabat et al., supra). For example, KOL and NEWM can be used for the heavy chain, REI can be used for the light chain and EU, LAY and POM can be used for both the heavy chain and the light chain. Alternatively, human germline sequences may be used; these are available at: http://vbase.mrc-cpe.cam.ac.uk/. In a further alternative a database of affinity matured human V region sequences may be used as a framework.

In a CDR-grafted antibody of the present invention, the acceptor heavy and light chains do not necessarily need to be derived from the same antibody and may, if desired, comprise composite chains having framework regions derived from different chains.

Also, in a CDR-grafted antibody of the present invention, the framework regions need not have exactly the same sequence as those of the acceptor antibody. For instance, unusual residues may be changed to more frequently-occurring residues for that acceptor chain class or type. Alternatively, selected residues in the acceptor framework regions may be changed so that they correspond to the residue found at the same position in the donor antibody (see Reichmann et al., 1998, Nature, 332, 323-324). Such changes should be kept to the minimum necessary to recover the affinity of the donor antibody. A protocol for selecting residues in the acceptor framework regions which may need to be changed is set forth in WO 91/09967.

Donor residues are residues from the donor antibody, i.e. the antibody from which the CDRs were originally derived, which may in one embodiment of the present invention are derived from rat, mouse or rabbit antibodies and may be incorporated into the final antibody or fragment as required.

In one embodiment, the antibody (or fragment such as a Fab or Fab' fragment) is a monoclonal, fully human, humanized or chimeric antibody fragment. In one embodiment the antibody Fab or Fab' fragments are fully human or humanised.

Thus antibodies for use in the present invention may therefore comprise a complete antibody molecule having full length heavy and light chains or a fragment thereof and may be, but are not limited to Fab, modified Fab, Fab', $F(ab')_2$, Fv, single domain antibodies (such as VH, VL, VHH, IgNAR V domains), scFv, bi, tri or tetra-valent antibodies, bis-scFv, diabodies, triabodies, tetrabodies and epitope-binding fragments of any of the above (see for example Holliger and Hudson, 2005, Nature Biotech. 23(9):1126-1136; Adair and Lawson, 2005, Drug Design Reviews - Online 2(3), 209-217). The methods for creating and manufacturing these antibody fragments are well known in the art (see for example Verma et al., 1998, Journal of Immunological Methods, 216, 165-181). Other antibody fragments for use in the present invention include the Fab and Fab' fragments described in International patent applications WO2005/003169, WO2005/003170 and WO2005/003171. Multi-valent antibodies may comprise multiple specificities or may be monospecific (see for example WO 92/22853 and WO05/113605).

In one example the antibodies for use in the present invention may be derived from a camelid, such as a camel or llama. Camelids possess a functional class of antibodies devoid of light chains, referred to as heavy chain antibodies (Hamers *et al.*, 1993, Nature, 363, 446-448; Muyldermans, *et al.*, 2001, Trends. Biochem.Sci. 26, 230-235). The antigen-combining site of these heavy-chain antibodies is limited to only three hypervariable loops (H1-H3) provided by the N-terminal variable domain (VHH). The first crystal structures of VHHs revealed that the H1 and H2 loops are not restricted to the known canonical structure classes defined for conventional antibodies (Decanniere, *et al.*, 2000, J. Mol.Biol, 300, 83-91). The H3 loops of VHHs are on average longer than those of conventional antibodies (Nguyen *et al.*, 2001, Adv. Immunol., 79, 261-296). A large fraction of dromedary heavy chain antibodies have a preference for binding into active sites of enzymes against which they are raised (Lauwereys *et al.*, 1998, EMBO J, 17, 3512-3520). In one case, the H3 loop was shown to protrude from the remaining paratope and insert in the active site of the

hen egg white lysozyme (Desmyter *et al.*, 1996, Nat.Struct.Biol.3, 803-811 and De Genst *et al.*, 2006, PNAS, 103, 12, 4586-4591 and WO97049805).

It has been suggested that these loops can be displayed in other scaffolds and CDR libraries produced in those scaffolds (See for example WO03050531 and WO97049805).

In one example the antibodies for use in the present invention may be derived from a cartilaginous fish, such as a shark. Cartilaginous fish (sharks, skates, rays and chimeras) possess an atypical immunoglobulin isotype known as IgNAR. IgNAR is an H-chain homodimer that does not associate with light chain. Each H chain has one variable and five constant domains. IgNAR V domains (or V-NAR domains) carry a number of non canonical cysteines that enable classification into two closely related subtypes, I and II. Type II V regions have an additional cysteine in CDRs 1 and 3 which have been proposed to form a domain-constraining disulphide bond, akin to those observed in camelid VHH domains. The CDR3 would then adopt a more extended conformation and protrude from the antibody framework akin to the camelid VHH. Indeed, like the VHH domains described above, certain IgNAR CDR3 residues have also been demonstrated to be capable of binding in the hen egg white lysozyme active site (Stanfield *et al.*, 2004, Science, 305, 1770-1773.

Examples of methods of producing VHH and IgNAR V domains are described in for example, Lauwereys *et al*, 1998, EMBO J. 1998, 17(13), 3512-20; Liu *et al.*, 2007, BMC Biotechnol., 7, 78; Saerens et al., 2004, J. Biol. Chem., 279 (5), 51965-72.

Antibodies for use in the present invention include whole antibodies of any suitable class for example, IgA, IgD, IgE, IgG or IgM or subclass such as IgG1, IgG2, IgG3 or IgG4. and functionally active fragments or derivatives thereof and may be, but are not limited to, monoclonal, clonal, humanised, fully human or chimeric antibodies.

In one embodiment the constant region employed, in the antibody or certain fragments thereof according to the disclosure, is a hybrid constant region or mutated constant region. Hybrid constant regions comprises portions or domains from two or more distinct constant regions, for example two or more distinct human constant regions.

Examples of hybrid constant regions include those disclosed in US2007/0041972, where at least CH1 and the hinge region are derived from one or more IgG2 antibodies and at least a portion of the CH2 and CH3 regions are derived from one or more IgG4 CH2 and CH3 regions. Eculizimumab (Alexion Pharmaceuticals) is a humanised anti-human C5 mAb for paroxysmal nocturnal hemoglobinuria comprising a hybrid constant region. It has a hybrid chain of IgG2 derived CH1 and hinge with IgG4 derived CH2 and CH3 domains. It does not bind FcγR nor does it activate complement. It also has low immunogenicity (low titres of anti-Eculizimumab antibodies detected in only 3 of 196 (3%) patients).

WO 2008/090958 discloses certain hybrid constant regions comprising a chain of CH1, hinge and CH2 from IgG1 and a CH3 domain from IgG3. The hybrid has a higher CDC activity than that of an IgG1 or IgG3 antibody and a protein A-binding activity equivalent to that of IgG1.

Further hybrid constant regions are disclosed in Tao et al., (S.L.Morrison's group) J. Exp.Med 173 1025-1028, 1991. This paper contains many IgG domain swaps from all classes but the key hybrids are g1g4 and g4g1, each joined in the CH2 domain. IgG (1-1-1/4-4) is completely unable to activate complement in contrast to IgG1. However, IgG(4-4-4/1-1) showed significant activity compared with IgG4 but was slightly impaired compared with IgG1. The key difference seems to be the hinge and many papers have since demonstrated that the hinge modulates but does not mediates complement activation.

Tao et al., (S.L.Morrison's group) J. Exp.Med 178 661-667, 1993 discloses structural features of human IgG that determine isotype-specific differences in complement activation. Ser331 (CH2) in IgG4 prevents C1q binding and complement activation. Mutagenesis of Ser331 to Pro in IgG4 and IgG (1-1-1/4-4) allows binding and activation but at a lower level than that of IgG1. Interestingly P331S in IgG1 allows binding but not activation.

Zucker et al., Canc Res 58 3905-3908 1998 employs Chimeric human-mouse IgG abs with shuffled constant region exons to demonstate that muliple domains contribute to *in vivo* half-life. In particular this article examines half-life of IgG (1-1-1/4-4) hybrid

and others. In SCID mice, IgG (1-1-1/4-4) has a significantly longer half-life than IgG4 but slightly less than IgG1. IgG (4-4-4/1-1) has the longest half-life.

An example of a mutated constant region includes that employed in Abatacept, which is a fusion of human CTLA-4 with IgG1 hinge-Fc. The hinge was altered from CPPC to SPPS. The latter is O-gly. The mutated constant region does not mediate ADCC or CDC and has low immunogenicity (3% incidence).

The hinge is thought to potentially have a role in complement activiation. The functional hinge, deduced from crystallographic studies, extends from 216-237 of IgG1 and consists of **EPKSCDKTHT**CPPCPAPELLGG (SEQ ID NO: 106) **upper**, middle and lower hinge respectively. In one embodiment an antibody or fragment according to the disclosure comprises a functional hinge.

Mutations/modifications to the constant region may, for example result in increased stability, for example US 2004/0191265 discloses mutagenesis of IgG1 hinge, which increased the stability of an IgG by introducing one or more amino acid modifications in the hinge region at positions 233-239 or 249 of human IgG1. This provided reduced degradation upon heating to 55°C for one week.

Alternatively, modification may be effected by making point mutations in labile amino acids (e.g., histidine or threonine) or reactive amino acids (e.g., lysine or glutamic acid) in the upper hinge portion (human IgG1 residues 226-243 and corresponding residues in other IgG subtypes and/or immunoglobulins from other species) and/or in the flanking CH1 and/or CH2 sequences (human IgG1 residue 249 and corresponding residues in other IgG subtypes and/or immunoglobulins from other species).

Monoclonal antibodies may be prepared by any method known in the art such as the hybridoma technique (Kohler & Milstein, *Nature*, 1975, 256, 495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al., Immunology Today*, 1983, 4, 72) and the EBV-hybridoma technique (Cole *et al.,* "Monoclonal Antibodies and Cancer Therapy", pp. 77-96, Alan R. Liss, Inc., 1985).

Antibodies for use in the invention may also be generated using single lymphocyte antibody methods by cloning and expressing immunoglobulin variable region cDNAs generated from single lymphocytes selected for the production of specific antibodies by, for example, the methods described by Babcook, J. *et al.*, *Proc. Natl. Acad. Sci. USA*, 1996, 93(15), 7843-7848, WO 92/02551, WO2004/051268 and WO2004/106377.

Humanized antibodies are antibody molecules from derived non-human species having one or more complementarity determining regions (CDRs) from the non-human species and a framework region from a human immunoglobulin molecule (see, for example, US 5,585,089).

The antibodies for use in the present invention can also be generated using various phage display methods known in the art and include those disclosed by Brinkman *et al.*, *J. Immunol. Methods*, 1995, 182, 41-50; Ames *et al.*, *J. Immunol. Methods*, 1995, 184, 177-186; Kettleborough *et al. Eur. J. Immunol.*, 1994, 24, 952-958; Persic *et al.*, *Gene*, 1997 187, 9-18; and Burton *et al.*, *Advances in Immunology*, 1994, 57, 191-280; WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; and WO 95/20401; and US 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743; and 5,969,108. Also, transgenic mice, or other organisms, including other mammals, may be used to generate humanized antibodies.

Fully human antibodies are those antibodies in which the variable regions and the constant regions (where present) of both the heavy and the light chains are all of human origin, or substantially identical to sequences of human origin, not necessarily from the same antibody. Examples of fully human antibodies may include antibodies produced for example by the phage display methods described above and antibodies produced by mice in which the murine immunoglobulin variable and/or constant region genes have been replaced by their human counterparts eg. as described in general terms in EP0546073 B1, US 5,545,806, US 5,569,825, US 5,625,126, US 5,633,425, US 5,661,016, US5,770,429, EP 0438474 B1 and EP0463151 B1.

The antibody or fragment for use in the present invention may be obtained from any whole antibody, especially a whole monoclonal antibody, using any suitable enzymatic cleavage and/or digestion techniques, for example by treatment with pepsin.

Alternatively, or in addition the antibody starting material may be prepared by the use of recombinant DNA techniques involving the manipulation and re-expression of DNA encoding antibody variable and/or constant regions. Standard molecular biology techniques may be used to modify, add or delete amino acids or domains as desired. Any alterations to the variable or constant regions are still encompassed by the terms 'variable' and 'constant' regions as used herein.

As discussed, antibody fragment "starting material" may be obtained from any species including for example mouse, rat, rabbit, hamster, shark, camel, llama, goat or human. Parts of the antibody fragment may be obtained from more than one species, for example the antibody fragments may be chimeric. In one example, the constant regions are from one species and the variable regions from another. The antibody fragment starting material may also be modified. In another example, the variable region of the antibody fragment has been created using recombinant DNA engineering techniques. Such engineered versions include those created for example from natural antibody variable regions by insertions, deletions or changes in or to the amino acid sequences of the natural antibodies. Particular examples of this type include those engineered variable region domains containing at least one CDR and, optionally, one or more framework amino acids from one antibody and the remainder of the variable region domain from a second antibody. The methods for creating and manufacturing these antibody fragments are well known in the art (see for example, Boss et al., US 4,816,397; Cabilly et al., US 6,331,415; Shrader et al., WO 92/02551; Ward et al., 1989, Nature, 341, 544; Orlandi et al., 1989, Proc.Natl.Acad.Sci. USA, 86, 3833; Riechmann et al., 1988, Nature, 322, 323; Bird et al, 1988, Science, 242, 423; Queen et al., US 5,585,089; Adair, WO91/09967; Mountain and Adair, 1992, Biotechnol. Genet. Eng. Rev, 10, 1-142; Verma et al., 1998, Journal of Immunological Methods, 216, 165-181).

Thus in one embodiment constant region domains may be human IgA, IgD, IgE, IgG or IgM domains. In particular, human IgG constant region domains may be used, especially of the IgG1 and IgG3 isotypes when the antibody molecule is intended for therapeutic uses and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the antibody molecule is intended for therapeutic purposes and antibody effector functions are not required. It will be appreciated that sequence variants of these constant region domains may also be used. For example IgG4 molecules in which the serine at position 241 has been changed to proline as described in Angal *et al.*, Molecular Immunology, 1993, 30 (1), 105-108 may be used. It will also be understood by one skilled in the art that antibodies may undergo a variety of posttranslational modifications. The type and extent of these modifications often depends on the host cell line used to express the antibody as well as the culture conditions. Such modifications may include variations in glycosylation, methionine oxidation, diketopiperazine formation, aspartate isomerization and asparagine deamidation. A frequent modification is the loss of a carboxy-terminal basic residue (such as lysine or arginine) due to the action of carboxypeptidases (as described in Harris, RJ. *Journal of Chromatography* 705:129-134, 1995).

In one embodiment the antibody or fragment light chain comprises a CL domain, either kappa or lambda.

The term 'antibody' of fragment as used herein may also include binding agents which comprise one or more CDRs incorporated into a biocompatible framework structure. In one example, the biocompatible framework structure comprises a polypeptide or portion thereof that is sufficient to form a conformationally stable structural support, or framework, or scaffold, which is able to display one or more sequences of amino acids that bind to an antigen (e.g. CDRs, a variable region etc.) in a localised surface region. Such structures can be a naturally occurring polypeptide or polypeptide 'fold' (a structural motif), or can have one or more modifications, such as additions, deletions or substitutions of amino acids, relative to a naturally occurring polypeptide or fold. These scaffolds can be derived from a polypeptide of any species (or of more than one species), such as a human, other mammal, other vertebrate, invertebrate, plant, bacteria or virus.

Typically the biocompatible framework structures are based on protein scaffolds or skeletons other than immunoglobulin domains. For example, those based on fibronectin, ankyrin, lipocalin, neocarzinostain, cytochrome b, CP1 zinc finger, PST1, coiled coil, LACI-D1, Z domain and tendramisat domains may be used (See for example, Nygren and Uhlen, 1997, Current Opinion in Structural Biology, 7, 463-469).

In one embodiment the overall charge of the antibody or fragment is neutral.

In one embodiment the overall charge of the antibody or fragment is negative.

In one embodiment the overall charge of the antibody or fragment is positive.

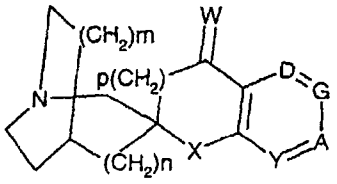
In one embodiment the CDRs, may be grafted or engineered into an alternative type of scaffold, for example a fibronectin or actin-binding repeats.

In one embodiment the antibody or fragment comprises an effector molecule, such as a polymer, toxin including biotoxins such as venom or chemical inhibitor conjugated thereto.

Biotoxins and venom are natural modulators, such as blockers of cell signalling. When conjugated to an antibody or fragment according to the invention then they can be used to augment the functional effect on the ion channel, whilst maintaining the selectivity provided by the antibody. Tarantula venom peptide ProTxII has, for example be shown to selectively inhibit $Na_v1.7$, see for example Mol Pharmacol 74:1476-1484, 2008. In one embodiment the toxin is botulinum toxin, for example botulinum toxin A. Other toxins include tetrodotoxin and saxitoxin.

In one embodiment the antibody or fragment is conjugated to an aptamer. Aptamers are single stranded oligonucleotide sequences that adopt secondary and tertiary structures and that are able to bind and modulate protein activity. The conjugation of such structures to antibodies will lead to a target specific modulation of the ion channel (direct effect).

In one embodiment the antibody or fragment is conjugated to chemical inhibitor such as a synthetic chemical inhibitor of an ion channel. Examples of chemical inhibitors include compounds of formula (I):



I

wherein n is 0 or 1;
m is 0 or 1;
p is 0 or 1;
Y is CH, N or NO
X is oxygen or sulfur;
W is oxygen, H_2 or F_2;
A is N or $C(R^2)$;
G is N or $C(R^3)$;
D is N or $C(R^4)$;

with the proviso that no more than one of A, G, and D is nitrogen but at least one of Y, A, G, and D is nitrogen or NO;
R^1 is hydrogen or C_1-C_4 alkyl;
R^2, R^3, and R^4 are independently hydrogen, halogen, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, aryl, heteroaryl, OH, OC_1-C_4 alkyl, CO_2R^1, -CN, $-NO_2$, $-NR^5R^6$, $-CF_3$, $-OSO_2CF_3$, or R^2 and R^3, or R^3 and R^4, respectively, may together form another six membered aromatic or heteroaromatic ring sharing A and G, or G and D, respectively containing between zero and two nitrogen atoms, and substituted with one to two of the following substituents: independently hydrogen, halogen, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, aryl, heteroaryl, OH, OC_1-C_4 alkyl, CO_2R^1, -CN, $-NO_2$, $-NR^5R^6$, $-CF_3$, OSO_2CF_3 ;

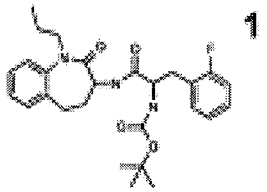
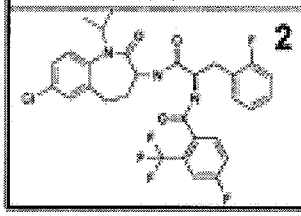
R^5 and R^6 are independently hydrogen, C_1-C_4 alkyl, $C(O)R^7$, $C(O)NHR^8$, $C(O)OR^9$, SO_2R^{10} or may together be $(CH_2)_j(CH_2)_kQ(CH_2)_k$ where Q is O, S, NR^{11}, or a bond;
j is 2 to 7;
k is 0 to 2;
R^7, R^8, R^9, R^{10}, and R^{11} are independently C_1-C_4 alkyl, aryl, or heteroaryl,
or an enantiomer thereof, and the pharmaceutically acceptable salts thereof is a potent ligand for nicotinic acetylch receptors.

See EP0996622.

Bioorg Med Chem (2003) 11: 2099-113. RA Hill, S Rudra, B Peng, DS Roane, JK Bounds, Y g, A Adloo, T Lu, discloses certain hydroxyl substituted sulfonylureas as inhibitors.

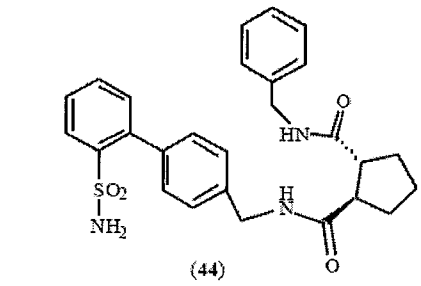
4,4-Diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS) has been used as an anion-transport inhibitor.

The following are also chemical inhibitors of $Na_v1.7$

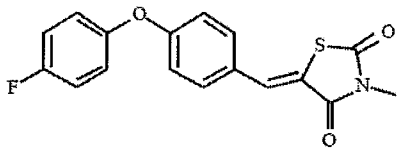
	FRET-based Assay (μM)			Electrophysiology (μM)	
	hNav1.5	hNav1.7	hNav1.8	*hNav1.5*	*hNav1.7*
1 	0.02	0.03	0.27	0.17	0.37
2 	0.18	0.03	0.30	4.30	0.55

The compound labeled 2 in the table above *N*-[(R)-1-(R)-7-chloro-1-isopropyl-2-oxo-2,3,4,5-tetrahydro-1*H*-benzo[b]azepine-3-ylcarbamoyl)-2-(2-fluorophenyl)-ethyl]-4-fluoro-2-trifluoromethyl-benzamide is discussed in a paper by McGowan *et al* Anesthesia and Analgesia Vol 109, No. 3, September 2009 (entitled A Peripherally Acting $Na_v1.7$ Sodium Channel Blocker Reverses Hyperalgesia and Allodynia on Rat Models of Inflammatory and Neuropathic Pain).

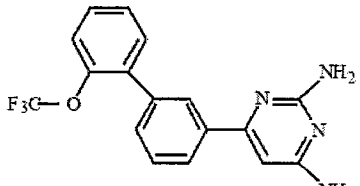
Tarnawa et al (2007) (Blockers of voltage-gated sodium channels for the treatment of central nervous system diseases, Recent Patents on CNS Drug Discovery, 2:57) reviewed the more recent medicinal chemistry of sodium channel blockers. Several old drugs such as lidocaine, mexiletine, carbamazepine, phenytoin, lamotrigine and newly developed drugs such as lacosamide, oxcarbazepine, crobenetine, ralfinamide are sodium channel blockers that have proved to be effective in the treatment of various types of chronic pain in animal models, and some of them are used clinically also. Some other examples of chemical modulators of voltage-gated sodium channels are listed below:



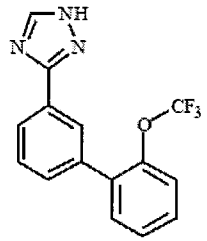
(44)



(45)

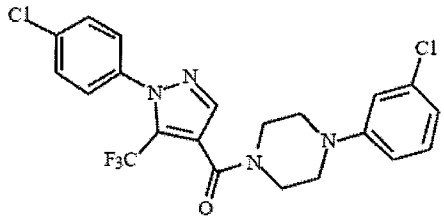


(46)



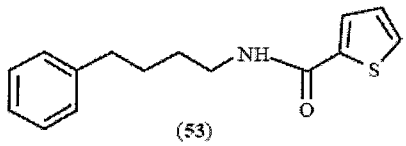
(47)

Fig. (15a). Compounds with a combination of aromatic and heteroaromatic rings, patented by Merck.

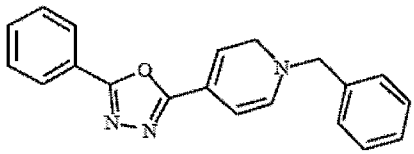


(52)

Fig. (16). Biarylcarboxamide compounds patented by Atkinson.

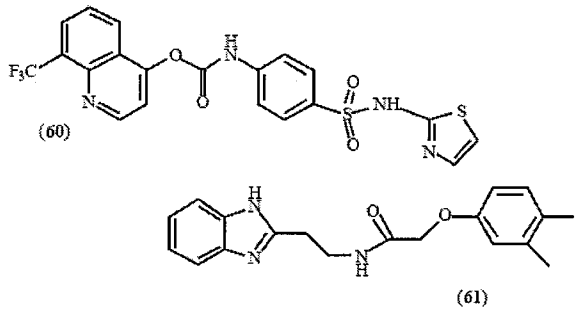


(53)



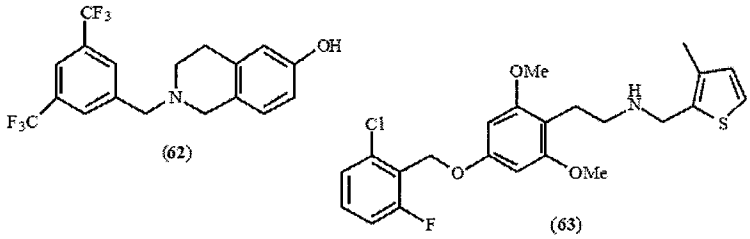
(54)

Fig. (17). Compounds with a combination of aromatic and heteroaromatic rings, patented by Ehring.



(60) (61)

Fig. (18b). Compounds with combined aromatic and heteroaromatic rings patented by Vertex.

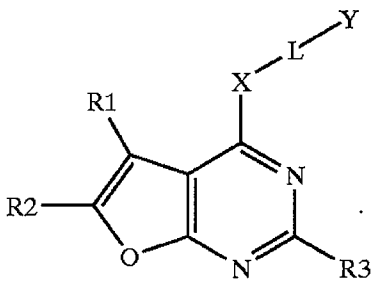


(62) (63)

Fig. (19). Compounds with combined aromatic and heteroaromatic rings patented by Ionix.

Other references describing chemical modulators of voltage-gated sodium channels: Anger et al. (2001) J. Med. Chem. 44(2):115; Hoyt et al. (2007), Bioorg. Med. Chem. Lett. 17:6172; Yang et al. (2004), J. Med. Chem. 47:1547; Benes et al. (1999) J. Med. Chem. 42:2582

US7456187 discloses certain potassium channel inhibitors of formula:



Wherein

R_1 is aryl, heteroaryl, cycloalkyl or alkyl;

R_2 is H, alkyl, nitro, —CO_2R_7, $CONR_4R_5$ or halo;

R_3 is H, NR_4R_5, $NC(O)R_8$, halo, trifluoromethyl, alkyl, nitrile or alkoxy;

R_4 and R_5 may be the same or different, and may be H, alkyl, aryl, heteroaryl or cycloalkyl; or R_4 and R_5 may together form a saturated, unsaturated or partially saturated 4 to 7 member ring, wherein said ring may optionally comprise one or more further heteroatoms selected from N, O or S;

X is O, S or NR_6;

R_6 is H or alkyl;

R_7 is hydrogen, methyl or ethyl;

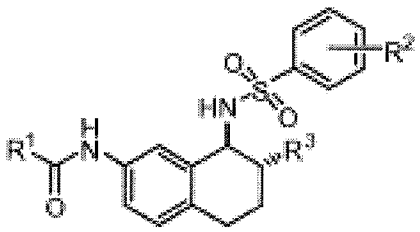
R_8 is methyl or ethyl;

L is $(CH_2)_n$, where n is 1, 2 or 3; and

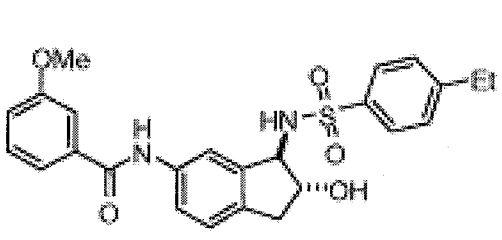
Y is aryl, a heterocyclic group, alkyl, alkenyl or cycloalkyl;

or pharmaceutically acceptable salts thereof.

Bioorganic and Medical Chemistry Letters Vol 19, Issue 11, 1 June 2009 pages 3063-3066 discloses certain inhibitors of formula:

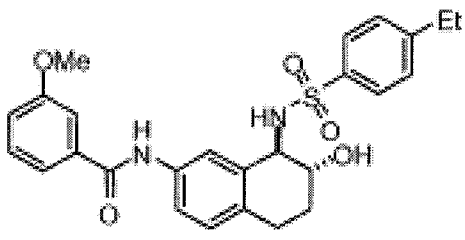


more specifically:



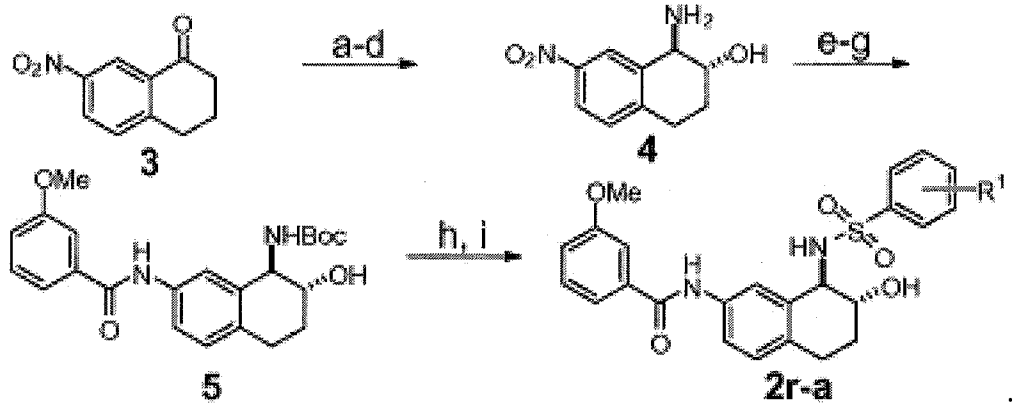
1

Kv1.5 IC_{50} 0.16 μM

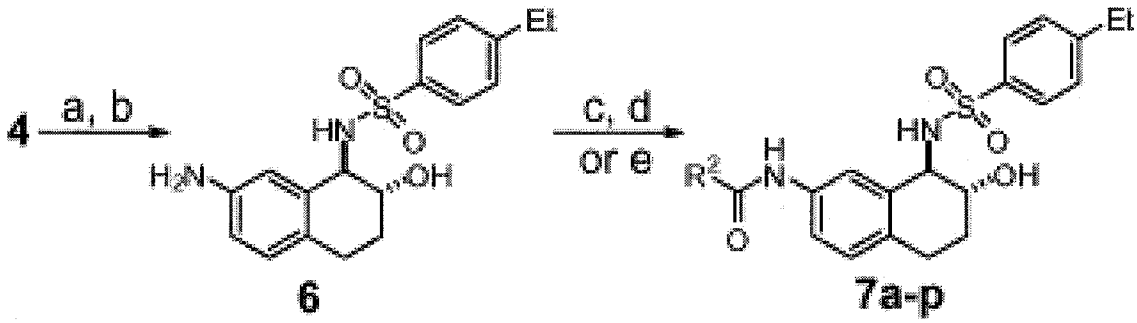


2a

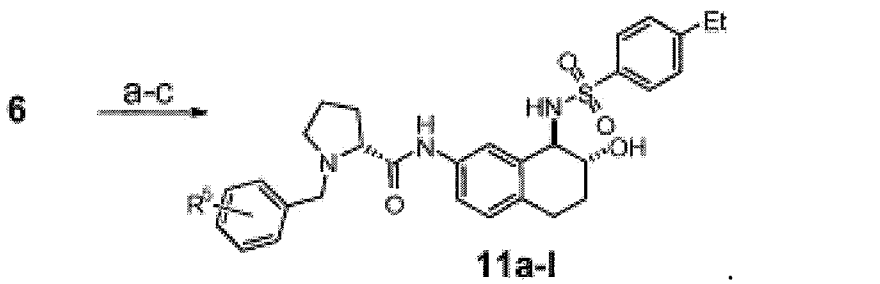
Kv1.5 IC_{50} 0.44 μM ;



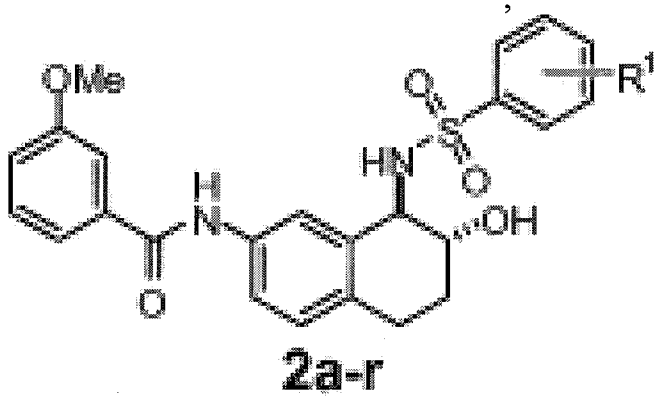
;



;



;



.

In one embodiment the entity conjugated to the antibody or fragment changes the overall charge of the molecule.

The term effector molecule as used herein includes, for example, antineoplastic agents, drugs, toxins, biologically active proteins, for example enzymes, other antibody or antibody fragments, synthetic or naturally occurring polymers, nucleic acids and fragments thereof e.g. DNA, RNA and fragments thereof, radionuclides, particularly radioiodide, radioisotopes, chelated metals, nanoparticles and reporter groups such as fluorescent compounds or compounds which may be detected by NMR or ESR spectroscopy.

Examples of effector molecules may include cytotoxins or cytotoxic agents including any agent that is detrimental to (*e.g.* kills) cells. Examples include combrestatins, dolastatins, epothilones, staurosporin, maytansinoids, spongistatins, rhizoxin, halichondrins, roridins, hemiasterlins, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof.

Effector molecules may also include, but are not limited to, antimetabolites (*e.g.* methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.* mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.* daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.* dactinomycin (formerly actinomycin), bleomycin, mithramycin, anthramycin (AMC), calicheamicins or duocarmycins), and anti-mitotic agents (*e.g.* vincristine and vinblastine).

Other effector molecules may include chelated radionuclides such as ^{111}In and ^{90}Y, Lu^{177}, Bismuth^{213}, Californium^{252}, Iridium^{192} and Tungsten^{188}/Rhenium^{188}; or drugs such as but not limited to, alkylphosphocholines, topoisomerase I inhibitors, taxoids and suramin.

Other effector molecules include proteins, peptides and enzymes. Enzymes of interest include, but are not limited to, proteolytic enzymes, hydrolases, lyases, isomerases, transferases. Proteins, polypeptides and peptides of interest include, but are not limited to, immunoglobulins, toxins such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin.

Other effector molecules may include detectable substances useful for example in diagnosis. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive nuclides, positron emitting metals (for use in positron emission tomography), and nonradioactive paramagnetic metal ions. See generally U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics. Suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; suitable prosthetic groups include streptavidin, avidin and biotin; suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride and phycoerythrin; suitable luminescent materials include luminol; suitable bioluminescent materials include luciferase, luciferin, and aequorin; and suitable radioactive nuclides include ^{125}I, ^{131}I, ^{111}In and ^{99}Tc.

In another example the effector molecule may increase the half-life of the antibody *in vivo*, and/or reduce immunogenicity of the antibody and/or enhance the delivery of an antibody across an epithelial barrier to the immune system. Examples of suitable effector molecules of this type include polymers, albumin, albumin binding proteins or albumin binding compounds such as those described in WO05/117984.

Where the effector molecule is a polymer it may, in general, be a synthetic or a naturally occurring polymer, for example an optionally substituted straight or branched chain polyalkylene, polyalkenylene or polyoxyalkylene polymer or a branched or unbranched polysaccharide, e.g. a homo- or hetero- polysaccharide.

Specific optional substituents which may be present on the above-mentioned synthetic polymers include one or more hydroxy, methyl or methoxy groups.

Specific examples of synthetic polymers include optionally substituted straight or branched chain poly(propyleneglycol) poly(vinylalcohol) or derivatives thereof, especially optionally substituted poly(ethyleneglycol) such as methoxypoly(ethyleneglycol) or derivatives thereof.

Suitable polymers include a polyalkylene polymer, such as a poly(ethyleneglycol) or, especially, a methoxypoly(ethyleneglycol) or a derivative thereof, and especially with a molecular weight in the range from about 15000Da to about 40000Da.

The size of the polymer may be varied as desired, but will generally be in an average molecular weight range from 500Da to 50000Da, for example from 5000 to 40000Da such as from 20000 to 40000Da. The polymer size may in particular be selected on the basis of the intended use of the product for example ability to localize to certain tissues such as tumors or extend circulating half-life (for review see Chapman, 2002, Advanced Drug Delivery Reviews, 54, 531-545). Thus, for example, where the product is intended to leave the circulation and penetrate tissue, for example for use in the treatment of a tumour, it may be advantageous to use a small molecular weight polymer, for example with a molecular weight of around 5000Da. For applications where the product remains in the circulation, it may be advantageous to use a higher molecular weight polymer, for example having a molecular weight in the range from 20000Da to 40000Da.

In one embodiment the PEG employed is releasable PEG, for example as supplied by Enzon pharmaceuticals.

In one example antibodies for use in the present invention are attached to poly(ethyleneglycol) (PEG) moieties. In one particular example the antibody is an antibody fragment and the PEG molecules may be attached through any available amino acid side-chain or terminal amino acid functional group located in the antibody fragment, for example any free amino, imino, thiol, hydroxyl or carboxyl group. Such amino acids may occur naturally in the antibody fragment or may be engineered into the fragment using recombinant DNA methods (see for example US 5,219,996; US 5,667,425; WO98/25971). In one example the antibody molecule of the present invention is a modified Fab fragment wherein the modification is the addition to the

C-terminal end of its heavy chain one or more amino acids to allow the attachment of an effector molecule. Suitably, the additional amino acids form a modified hinge region containing one or more cysteine residues to which the effector molecule may be attached. Multiple sites can be used to attach two or more PEG molecules.

In one embodiment the Fab or Fab' is PEGylated with one or two PEG molecules.

In one embodiment a PEG molecule is linked to a cysteine 171 in the light chain, for example see WO2008/038024 incorporated herein by reference.

In one the Fab or Fab' is PEGylated through a surface accessible cysteine.

Suitably PEG molecules are covalently linked through a thiol group of at least one cysteine residue located in the fusion protein. Each polymer molecule attached to the fusion protein may be covalently linked to the sulfur atom of a cysteine residue located in the protein. The covalent linkage will generally be a disulphide bond or, in particular, a sulfur-carbon bond. Where a thiol group is used as the point of attachment appropriately activated PEG molecules, for example thiol selective derivatives such as maleimides and cysteine derivatives may be used. An activated PEG molecule may be used as the starting material in the preparation of polymer-fusion protein containing molecules as described above. The activated PEG molecule may be any polymer containing a thiol reactive group such as an α-halocarboxylic acid or ester, e.g. iodoacetamide, an imide, e.g. maleimide, a vinyl sulphone or a disulphide. Such starting materials may be obtained commercially (for example from Nektar, formerly Shearwater Polymers Inc., Huntsville, AL, USA) or may be prepared from commercially available starting materials using conventional chemical procedures. Particular PEG molecules include 20K methoxy-PEG-amine (obtainable from Nektar, formerly Shearwater; Rapp Polymere; and SunBio) and M-PEG-SPA (obtainable from Nektar, formerly Shearwater).

Effector molecules such a PEG molecules may be attached to antibodies or by a number of different methods, including through aldehyde sugars or more commonly through any available amino acid side-chain or terminal amino acid functional group

located in the antibody fragment, for example any free amino, imino, thiol, hydroxyl or carboxyl group. The site of attachment of effector molecules can be either random or site specific.

Random attachment is often achieved through amino acids such as lysine and this results in effector molecules, such as PEG molecules, being attached at a number of sites throughout the antibody fragment depending on the position of the lysines. While this has been successful in some cases the exact location and number of effector molecules, such as PEG molecules, attached cannot be controlled and this can lead to loss of activity for example if too few are attached and/or loss of affinity if for example they interfere with the antigen binding site (Chapman 2002 Advanced Drug Delivery Reviews, 54, 531-545). As a result, controlled site specific attachment of effector molecules, such as PEG molecules, is usually the method of choice.

Site specific attachment of effector molecules, such as PEG molecules, is most commonly achieved by attachment to cysteine residues since such residues are relatively uncommon in antibody fragments. Antibody hinges are popular regions for site specific attachment since these contain cysteine residues and are remote from other regions of the fusion protein likely to be involved in antigen binding. Suitable hinges either occur naturally in the fragment or may be created using recombinant DNA techniques (See for example US 5,677,425; WO98/25971; Leong et al., 2001 Cytokine, 16, 106-119; Chapman et al., 1999 Nature Biotechnology, 17, 780-783). Alternatively, or in addition, site-specific cysteines may also be engineered into the antibody fragment for example to create surface exposed cysteine(s) for effector molecule attachment (US 5,219,996).

Thus in one embodiment the PEG molecule is attached to a surface exposed cysteine.

A surface exposed cysteine (free cysteine) as employed herein is intended to refer to cysteine, that when the protein is in a "natual" folded conformation, is accessible for conjugating an effector molecule, such as a PEG molecule thereto. Examples of how to engineer free cysteines of this type are also provided in US7,521,541.

Specific naturally occurring polymers include lactose, amylose, dextran, glycogen or derivatives thereof.

"Derivatives" as used herein is intended to include reactive derivatives, for example thiol-selective reactive groups such as maleimides and the like. The reactive group may be linked directly or through a linker segment to the polymer. It will be appreciated that the residue of such a group will in some instances form part of the product as the linking group between the fusion protein and the polymer.

The present invention also provides isolated DNA encoding an antibody described herein or a fragment thereof of a heavy or light chain thereof.

In a further aspect there is provided a vector comprising said DNA.

General methods by which the vectors may be constructed, transfection methods and culture methods are well known to those skilled in the art. In this respect, reference is made to "Current Protocols in Molecular Biology", 1999, F. M. Ausubel (ed), Wiley Interscience, New York and the Maniatis Manual produced by Cold Spring Harbor Publishing.

In a further aspect there is provided a host cell comprising said vector and/or DNA.

Any suitable host cell/vector system may be used for expression of the DNA sequences encoding the fusion protein molecule of the present invention. Bacterial, for example *E. coli*, and other microbial systems may be used or eukaryotic, for example mammalian, host cell expression systems may also be used. Suitable mammalian host cells include CHO, myeloma or hybridoma cells.

The present invention also provides a process for the production of an antibody or fragment thereof according to the present invention comprising culturing a host cell containing a vector (and/or DNA) of the present invention under conditions suitable for leading to expression of protein from DNA encoding the antibody or fragment thereof, and isolating the same.

For production of products comprising both heavy and light chains, the cell line may be transfected with two vectors, a first vector encoding a light chain polypeptide and a second vector encoding a heavy chain polypeptide. Alternatively, a single vector may be used, the vector including sequences encoding light chain and heavy chain polypeptides.

In one aspect there is provided a method of generating antibodies employing peptides whose sequence are derived from E1 extracellular regions of an ion channel involved in modulation of pain, for example $Na_v1.7$. Such peptides, are used both in immunization protocols to raise polyclonal antibodies and/or in screening or panning protocols to select specific anti-ion channel antibodies, for example anti-$Na_v1.7$ antibodies. One embodiment uses peptides that correspond to discrete sequences of the aforementioned extracellular regions where there is maximum dissimilarity to other ion channels and isoforms thereof.

Based on the amino acid sequence numbering of the $Na_v1.7$ sequence deposited in the Swiss Prot database as human protein SCN9A (accession no. Q15858), these sequences are as follows:

domain A amino acids 146-153,

domain B amino acids 764-774,

domain C amino acids 1216-1224, and

domain D amino acids 1535-1545 are regions of particular difference/distinction and thus may be particularly suitable for generating antibodies.

The selected peptides may be conventional linear peptides or cyclic peptides and comprise at least 4 consecutive amino acids from the above sequences. In either case the peptide is designed to include a single functional group that is used for subsequent conjugation to carrier protein or reporter group. The said functional group may be a side chain thiol of a cysteine residue, a C-terminal carboxyl or side chain carboxyl of an aspartic acid or glutamic acid residue or primary amine of an N-terminal amino group or lysine side chain residue.

The amino acid residue bearing the said functional group may correspond to the native $Na_v1.7$ sequence or may be additional to the native $Na_v1.7$ sequence. The

position of this residue in the $Na_v1.7$ peptide sequence may be at either terminus of the sequence or at any internal position.In one embodiment the peptide is a linear peptide, for example, containing any of the following sequences, wherein the domain A, B, C or D of $Na_v1.7$, from which the peptide is derived is denoted in brackets. The cysteines which are underlined in the peptides are non-naturally occurring cysteine residues in the ion channel.: The cysteine residues in the following peptides may be used to attach a carrier protein.

CEHHPMTEEFKN,	(BE1)	(SEQ ID NO: 107)
EHHPMTEEFKNC,	(BE1)	(SEQ ID NO: 108)
CPMTEEFKN,	(BE1)	(SEQ ID NO: 109)
PMTEEFKNC,	(BE1)	(SEQ ID NO: 110)
CEDIYIERKKTIKI,	(CE1)	(SEQ ID NO: 111)
EDIYIEERKKTIKIC,	(CE1)	(SEQ ID NO: 112)
CIERKKTIKI,	(CE1)	(SEQ ID NO: 113)
IERKKTIKIC,	(CE1)	(SEQ ID NO: 114)
CERKKTIKI,	(CE1)	(SEQ ID NO: 115)
ERKKTIKIC,	(CE1)	(SEQ ID NO: 116)
CEKEGQSQHMTE,	(DE1)	(SEQ ID NO: 117)
EKEGQSQHMTEC.	(DE1)	(SEQ ID NO: 118)

These sequences may be capped at either N-terminal or C-terminal with for example an Nαacetyl or amide group respectively.

Other Nav1.7 sequences include:

AE1	MT**MNNP**PDW	(SEQ ID NO: 119)
BE1	MEHHPM**TEEFK**N	(SEQ ID NO: 120)
CE1	**IERKKTIKI**	(SEQ ID NO: 121)
DE1	E**KEGQSQHMTE**	(SEQ ID NO: 122)

In one embodiment of the present invention the $Na_v1.7$ peptide used in generating the anti-E1 ion channel antibody has an amino acid sequence selected from the group consisting of SEQ ID NOs:107 to 122. In a further embodiment, the $Na_v1.7$ peptide used in generating the anti-E1 ion channel antibody has an amino acid sequence selected from the group consisting of SEQ ID NOs:107, 108, 110 to 112, 114 to 116 and 119 to 127.

Immunogens from other E1 loops of ion channels may similarly be prepared, for example the respective immunogen will thus comprise of at least four consecutive amino acid residues, for example shown in bold type below and additional residues at either N-terminal and / or C-terminal ends in the sequence order as shown. Again the selected peptide may be capped at the N-terminus by a functional group such as an N-acetyl and / or at the C-terminal by a functional group such as an amide. The peptide may be synthesized as a linear peptide or a cyclic peptide. In either case the peptide is designed to contain a single unique functional group for covalent attachment to a macromolecular carrier such as a xenogenic protein.

If the peptide does not contain an aspartate, glutamate or lysine residue and is capped at the N-terminal, then the unique functional group may be the C-terminal carboxylic acid which may be derivatized directly for coupling to the carrier via amide chemistry.

If the peptide does not contain a lysine residue then the unique functional group may be the N-terminal amino group which can be derivatized to introduce a further reactive group such as a maleimide.

If the peptide contains a single cysteine residue then the side chain thiol may be the unique functional group which can be coupled to carrier via maleimide chemistry.

A unique functional group may be incorporated by an additional residue (either natural or non-natural amino acid) e.g. a cysteine, at either terminus to allow specific coupling.

Examples of sequence from certain ion channels are listed below, wherein the domain A, B, C or D from which the peptide is derived is denoted for each peptide before the sequence:

Domain	**Sequence**
Nav1.3	

AE1	MT**LSNP**PDW	(SEQ ID NO: 131)
BE1	MEHYPM**TEQFSS**	(SEQ ID NO: 132)
CE1	IE**QRKTIKT**	(SEQ ID NO: 133)
DE1	ETDDQ**GKYMT**L	(SEQ ID NO: 134)

Accordingly, in one embodiment the $Na_v1.3$ peptide used in generating the anti-E1 ion channel antibody has an amino acid sequence selected from the group consisting of SEQ ID NOs:131 to 134.

Nav1.6

AE1	MT**FSNP**PDW	(SEQ ID NO: 135)
BE1	MEHHPM**TPQFEH**	(SEQ ID NO: 136)
CE1	IE**QRKTIRT**	(SEQ ID NO: 137)
DE1	ETDTQ**SKQMEN**	(SEQ ID NO: 138)

In one embodiment of the present invention the $Na_v1.6$ peptide used in generating the anti-E1 ion channel antibody has an amino acid sequence selected from the group consisting of SEQ ID NOs:135 to 138.

Nav1.8

AE1	CMT**RTDL**PEK	(SEQ ID NO: 139)
BE1.1	MEHHGM**SPTFEA**	(SEQ ID NO: 140)
BE1.2	MEHYPM**TDAFDA**	(SEQ ID NO: 141)
CE1.1	L**DQKPTV**KA	(SEQ ID NO: 142)
CE1.2	L**EEKPRV**KS	(SEQ ID NO: 143)
DE1.1	TDDQ**SEEKTK**	(SEQ ID NO: 144)
DE1.2	TDNQ**SEEKTK**	(SEQ ID NO: 145)

In one embodiment of the present invention the $Na_v1.8$ peptide used in generating the anti-E1 ion channel antibody has an amino acid sequence selected from the group consisting of SEQ ID NOs:139 to 145.

Nav1.9

AE1	**MATGPAKNSNSNN**TD	(SEQ ID NO: 146)
BE1	MEHH**KMEASFEK**	(SEQ ID NO: 147)
CE1	VHLE**NQPKIQE**	(SEQ ID NO: 148)
DE1	E**SYNQPKAMKS**	(SEQ ID NO: 149)

In one embodiment of the present invention the $Na_v1.9$ peptide used in generating the anti-E1 ion channel antibody has an amino acid sequence selected from the group consisting of SEQ ID NOs:146 to 149.

HCN1 and HCN2

HCN1 E1	**TEQTTTP**	(SEQ ID NO: 150)
HCN2 E1	K**DETTA**P	(SEQ ID NO: 151)

In one embodiment of the present invention the HCN1 or HCN2 peptide used in generating the anti-E1 ion channel antibody has an amino acid sequence of SEQ ID NOs:150 or 151.

In one aspect there is provided a method of generating an antibody employing a cyclic peptides.

A cyclic peptide as employed herein is a peptide where a sequence of amino acids are joined by a bond, such as a disulfide bond, thereby forming a loop or circle with no discernable start and/or finish. The cyclic peptide may be formed from a corresponding linear peptide by various means such as but not limited to the following: C-terminal carboxyl group ligation to the N-terminal alpha amino group to form a peptide bond; alternatively side chain carboxyl groups (of aspartic or glutamic acid residues) may be ligated to the side chain amino group of lysine or the N-terminal alpha amino group or the C-terminal carboxyl group may be ligated to the side chain amino group of lysine; disulphide bond formation between side chains thiols of two cysteine residue separated from each other by at least three residues in the linear sequence. It may be desirable to form the "ring completing bond" in an area of overlap in the linear sequence. Area of overlap as employed herein is intended to refer to where there is a repeat of two or more amino acids occurring in the sequence. Thus a sequence of overlap as employed herein is intended to refer to where there is some commonality in the sequence, for example at least two, such as 3 or 4 amino acids are located in the same order in the sequence in two separate locations. These regions of overlap can be aligned and ligated such that an amino acid in one location replaces the corresponding amino acid in the second location to form the cyclised peptide.

Thus in one embodiment the peptide is cyclised by forming an amide bond.
In one embodiment the peptide is cyclised by forming a disulfide bond.

In one embodiment the sequence is ligated in a region of overlap in the linear sequence.

Cyclic peptides may be synthesized using any suitable method known in the art. In one embodiment the cyclic peptide is synthesized using protecting groups to prevent reactions of the amino acid side chains (Barlos, K.; Gatos, D.; Kutsogianni, S.; Papaphotiou, G.; Poulos, C.; Tsegenidis, T. Int. J. Pept. Protein Res. 1991, Vol 38, Issue 6 p 562-568) followed by cyclization and removal of the protecting groups (Kessler H et al., 1989, Computer Aided Drug Design, p 461-484; Dekker M et al, 1990, J. Peptide Research, 35, p 287-300; Gurrath M. et al., 1992, Eur. J. Biochem., 210, 911-921; Izumiya N. et al., 1981, Biopolymers, 20, 1785-1791; Brady S. F. et al., 1983, in Peptides, Structure and Function, Proceedings of the Eighth American Peptide Symposium, Ed. V. J. Hruby and D. H. Rick, pp. 127-130, Pierce Chemical Company, Rockford, Illinois; He J. X. et al., 1994, Lett. Peptide Sci., 1, 25-30).

Surprisingly functionally modifying antibodies can be generated employing very short cyclic peptide sequence, for example containing only 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 or 19 amino acids. This may be due the rigidity provided by cyclising the peptide.

In one embodiment the cyclised peptide comprising a fragment of at least 4 consecutive amino acids from Na_v 1.7, for example is selected from, wherein the domain A, B, C or D and the extracellular loop E1, E2 or E3, from which the peptide is derived is denoted in brackets:

MTMNNPP	(AE1)	(SEQ ID NO: 123)
PMTEEFKN,	(BE1)	(SEQ ID NO: 124)
IERKKTIKI and / or	(CE1)	(SEQ ID NO: 125)
EKEGQSQHMTE.	(DE1)	(SEQ ID NO: 126)

In one embodiment the cyclised peptide comprising a fragment of at least 4 consecutive amino acids from Na_v 1.7, and one additional cysteine residue for attachment to carrier, for example is selected from, wherein the domain A, B, C or D and the extracellular loop E1, E2 or E3, from which the peptide is derived is denoted in brackets:

CTMNNPP	(AE1)	(SEQ ID NO: 127)
CPMTEEFKN,	(BE1)	(SEQ ID NO: 128)
CIERKKTIKI, and/or	(CE1)	(SEQ ID NO: 129)
CEKEGQSQHMTE.	(DE1)	(SEQ ID NO: 130)

Accordingly, in one embodiment of the present invention the $Na_v1.7$ cyclic peptide used in generating the anti-E1 ion channel antibody has an amino acid sequence selected from the group consisting of SEQ ID NOs:123 to 130.

To prepare immunogens for the purpose of raising anti-ion channel antibodies in a host animal each peptide requires covalent conjugation to a carrier protein. The carrier protein is selected on the basis of its 'foreigness' to the host species; thus for immunization of rabbits or rodents examples of suitable carrier proteins are keyhole limpet hemocyanin (KLH), ovalbumin (OVA) and bovine serum albumun (BSA). Each of the above peptides, whether linear or cyclic, may be conjugated through the cysteine thiol to one of each of the above proteins, where the lysine side chain amino groups of the latter have been covalently modified with a maleimide functionality to yield respectively:

- KLH-maleimide,
- Ovalbumin-maleimide, or
- BSA-maleimide.

The present disclosure explicitly envisages each one of the peptides described herein in separate conjugations with each one of the carriers listed above, i.e. 45 different molecules are specifically provided for immunizing a host, for example KLH-CEKEGQSQHMTE (cyclic) (SEQ ID NO: 130) or BSA-CEKEGQSQHMTE (cyclic) (SEQ ID NO: 130). Accordingly, any of the peptides having an amino acid sequence selected from SEQ ID NOs: 107 to 151 may be conjugated with each of the carrier proteins listed above.

As described above, the carrier protein may be conjugated through a unique functional group such as a cysteine residue. However, any alternative naturally occurring or non-naturally occurring residue may be used in place of a cysteine

residue in order to conjugate the peptide to the carrier protein. An example of a non-naturally occurring residue which may be used in place of cysteine is a homocysteine residue, which is a homologue of cysteine which further comprises an additional methylene group in the side chain. Accordingly, any of the peptides having an amino acid sequence selected from SEQ ID NOs: 107 to 151, which comprise a cysteine residue may be modified to replace the cysteine residue with an alternative suitable naturally occurring or non-naturally occurring residue for conjugation to the carrier protein, such as a homocysteine residue.

The present disclosure also extends to novel peptides disclosed herein and compositions comprising same.

Generally between 0.001 and 1 mg of each peptide-carrier protein are required for each immunization dose per host animal.

Alternative immunogens suitable for raising function modifying antibodies include: relevant full length human ion channels, for example $Na_v1.7$, truncations thereof including individual sub-domains and truncations of sub-domains; chimeric molecules with regions of ion channel fused to regions other transmembrane proteins to aid expression or present extracellular loops to the immune system and mutations of ion chanbels to constrain regions of the ion channel in a desired conformation.

These immunogens may be expressed in mammalian cells for direct cell immunization or purification of protein for immunization.

These immunogens may be expressed in E.coli or cell-free expression systems for purification of protein for immunization.

Purified protein may be integrated into lipid vesicles or micelles for immunisation.

These ion channel versions may also be generated as lipoparticles for immunization.

In addition any of the above immunogens can be utilized as screening tools for identifying function-modifying antibodies.

Thus in one aspect there is provided a method of generating antibodies in a host by immunizing, for example with at least one ion channel E1 peptide-carrier protein conjugate or several different peptides (wherein at least one is E1) conjugated separately or as a mixture conjugated to the same carrier protein.

In one embodiment the method involves one, two, three, four or five immunizations.

In one embodiment the method involves at least two, such as two or three immunizations with the respective conjugates peptide(s).

In one embodiment the second immunization employs a different conjugate, wherein the peptide(s) is (are) common but the carrier protein is different to the carrier protein employed in the first immunization.

Thus in one embodiment the third immunization employs a different conjugate wherein the peptide(s) is (are) common to that of the first and second immunization, but the carrier protein is different to that employed in the first and/or second immunization. Unwanted antibody specificities against the carrier protein may in this way be minimized.

Suitable carrier protein combinations for sequential immunization include, KLH and Ovalbumin and BSA in any order.

Varying the carrier may be advantageous in optimizing the response to the peptide.

Each immunization will generally also include the administration of an adjuvant to stimulate immune responses. Suitable adjuvants include Freud's complete or incomplete adjuvant, and adjuvants comprising, alum, QS21, MPL and/or CPG.

The method may further comprises a step of separating antibodies or antibody producing cells from the host.

In one embodiment the host is a rodent such as a mouse or a rat, camel, llama, porcine, canine, primate, shark or rabbit, in particular a rabbit.

The aforementioned peptides may also be conjugated to a reporter group. The reporter group may for instance be biotin, a fluorescent group or enzyme tag, or any group which will allow detection or isolation of anti- ion channel antibodies. Reporter group- peptide conjugates may be used to screen the resulting polyclonal sera and monoclonal antibodies. For example, a screening ELISA may comprise streptavidin coated microwells and captured biotinylated peptide. Titrations of immune sera over this would result in peptide specific antibody binding to the surface, which in turn could be revealed by a fourth anti-species (such as anti-rabbit) IgG-peroxidase layer.

Reporter group- peptide conjugates may also be used to isolate specific ion channel antibody like modalities by library based techniques such as phage panning.

The method may also include a further purification step, for example to provide polyclonal or monoclonal antibodies.

The methods may also comprise the step of producing recombinant clonal antibodies derived from said immunizations.

Before clonal antibodies can be prepared recombinantly part, such as the variable regions, or all of the antibody may need to be cloned and/or sequenced.

The disclosure also extends to antibody producing cells and antibodies obtainable or obtained from the method herein.

The disclosure also extends to an antibody or suitable antibody fragment obtainable or obtained employing the method herein.

The disclosure also includes pharmaceutical compositions comprising antibodies or fragments herein.

The pharmaceutical compositions suitably comprise a therapeutically effective amount of the antibody of the invention. The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent needed to treat, ameliorate or prevent a targeted disease or condition, or to exhibit a detectable therapeutic or preventative effect. For any antibody, the therapeutically effective amount can be estimated initially either in cell culture assays or in animal models, usually in rodents, rabbits, dogs, pigs or primates. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

The precise therapeutically effective amount for a human subject will depend upon the severity of the disease state, the general health of the subject, the age, weight and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities and tolerance/response to therapy. This amount can be determined by routine experimentation and is within the judgement of the clinician. Generally, a therapeutically effective amount will be from 0.01 mg/kg to 50 mg/kg, for example 0.1 mg/kg to 20 mg/kg. Pharmaceutical compositions may be conveniently presented in unit dose forms containing a predetermined amount of an active agent of the invention per dose.

Compositions may be administered individually to a patient or may be administered in combination (e.g. simultaneously, sequentially or separately) with other agents, drugs or hormones.

The dose at which the antibody or fragment of the present invention is administered depends on the nature of the condition to be treated, the extent of the disease and/or symptoms present and on whether the antibody or fragment is being used prophylactically or to treat an existing condition.

The frequency of dose will depend on the half-life of the antibody molecule and the duration of its effect. If the antibody molecule has a short half-life (e.g. 2 to 10 hours) it may be necessary to give one or more doses per day. Alternatively, if the antibody molecule has a long half life (e.g. 2 to 15 days) it may only be necessary to give a dosage once per day, once per week or even once every 1 or 2 months.

The pharmaceutically acceptable carrier should not itself induce the production of antibodies harmful to the individual receiving the composition and should not be toxic. Suitable carriers may be large, slowly metabolised macromolecules such as proteins, polypeptides, liposomes, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers and inactive virus particles.

Pharmaceutically acceptable salts can be used, for example mineral acid salts, such as hydrochlorides, hydrobromides, phosphates and sulphates, or salts of organic acids, such as acetates, propionates, malonates and benzoates.

Pharmaceutically acceptable carriers in therapeutic compositions may additionally contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents or pH buffering substances, may be present in such compositions. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries and suspensions, for ingestion by the patient.

Suitable forms for administration include forms suitable for parenteral administration, e.g. by injection or infusion, for example by bolus injection or continuous infusion. Where the product is for injection or infusion, it may take the form of a suspension, solution or emulsion in an oily or aqueous vehicle and it may contain formulatory agents, such as suspending, preservative, stabilising and/or dispersing agents. Alternatively, the antibody molecule may be in dry form, for reconstitution before use with an appropriate sterile liquid.

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals. However, in one or more embodiments the compositions are adapted for administration to human subjects.

Suitably in formulations according to the present disclosure, the pH of the final formulation is not similar to the value of the isoelectric point of the antibody or fragment, for example if the pH of the formulation is 7 then a pI of from 8-9 or above may be appropriate. Whilst not wishing to be bound by theory it is thought that this

may ultimately provide a final formulation with improved stability, for example the antibody or fragment remains in solution.

The pharmaceutical compositions of this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, transcutaneous (for example, see WO98/20734), subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, intravaginal or rectal routes. Hyposprays may also be used to administer the pharmaceutical compositions of the invention. Typically, the therapeutic compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared.

Direct delivery of the compositions will generally be accomplished by injection, subcutaneously, intraperitoneally, intravenously or intramuscularly, or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Dosage treatment may be a single dose schedule or a multiple dose schedule.

It will be appreciated that the active ingredient in the composition will be a protein molecule. As such, it will be susceptible to degradation in the gastrointestinal tract. Thus, if the composition is to be administered by a route using the gastrointestinal tract, the composition will need to contain agents which protect the antibody from degradation but which release the antibody once it has been absorbed from the gastrointestinal tract.

A thorough discussion of pharmaceutically acceptable carriers is available in Remington's Pharmaceutical Sciences (Mack Publishing Company, N.J. 1991).

In one embodiment the formulation is provided as a formulation for topical administrations including inhalation.

Suitable inhalable preparations include inhalable powders, metering aerosols containing propellant gases or inhalable solutions free from propellant gases. Inhalable powders according to the disclosure containing the active substance may

consist solely of the abovementioned active substances or of a mixture of the abovementioned active substances with physiologically acceptable excipient.

These inhalable powders may include monosaccharides (e.g. glucose or arabinose), disaccharides (e.g. lactose, saccharose, maltose), oligo- and polysaccharides (e.g. dextranes), polyalcohols (e.g. sorbitol, mannitol, xylitol), salts (e.g. sodium chloride, calcium carbonate) or mixtures of these with one another. Mono- or disaccharides are suitably used, the use of lactose or glucose, particularly but not exclusively in the form of their hydrates.

Particles for deposition in the lung require a particle size less than 10 microns, such as 1-9 microns for example from 0.1 to 5 µm, in particular from 1 to 5 µm. The particle size of the active ingredient (such as the antibody or fragment) is of primary importance.

The propellant gases which can be used to prepare the inhalable aerosols are known in the art. Suitable propellant gases are selected from among hydrocarbons such as n-propane, n-butane or isobutane and halohydrocarbons such as chlorinated and/or fluorinated derivatives of methane, ethane, propane, butane, cyclopropane or cyclobutane. The abovementioned propellent gases may be used on their own or in mixtures thereof.

Particularly suitable propellant gases are halogenated alkane derivatives selected from among TG 11, TG 12, TG 134a and TG227. Of the abovementioned halogenated hydrocarbons, TG134a (1,1,1,2-tetrafluoroethane) and TG227 (1,1,1,2,3,3,3-heptafluoropropane) and mixtures thereof are particularly suitable.

The propellant-gas-containing inhalable aerosols may also contain other ingredients such as cosolvents, stabilisers, surface-active agents (surfactants), antioxidants, lubricants and means for adjusting the pH. All these ingredients are known in the art.

The propellant-gas-containing inhalable aerosols according to the invention may contain up to 5 % by weight of active substance. Aerosols according to the invention contain, for example, 0.002 to 5 % by weight, 0.01 to 3 % by weight, 0.015 to 2 % by

weight, 0.1 to 2 % by weight, 0.5 to 2 % by weight or 0.5 to 1 % by weight of active ingredient.

Alternatively topical administrations to the lung may also be by administration of a liquid solution or suspension formulation, for example employing a device such as a nebulizer, for example, a nebulizer connected to a compressor (e.g., the Pari LC-Jet Plus(R) nebulizer connected to a Pari Master(R) compressor manufactured by Pari Respiratory Equipment, Inc., Richmond, Va.).

The antibody or fragment of the invention can be delivered dispersed in a solvent, e.g., in the form of a solution or a suspension. It can be suspended in an appropriate physiological solution, e.g., saline or other pharmacologically acceptable solvent or a buffered solution. Buffered solutions known in the art may contain 0.05 mg to 0.15 mg disodium edetate, 8.0 mg to 9.0 mg NaCl, 0.15 mg to 0.25 mg polysorbate, 0.25 mg to 0.30 mg anhydrous citric acid, and 0.45 mg to 0.55 mg sodium citrate per 1 ml of water so as to achieve a pH of about 4.0 to 5.0. A suspension can employ, for example, lyophilised antibody.

The therapeutic suspensions or solution formulations can also contain one or more excipients. Excipients are well known in the art and include buffers (e.g., citrate buffer, phosphate buffer, acetate buffer and bicarbonate buffer), amino acids, urea, alcohols, ascorbic acid, phospholipids, proteins (e.g., serum albumin), EDTA, sodium chloride, liposomes, mannitol, sorbitol, and glycerol. Solutions or suspensions can be encapsulated in liposomes or biodegradable microspheres. The formulation will generally be provided in a substantially sterile form employing sterile manufacture processes.

This may include production and sterilization by filtration of the buffered solvent/solution used for the for the formulation, aseptic suspension of the antibody in the sterile buffered solvent solution, and dispensing of the formulation into sterile receptacles by methods familiar to those of ordinary skill in the art.

Nebulizable formulation according to the present disclosure may be provided, for example, as single dose units (e.g., sealed plastic containers or vials) packed in foil

envelopes. Each vial contains a unit dose in a volume, e.g., 2 ml, of solvent/solution buffer.

The fusion protein molecule of the present disclosure are thought to be suitable for delivery via nebulisation.

The antibodies and fragments of the present disclosure may be suitable for treating pain, for example neuropathic pain including painful diabetic neuropathy (PDN), post-herpetic neuropathy (PHN) or trigeminal neuralgia (TN). Other causes of neuropathic pain include spinal cord injuries, multiple sclerosis, phantom limb pain, post-stroke pain and HIV-associated pain. Conditions such as chronic back pain, osteoarthritis and cancer may also result in the generation of neuropathic-related pain and thus are potentially suitable for treatment with an antibody or fragment according to the present disclosure..

In one embodiment the antibodies or fragments according the invention are suitable for the treatment or prophylaxis of pain, including somatic pain, visceral pain, neuropathic pain, nociceptive pain, acute pain, chronic pain, breakthrough pain and/or inflammatory pain.

In one embodiment antibodies or fragments according the invention are suitable for the treatment or prophylaxis of one ore more of the following pain types: allodynia, anaesthesia dolorosa, anginal pain, breakthrough pain, complex regional pain syndrome I, complex regional pain syndrome II, hyperalgesia, hyperpathia, idiopathic pain, malignant pain, paresthesia, phantom limb pain, psychogenic pain.

In one embodiment the antibody or fragment according to the disclosure is useful in the treatment of asthma, airway hyper-reactivity in asthma, chronic cough, for example in asthma and/or chronic obstructive airways.

In one embodiment the antibody or fragment according to the disclosure is useful in the treatment inflammation, osteoarthritis, rheumatoid arthritis and/or pain associated with any of the same.

In one embodiment the antibody or fragment according to the disclosure is useful in the treatment of pain associated with acute injuries, for examples wounds such as lacerations, incisions, burns, bullet and/or shrapnel injuries.

As discussed the antibody or fragment according the present disclosure is likely to be useful in the treatment of pain, such as acute pain and chronic pain, neuropathic pain, nociceptive pain, visceral pain, back pain and pain associated with disease and degeneration.

The pain may result from one or more causes, including, but not restricted to peripheral neuropathy, central neuropathy, nerve compression or entrapment syndromes such as carpal tunnel syndrome, tarsus tunnel syndrome, ulnar nerve entrapment, compression radiculopathy, lumbar spinal stenosis, sciatic nerve compression, spinal root compression, intercostal neuralgia, compression radiculopathy and radicular low back pain, spinal root lesions, back pain, neuritis, automimmune diseases, postoperative pain, dental pain, direct trauma, inflammation, HIV infection, small pox infection, herpes infection, toxic exposure, invasive cancer, chemotherapy, radiotherapy, hormonal therapy, foreign bodies, burns, congenital defect, phantom limb pain, rheumatoid arthritis, osteoarthritis, fracture pain, gout pain, fibromyalgias, multiple sclerosis, pain associated with diarrhea, irritable bowel syndrome, migraine, encephalitis, diabetes, chronic alcoholism, hypothyroidism, uremia and vitamin deficiencies. Thus the antibody or fragment according to the present disclosure may be useful in the treatment or amelioration of symptoms of one or more of the above indications.

In one embodiment an antibody or fragment according to the present disclosure is employed as a standard in an assay for screening for ion channel inhibitors.

Comprising in the context of the present specification is intended to meaning including.

Where technically appropriate embodiments of the invention may be combined.

Embodiments are described herein as comprising certain features/elements. The disclosure also extends to separate embodiments consisting or consisting essentially of said features/elements.

The present invention is further described by way of illustration only in the following examples, which refer to the accompanying Figures, in which:

Examples

Therapeutic Antibody Generation/Selection for $Na_v1.7$

Peptides were supplied by Peptide Protein Research Ltd., Fareham, U.K., and linear peptides were synthesized by Fmoc solid phase peptide chemistry according to the method of Atherton and Sheppard (1989). *Solid Phase peptide synthesis: a practical approach.* Oxford, England: IRL Press. N to C terminal cyclic peptides were synthesised as side chain protected peptides according to the method of Barlos et al Int. J. Pept. Protein Res. 1991 and cyclisation was carried out in solution phase followed by side chain deprotection according to the method of Kessler H et al., 1989, in Computer-aided drug design, methods and applications, Ed. T. J. Perun and C. L. Probst, pp. 461-484, Marcel Dekker, New-York; Toniolo C., 1990, Int. J. Pept. Protein Res., 35, 287-300; Gurrath M. et al., 1992, Eur. J. Biochem., 210, 911-921; Izumiya N. et al., 1981, Biopolymers, 20, 1785-1791; Brady S. F. et al., 1983, in Peptides, Structure and Function, Proceedings of the Eighth American Peptide Symposium, Ed. V. J. Hruby and D. H. Rick, pp. 127-130, Pierce Chemical Company, Rockford, Illinois; He J. X. et al., 1994, Lett. Peptide Sci., 1, 25-30.

Rabbits were immunised with combinations of human $Na_v1.7$ peptides conjugated to either KLH, OVA or BSA (Table 1). Following 5 subcutaneous immunisations (KLH, OVA, BSA, KLH, OVA), animals were sacrificed and PBMC, spleen and bone marrow harvested. Sera was tested for binding to human biotinylated peptide in ELISA.

Table 1. $Na_v1.7$ peptide immunogens

Rabbit	Peptides	Peptide Sequence
3823 and	B11,	B11 – CPMTEEFKN (cyclic) (SEQ ID

3824		NO: 128)
5825 and 5826	C11,	C11 – CIERKKTIKI (cyclic) (SEQ ID NO: 129)
5827 and5828	D11,	D11 - Nαacetyl-EKEGQSQHMTEC-amide (SEQ ID NO: 118)

The table shows immunised rabbit number, peptide combination employed for immunisation and peptide sequence. B11 is a peptide from loop E1 in domain B. C11 is a peptide from loop E1 in domain C. D11 is a peptide from loop E1 in domain D.

SLAM was performed using substantially the methods described in Tickle et al. 2009 (JALA, Vol. 14, number 5, p303-307). Briefly, SLAM cultures were set up using rabbit splenocytes or PBMC and supernatants were first screened for their ability to bind biotinylated peptide in a bead-based assay in the FMAT. This was a homogeneous assay using biotinylated human peptide bound to streptavidin beads (Bangs Laboratories) and revealing binding using a goat anti-rabbit Fc-Cy5 conjugate (Jackson immunoResearch). Positives from this screen were then put through a negative screen to identify non-specific antibodies. This used streptavidin beads with no peptide or with an irrelevant peptide, revealing binding with a goat anti-rabbit Fc-Cy5 conjugate (Jackson ImmunoResearch), to identify the peptide specific binders.

From 10 SLAM experiments, a number of, B11-specific, C11-specific and D11-specific antibody-containing wells were identified using the screens described above.

Single B cell isolation via the fluorescent foci method and subsequent variable region gene cloning from a number of these wells successfully yielded heavy and light chain variable region gene pairs following reverse transcription (RT)-PCR. These V-region genes were cloned as rabbit IgG1 full-length antibodies and re-expressed in a HEK-293 transient expression system.

Sequence analysis of cloned v-regions revealed the presence of a number of unique families of anti-human B11-specific (see table 2 below). DNA and amino acid

sequences of these antibodies are shown in the Figures. Antibodies were expressed in a transient CHO system and subsequently purified to allow further characterisation *in vitro* and *in vivo*.

Table 2

UCB antibody number	Rabbit number	Peptide specificity
CA167_00983	3824	B11
CA167_00984	3824	B11
CA167_00985	3824	B11
CA167_01080	3824	B11
CA167_01081	3824	B11
CA167_01082	3824	B11
CA167_01083	3824	B11
CA167_01084	3824	B11
CA167_01085	3824	B11
CA167_01086	3824	B11

Figures 4-7 show sequences for anti- $Na_v1.7$ antibodies. The immunised rabbit number that the antibodies were derived from and their peptide specificities are detailed.

Procedure for h $Na_v1.7$ recording for antibody testing

Solutions and antibodies handling

Extracellular solution contained (in mM): 130 NaCl, 4 KCl, 1.5 $CaCl_2$, 1 $MgCl_2$, 30 glucose, 10 HEPES (pH 7.4 with Tris-Base, and 300 to 305 mOsmolar). Intracellular solution contained (in mM): 5 NaCl, 115 CsF, 20 CsCl, 110 HEPES, 10 EGTA free acid (pH 7.2 with CsOH, and 290 to 295 mOsmolar) and was either made fresh or kept frozen. Extracellular and intracellular solutions were filtered prior to use. Antibodies were directly diluted in extracellular solution and were freshly (no more than 15min) prepared before transfer to a 96-well polypropylene compound plate (Sarsted, #83.1835.500). For the experiments using selective peptide, antibodies and peptides, at equal concentrations, were preincubated at least 30-min at 4°C prior Patch Clamp experiments.

Cell preparation

HEK293 cells stably expressing the human $Na_v1.7$ channel (type IX voltage-gated sodium channel alpha subunit) were purchased from Upstate (Upstate, Millipore, cat.#CYL3011). Cells were cultured in T-75 (BD BioCoat™ Collagen I Cellware, Becton Dickinson Labware, Bedford, MA, #356485) flasks coated with collagen type

I using standard culture medium DMEM-F12 with-Glutamine (Invitrogen, #11320) containing 10% FBS (Lonza, #DE14-802F), 1% penicillin + streptomycin (Lonza, DE17-603E), 1% non essential amino acids (Lonza, BE13-114E) and 400µg/ml G418 (GIBCO, #10131-027). Cells were plated at a density of 15,000 cells/cm2 or 8,000 cells/cm2 density for 2 or 3 days respectively before being used on PatchXpress® 7000A (Axon instrument, new part of MDS Analytical Technologies). Cells confluence never exceeded 90%. The day of the experiment, cells were harvested using Accumax (Sigma, A7089). Briefly, cells were washed twice in PBS (Lonza, #BE12-516F) without calcium and magnesium, and a 1:4 dilution of Accumax solution was added and incubated for 1.5 to 2-min at 37°C. DMEM-F12 with 15mMHEPES and L-glutamine (Lonza, #BE12-719F) containing 10% FBS (recovery media) was added to quench Accumax digestion. The cells were subsequently centrifuged at 1,000 rpm for 5-min in 50ml falcon tube and pellets are resuspended in 10ml of recovery media. Cells are counted (CoulterZ2) and suspended at ~ 0.1 million cells/ml and transferred to a 15ml screw-cap tube for minimum 90 minutes at room temperature. Cells were then centrifuged for 60-s at 1,000rpm. The pellet was gently resuspended in 1,000 µl extracellular solution and centrifuged a second time for 30-s at 1,000 rpm. Pellet was resuspended in 150µL extracellular solution and immediately tested on the PatchXpress®.

PatchXpress® procedures

The AVIVA Biosciences SealChip16™ electrode arrays (purchased from Axon Instruments, Union City, CA) were manually placed in the holder of the PatchXpress® system and automatically prepared for application of the cells. Intracellular solution was injected into the bottom of each chamber, and extracellular solution was perfused into the top of the chambers through the 16-nozzle wash station. Throughout this period, the pressure controller maintained a positive pressure (+10 mmHg) from the intracellular side to keep the hole free of debris. Cells were triturated by the integrated Cavro pipetting robot prior to addition of 4 µl (containing 10K-30K cells) to each well.

PatchXpress® h $Na_v1.7$ Assay

After 10-s, the pressure was switched from +4 to -30 mmHg to attract suspended cells to each of the 16 holes (electrodes). Seal formation was achieved by repeating negative pressure ramp from -1 to -35 mmHg at a rate of 1.6 mmHg / s every 36-s until a Giga Ohm Seal was obtained and verified for 20-s. Whole-cell access was

achieved by rupturing the patch of membrane over the hole using a ramp increase in negative pressure from -40 to -150 mmHg at a rate of 7.5 mmHg / s with a pipette potential of -80 mV. After whole cell configuration cells are washed with extracellular solution for 66-s to remove the excess cells in the well. The cell was allowed to dialyze for 5 min, during which the access resistance was monitored. From the time of whole-cell break-in to the end of the experiment, the cells were held at -80mV between voltage protocols. A time course protocol was applied to assess the antibody potencies on sodium current elicited by a depolarizing step from -80 mV to 0 mV for 20 milliseconds at 10 seconds interval. Whole cell compensation was automatically made before each trial starts and electrical access resistance (Ra) was corrected by 65%. Linear leak substraction was performed online using a P/N leak subtraction protocol (N=4) at the holding of -80mV.

After a stabilizing period (up to 10 min), a negative control solution (extracellular solution) was applied for 5-min, followed by two doses of antibodies. The interval between both additions of the same concentration of compound to a well was ~11-s. Antibody solution (45 µL) was added online (30 µL/s) at the desired concentration with permanent aspiration. Currents were monitored continuously during the 18-min exposure to the antibody.

Data analysis

Cells were not analyzed if:

(1) the membrane resistance was initially <200 MOhm,

(2) current amplitude <200pA,

(3) an access resistance no greater then 20 MOhm and

(4) no real stabilized current after negative control addition.

The current amplitude was measured using DataXpress2 software (Axon instruments) and rundown current correction was performed by linear or exponential fitting method on the measurement associated with the last 10-15 data points after the washout period and the last 10-15 data point after the negative control addition.

Current was normalized by the mean current corrected amplitude prior antibody addition. Current inhibition was estimated by the residual response after 18-min antibodies application. Data is given below in Table 3.

Table 3:

Antibody	Peptide	Concentration (µg/ml)	Nav1.7 inhibition (%)
CA167_00983	B11	25	41
CA167_00984	B11	25	9
CA167_00985	B11	25	12
CA167_01080	B11	25	46
CA167_01081	B11	25	33
CA167_01082	B11	25	10
CA167_01083	B11	25	16
CA167_01084	B11	25	27
CA167_01085	B11	25	27
CA167_01086	B11	25	31
R3824_B11	B11	25	68
R5825_C11	C11	25	20

Table 3: Inhibition of Nav 1.7 currents expressed in HEK cells.

Figure 1

Figure 1 shows the functional effects of selected antibodies (at 25µg/ml), in the presence or absence of specific peptide, on human Nav1.7 currents expressed in HEK cells. Nav1.7 currents were recorded by automated Patch Clamp using a repetitive stimulation protocol and data are presented as the normalized Nav1.7 current after the last stimulation. Selected antibodies were incubated in the presence of the specific peptide (25µg/ml) for 30 minutes at 4°C and then transferred to the PatchXpress system for Nav1.7 current recordings. The presence of the peptide systematically reverses the inhibitory effect of the antibody thus indicating that inhibition of Nav1.7 currents is mediated by a specific interaction of antibodies with the Nav1.7 extracellular loops.

Figure 3d (a)

Automated Patch Clamp analysis of recombinant human Nav1.7 channels expressed in HEK cells. 983 monoclonal antibody produces a dose-dependent inhibition of Nav1.7 currents. Data points represent the normalized peak current amplitudes after application of a repeated voltage step protocol (end point) in the presence of antibody.

Figure 3d (b)

Automated Patch Clamp analysis of recombinant human Nav1.7 channels expressed in HEK cells. 1080 monoclonal antibody produces a dose-dependent inhibition of

Nav1.7 currents. Data points represent the normalized peak current amplitudes after application of a repeated voltage step protocol (end point) in the presence of antibody.

Figure 3e

Automated Patch Clamp analysis of recombinant rat Nav1.7 channels expressed in HEK cells. 983 monoclonal antibody produces a dose-dependent inhibition of Nav1.7 currents. 1080 monoclonal antibody produces a ~26% inhibition of Nav1.7 currents at 25μg/ml. Data points represent the normalized peak current amplitudes after application of a repeated voltage step protocol (end point) in the presence of antibody.

Figure 3f

Kinetics of human Nav1.7 inhibition by 983 monoclonal antibody. HEK cells expressing recombinant human Nav1.7 channels are stimulated with a voltage step protocol at 0.1Hz for ~20 minutes. Data points represent the normalized peak current amplitudes (run down corrected) of Nav1.7 channels recorded every 10 seconds. Nav1.7 currents are reduced in the presence of the antibody (25μg/ml) but only when repeated activation of the channel at 0.1Hz is maintained. Stimulation of Nav1.7 channels only at the end of the protocol (and after incubation of antibody) does not produce an inhibition of the Nav1.7 current. Data suggest that specific inhibition by 983 monoclonal antibody requires repetitive activation (channel cycling) of the Nav1.7 channel protein.

Dorsal Route Ganglion *in vitro* testing

Primary culture preparation

Dorsal Root Ganglia were isolated from 1-2 wild-type rat pups, aged between postnatal day 1 and 3. Ganglia were washed in PBS after dissection and immediately placed into a DMEM (Lonza, #BE12-604F) solution containing 2mg/ml collagenase (Sigma-Aldrich, #C2674) and incubated at 37oC for approximately 45 minutes for enzymatic digestion. Collagenase solution was removed and replaced with DMEM supplemented with 10% Fetal Bovine Serum (Lonza, #DE14802F), 0.5mM L-Glutamine (Lonza, #BE17-605E), 1% Penicillin/Streptomycin (Lonza, #BE17-603E) and 20ng/ml nerve growth factor (NGF, Invitrogen). Ganglia were then mechanically triturated, centrifuged at 1000g for 5 minutes, and resuspended in the same culture medium. Dissociated cells were counted and diluted to a suspension of 100,000-

120,000 cells/ml on glass coverslips precoated with 50μg/ml poly-D-lysine (Sigma) and 30μg/ml laminin (Invitrogen) and incubated at 37°C, 5% CO_2 until ready for use.

Primary Culture Electrophysiology

Dissociated DRG were taken for use no more than two days in vitro (DIV) following preparation. Cells were visualized on an Olympus BX50WI upright microscope with an Ikegami ICD-42B CCD camera. Electrophysiological recordings were acquired using 5khz digital sampling and filtered at 3dB at a 3khz frequency on an Axopatch 1D (Molecular Devices) amplifier and converted to a digital signal using a Digidata 1322A analog-to-digital converter (Molecular Devices). All recordings were acquired using pClamp 10 software (Molecular Devices) and subsequently analyzed in Clampfit 10 (Molecular Devices). Recording electrodes were pulled from borosilicate glass pipettes on a Sutter p-97 horizontal pipette puller to a final resistance of 4.5-6MΩ and filled with an internal solution containing (in mM): 140 K-Methansulfonate, 5 NaCl, 1 CaCl2, 2 MgCl2, 11 EGTA, 10 HEPES, 2 Mg-ATP, and 1 Li-GTP; pH was adjusted to 7.2 with Tris-base, and osmolality was adjusted to 310 mOsm with sucrose. Bath solution contained (in mM): 130 NaCl, 25 glucose, 10 HEPES, 4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 1.25 $NaPO_4$; pH was adjusted to 7.35 with NaOH and osmolality was adjusted to 310 mOsm with sucrose. The liquid junction potential was calculated to be 14.2mV, all reported voltages have been corrected to compensate.

After formation of a tight seal (>1GΩ) by release of positive pressure and manual suction in voltage clamp mode, capacitative currents were compensated and the command voltage was set to -70mV. The cell membrane was ruptured and the cell allowed to dialyze intracellular solution for 5 minutes. Whole cell parameters were recorded after dialysis. Cells were rejected if whole cell capacitance was >35pF or a stable access resistance less than 3x electrode resistance could not be achieved. The amplifier was switched to current clamp mode and the resting membrane potential was recorded. The cell was then injected with a series of 1.5s duration, depolarizing current steps of increasing amplitude intended to evoke an action potential (AP) or train of APs. Cells that could not fire more than a single AP during a single step after depolarizing to a maximum of -35mV were rejected.

Cells were subsequently treated either with control or antibody solutions by fast bath perfusion directly on to the recorded cell for 90 seconds to sufficiently fill the

recording chamber, at which point both perfusion and aspiration were halted. The previous series of depolarizing current steps were repeatedly administered at two minute intervals over a period of 40 minutes, typically with a delay of 1.5s between individual steps to allow for membrane repolarization. Occasionally a constant current was injected if the resting membrane potential (RMP) adjusted over the course of the experiment in order to maintain a constant RMP of -65mV. Cells whose RMP deviated more than 20% in either the positive or negative direction or whose holding current changed more than 100pA during the course of the experiment were rejected. Individual holding currents and injected currents for each step were noted individually for each cell, as well as any electrophysiological parameters that were changed during the course of the experiment.

Data Analysis

Action Potentials (AP) were manually counted for each depolarizing step and the total number of evoked APs were summed for each time point. The number of APs at each time point were normalized in Microsoft Excel 2003 to the number of evoked APs at time = 0 and plotted as a function of time using Graphpad Prism 5.0 software. Each plotted data point represents the mean value of all recorded cells under the specified experimental condition, with error bars representing the calculated standard error.

Figure 3a Current clamp traces of evoked action potentials from representative DRG neurons before (time = 0) and following (time = 30 minutes) treatment.
potentials compared with vehicle or control antibody treated controls following antibody administration at time = 2 minutes.

Fig 3b: The antibody 983 (25μg/ml) significantly reduced the number of evoked action potentials compared with vehicle or control antibody treated controls following antibody administration at time = 2 minutes.

Fig3c: Electrophysiology (current clamp recordings) investigations on action potential firing in cultured rat dorsal root ganglion (DRG) neurons. 1080 monoclonal antibody, at a dose of 25 μg/ml, reduces the electrically induced spike frequency of DRG neurons. Data points represent the normalized spike frequency compared to initial frequency observed at time 0 before antibody application.

Isoform selectivity for 983 and 1080

Table 4. E1 peptides used for Nav isoform selectivity studies

Peptide Name	Ion channel	Sequence
B11.1	Nav 1.1	Cyclo[biotinyl-PEG-cysEHYPMTDHFNN] (SEQ ID NO: 152)
B11.2/3	Nav 1.2 and 1.3	Cyclo[biotinyl-PEG-cysEHYPMTEQFSS] (SEQ ID NO: 153)
B11.4	Nav 1.4	Cyclo[biotinyl-PEG-cysEHYPMTEHFDN] (SEQ ID NO: 154)
B11.5	Nav 1.5	Cyclo[biotinyl-PEG-cysEHYNMTSEFEE] (SEQ ID NO: 155)
B11.6	Nav 1.6	Cyclo[biotinyl-PEG-cysEHHPMTPQFEH] (SEQ ID NO: 156)
B11.7	Nav 1.7	Cyclo[biotinyl-PEG-cysPMTEEFKN] (SEQ ID NO: 128)
B11.8	Nav 1.8	Cyclo[biotinyl-PEG-cysEHHGMSPTFEA] (SEQ ID NO: 157)
B11.9	Nav 1.9	Cyclo[biotinyl-PEG-cysEHHKMEASFEK] (SEQ ID NO: 158)

Peptide binding ELISA

Nunc 96 well plates were coated overnight at 4°C in 5ug/ml Streptavidin (Jackson 016-000-114) 100ul/well in carbonate coating buffer. Plates were washed four times in PBS/tween and 200ul/well of block (1% BSA in PBS) was added for 1 hour at RT. Plates were washed four times in PBS/tween and 100ul/well of biotinylated peptide at 5ug/ml was added for 1 hour at RT. Plates were washed four times in PBS/tween and 100ul/well of antibody added (starting at 10ug/ml diluting in block in half logs down the plate) for 1 hour at RT. Plates were washed four times in PBS/tween and 100ul/ml goat anti rabbit Fc HRP (Jackson 111-036-046) added for 1 hour at RT. Plates were washed four times in PBS/tween and 100ul/well TMB (3,3',5,5' Tetramethylbenzidine) solution added. 50ul/well of NaF was added to stop reaction and absorbance read at 630nm.

Figure 3g shows ELISA data for antibody 983 binding to various cyclic Nav ion channel peptides Table 4

Figure 3h shows ELISA data for antibody 1080 binding to various cyclic Nav ion channel peptides Table 4.

Specific binding in both cases was only observed for the B11.7 peptide and no binding to equivalent loops from the other Nav ion channels was observed.

Claims:

1. An anti-E1 ion channel antibody or binding fragment thereof, which binds to an E1 extracellular loop of the ion channel, wherein said ion channel has a function in the modulation of pain, and said antibody or fragment is functionally modifying to said ion channel after binding thereto.

2. An anti-E1 ion channel antibody or binding fragment thereof according to claim 1, wherein the ion channel is a sodium ion channel selected from the group consisting of Na_v 1.3, 1.6, 1.7, 1.8 and 1.9.

3. An anti-E1 ion channel antibody or binding fragment thereof according to claim 2, wherein the antibody binds the E1 loop in domain A, domain B, domain C or domain D of the ion channel.

4. An anti-E1 ion channel antibody or binding fragment thereof according to claim 2 or claim 3 wherein the antibody or fragment is an anti- $Na_v1.7$ antibody or fragment.

5. An anti-E1 ion channel antibody or binding fragment thereof according to claim 4, wherein the antibody or fragment thereof binds to an amino acid sequence selected from the group consisting of SEQ ID NOs:107 to 130.

6. An anti-E1 ion channel antibody or binding fragment thereof according to claim 2 or claim 3, wherein the antibody or fragment thereof binds to a $Na_v1.3$ peptide having an amino acid sequence selected from the group consisting of SEQ ID NOs:131 to 134.

7. An anti-E1 ion channel antibody or binding fragment thereof according to claim 2 or claim 3, wherein the antibody or fragment thereof binds to a $Na_v1.6$ peptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 135 to 138.

8. An anti-E1 ion channel antibody or binding fragment thereof according to claim 2 or claim 3, wherein the antibody or fragment thereof binds to a $Na_v1.8$ peptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 139 to 145.

9. An anti-E1 ion channel antibody or binding fragment thereof according to claim 2 or claim 3, wherein the antibody or fragment thereof binds to a $Na_v1.9$ peptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 146 to 149.

10. An anti-E1 ion channel antibody or binding fragment thereof according to claim 1, wherein the antibody or fragment thereof binds to a potassium ion channel selected from the group consisting of K_v 2.1, K_v 2.2 and K_v 7.x.

11. An anti-E1 ion channel antibody or binding fragment thereof according to claim 1, wherein the antibody or fragment thereof binds to a hyperpolarisable ion channel selected from the group consisting of HCN1 and HCN2.

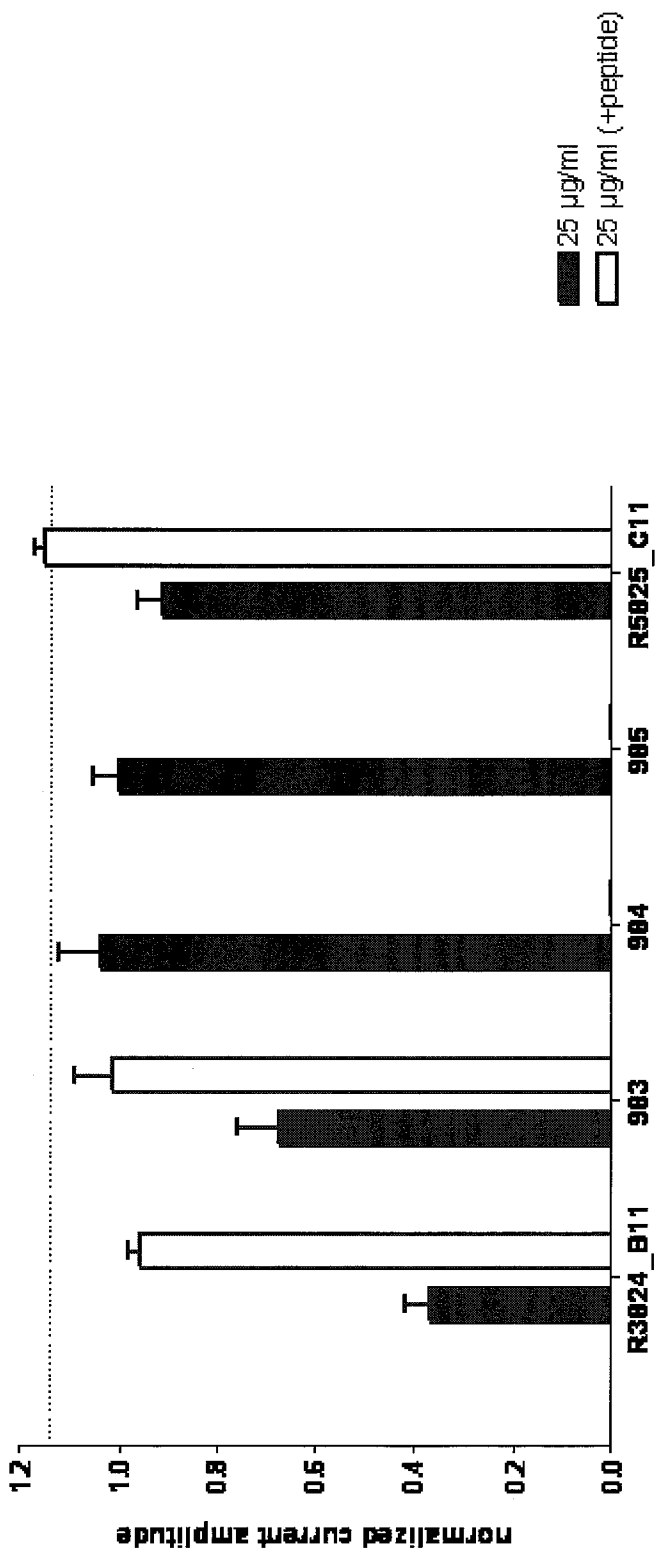
12. An anti-E1 ion channel antibody or binding fragment thereof according to claim 11, wherein the antibody or fragment thereof binds to a peptide having the amino acid sequence of SEQ ID NOs:150 or 151.

13. An anti-E1 ion channel antibody or binding fragment thereof according to claim 1, wherein the antibody or fragment thereof binds to a calcium ion channel selected from the group consisting of Ca_v 3.1, Ca_v 3.2, Ca_v 3.3, Ca_v 2.1, Ca_v 2.2 and Ca_v 2.3.

14. An anti-E1 ion channel antibody or binding fragment thereof according to claim 1, wherein the antibody or fragment thereof binds to a non-gated ion channel selected from the group consisting of TRPV1, TRPA1, ASIC1, TRPM8, TRPV3 and TRP4.

15. An antibody or fragment according to any one of claims 1 to 14, wherein the antibody inhibits the function of the relevant ion channel to which it is specific in an *in vitro* patch clamp assay by at least 5 percent.

Figure 1 Functional Effects of Selected Antibodies on Human Nav 1.7 current in HEK Cells



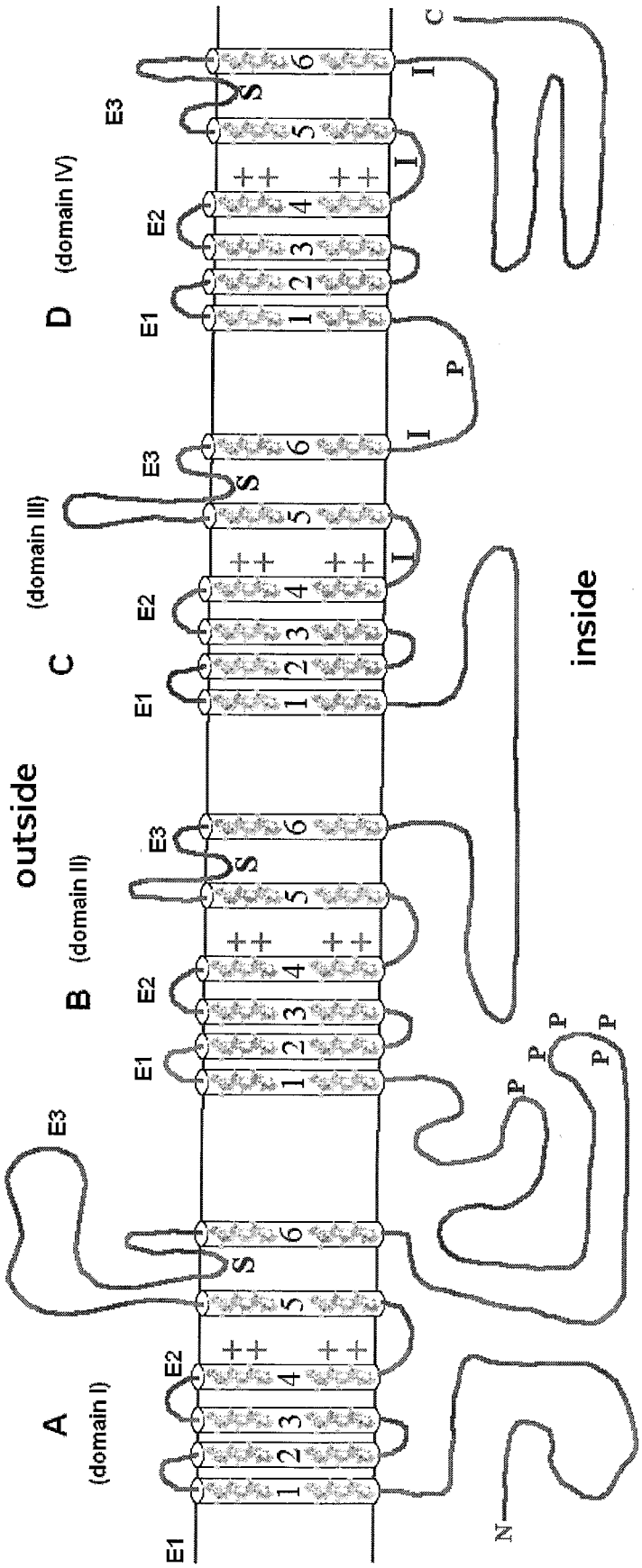

A
(domain I)
B
(domain II)
C
(domain III)
D
(domain IV)
outside
inside
E1
E2
E3
S
P
N
C

Figure 2a

Figure 2b

Domain A (SEQ ID NO: 101)
MAMLPPPGPQSFVHFTKQSLALIEQRIAERKSKEPKEEKKDDDEEAPKPSSDL
EAGKQLPFIYGDIPPGMVSEPLEDLDPYYADKKTFIVLNKGKTIFRFNATPALY
MLSPFSPLRRISIKILVHSLFSMLIMCTILTNCIFMTMNNPPDWTKNVEYTFTGI
YTFESLVKILARGFCVGEFTFLRDPWNWLDFVVIVFAYLTEFVNLGNVSALRT
FRVLRALKTISVIPGLKTIVGALIQSVKKLSDVMILTVFCLSVFALIGLQLFMGN
LKHKCFRNSLENNETLESIMNTLESEEDFRKYFYYLEGSKDALLCGFSTDSGQ
CPEGYTCVKIGRNPDYGYTSFDTFSWAFLALFRLMTQDYWENLYQQTLRAA
GKTYMIFFVVVIFLGSFYLINLILAVVAMAYEEQNQANIEEAKQKELEFQQML
DRLKKEQEEAEAIAAAAAEYTSIRRSRIMGLSESSSETSKLSSKSAKERRNRRK
KKNQKKLSSGEEKGDAEKLSKSESEDSIRRKSFHLGVEGHRRAHEKRLSTPNQ
SPLSIRGSLFSARRSSRTSLFSFKGRGRDIGSETEFADDEHSIFGDNESRRGSLFV
PHRPQERRSSNISQASRSPPMLPVNGKMHSAVDCNGVVSLVDGRSALMLPNG
QLLPEVIIDKATSDDSGTTNQIHKKRRCSSYLLSEDMLNDPNLRQRAMSRASIL
TNTVEELEESRQKCPPWWYRFAHKFLIWNCSPYWIKFKKCIY
Domain B (SEQ ID NO: 102)
FIVMDPFVDLAITICIVLNTLFMAMEHHPMTEEFKNVLAIGNLVFTGIFAAEM
VLKLIAMDPYEYFQVGWNIFDSLIVTLSLVELFLADVEGLSVLRSFRLLRVFKL
AKSWPTLNMLIKIIGNSVGALGNLTLVLAIIVFIFAVVGMQLFGKSYKECVCKI
NDDCTLPRWHMNDFFHSFLIVFRVLCGEWIETMWDCMEVAGQAMCLIVYM
MVMVIGNLVVLNLFLALLLSSFSSDNLTAIEEDPDANNLQIAVTRIKKGINYV
KQTLREFILKAFSKKPKISREIRQAEDLNTKKENYISNHTLAEMSKGHNFLKEK
DKISGFGSSVDKHLMEDSDGQSFIHNPSLTVTVPIAPGESDLENMNAEELSSDS
DSEYSKVRLNRSSSSECSTVDNPLPGEG
EEAEAEPMNSDEPEACFTDGCVRRFSCCQVNIESGKGKIWWNIRKTCYK
Domain C (SEQ ID NO: 103)
IVEHSWFESFIVLMILLSSGALAFEDIYIERKKTIKIILEYADKIFTYIFILEMLLK
WIAYGYKTYFTNAWCWLDFLIVDVSLVTLVANTLGYSDLGPISLRTLRALRP
LRALSRFEGMRVVVNALIGAIPSIMNVLLVCLIFWLIFSIMGVNLFAGKFYECI
NTTDGSRFPASQVPNRSECFALMNVSQNVRWKNLKVNFDNVGLGYLSLLQV
ATFKGWTIIMYAAVDSVNVDKQPKYEYSLYMYIYFVVFIIFGSFFTLNLFIGVII
DNFNQQKKKLGGQDIFMTEEQKKYYNAMKKLGSKKPQKPIPRPGNKIQGCIF
D
Domain D (SEQ ID NO: 104)
LVTNQAFDISIMVLICLNMVTMMVEKEGQSQHMTEVLYWINVVFIILFTGECV
LKLISLRHYYFTVGWNIFDFVVVIISIVGMFLADLIETYFVSPTLFRVIRLARIGR
ILRLVKGAKGIRTLLFALMMSLPALFNIGLLLFLVMFIYAIFGMSNFAYVKKE
DGINDMFNFETFGNSMICLFQITTSAGWDGLLAPILNSKPPDCDPKKVHPGSS
VEGDCGNPSVGIFYFVSYIIISFLVVVNMYIAVILENFSVATEESTEPLSEDDFE
MFYEVWEKFDPDATQFIEFSKLSDFAAALDPPLLIAKPNKVQLIAMDLPMVSG
DRIHCLDILFAFTKRVLGESGEMDSLRSQMEERFMSANPSKVSYEPITTTLKRK
QEDVSATVIQRAYRRYRLRQNVKNISSIYIKDGDRDDDLLNKKDMAFDNVNE
NSSPEKTDATSSTTSPPSYDSVTKPDKEKYEQDRTEKEDKGKDSKESKK

Figure 2c
Nav1.7 (SEQ ID NO: 105)

MAMLPPPGPQSFVHFTKQSLALIEQRIAERKSKEPKEEKKDDDEEAPKPSSDLEAGKQLPFIYGDIPP
GMVSEPLEDLDPYYADKKTFIVLNKGKTIFRFNATPALYMLSPFSPLRRISIKILVHSLFSMLIMCTI
LTNCIFMTMNNPPDWTKNVEYTFTGIYTFESLVKILARGFCVGEFTFLRDPWNWLDFVVIVFAYLTEF
VNLGNVSALRTFRVLRALKTISVIPGLKTIVGALIQSVKKLSDVMILTVFCLSVFALIGLQLFMGNLK
HKCFRNSLENNETLESIMNTLESEEDFRKYFYYLEGSKDALLCGFSTDSGQCPEGYTCVKIGRNPDYG
YTSFDTFSWAFLALFRLMTQDYWENLYQQTLRAAGKTYMIFFVVVIFLGSFYLINLILAVVAMAYEEQ
NQANIEEAKQKELEFQQMLDRLKKEQEEAEAIAAAAAEYTSIRRSRIMGLSESSSETSKLSSKSAKER
RNRRKKKNQKKLSSGEEKGDAEKLSKSESEDSIRRKSFHLGVEGHRRAHEKRLSTPNQSPLSIRGSLF
SARRSSRTSLFSFKGRGRDIGSETEFADDEHSIFGDNESRRGSLFVPHRPQERRSSNISQASRSPPML
PVNGKMHSAVDCNGVVSLVDGRSALMLPNGQLLPEVIIDKATSDDSGTTNQIHKKRRCSSYLLSEDML
NDPNLRQRAMSRASILTNTVEELEESRQKCPPWWYRFAHKFLIWNCSPYWIKFKKCIYFIVMDPFVDL
AITICIVLNTLFMAMEHHPMTEEFKNVLAIGNLVFTGIFAAEMVLKLIAMDPYEYFQVGWNIFDSLIV
TLSLVELFLADVEGLSVLRSFRLLRVFKLAKSWPTLNMLIKIIGNSVGALGNLTLVLAIIVFIFAVVG
MQLFGKSYKECVCKINDDCTLPRWHMNDFFHSFLIVFRVLCGEWIETMWDCMEVAGQAMCLIVYMMVM
VIGNLVVLNLFLALLLSSFSSDNLTAIEEDPDANNLQIAVTRIKKGINYVKQTLREFILKAFSKKPKI
SREIRQAEDLNTKKENYISNHTLAEMSKGHNFLKEKDKISGFGSSVDKHLMEDSDGQSFIHNPSLTVT
VPIAPGESDLENMNAEELSSDSDSEYSKVRLNRSSSSECSTVDNPLPGEGEEAEAEPMNSDEPEACFT
DGCVRRFSCCQVNIESGKGKIWWNIRKTCYKIVEHSWFESFIVLMILLSSGALAFEDIYIERKKTIKI
ILEYADKIFTYIFILEMLLKWIAYGYKTYFTNAWCWLDFLIVDVSLVTLVANTLGYSDLGPISLRTLR
ALRPLRALSRFEGMRVVVNALIGAIPSIMNVLLVCLIFWLIFSIMGVNLFAGKFYECINTTDGSRFPA
SQVPNRSECFALMNVSQNVRWKNLKVNFDNVGLGYLSLLQVATFKGWTIIMYAAVDSVNVDKQPKYEY
SLYMYIYFVVFIIFGSFFTLNLFIGVIIDNFNQQKKKLGGQDIFMTEEQKKYYNAMKKLGSKKPQKPI
PRPGNKIQGCIFDLVTNQAFDISIMVLICLNMVTMMVEKEGQSQHMTEVLYWINVVFIILFTGECVLK
LISLRHYYFTVGWNIFDFVVVIISIVGMFLADLIETYFVSPTLFRVIRLARIGRILRLVKGAKGIRTL
LFALMMSLPALFNIGLLLFLVMFIYAIFGMSNFAYVKKEDGINDMFNFETFGNSMICLFQITTSAGWD
GLLAPILNSKPPDCDPKKVHPGSSVEGDCGNPSVGIFYFVSYIIISFLVVVNMYIAVILENFSVATEE
STEPLSEDDFEMFYEVWEKFDPDATQFIEFSKLSDFAAALDPPLLIAKPNKVQLIAMDLPMVSGDRIH
CLDILFAFTKRVLGESGEMDSLRSQMEERFMSANPSKVSYEPITTTLKRKQEDVSATVIQRAYRRYRL
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TEKEDKGKDSKESKK

Figure 3A

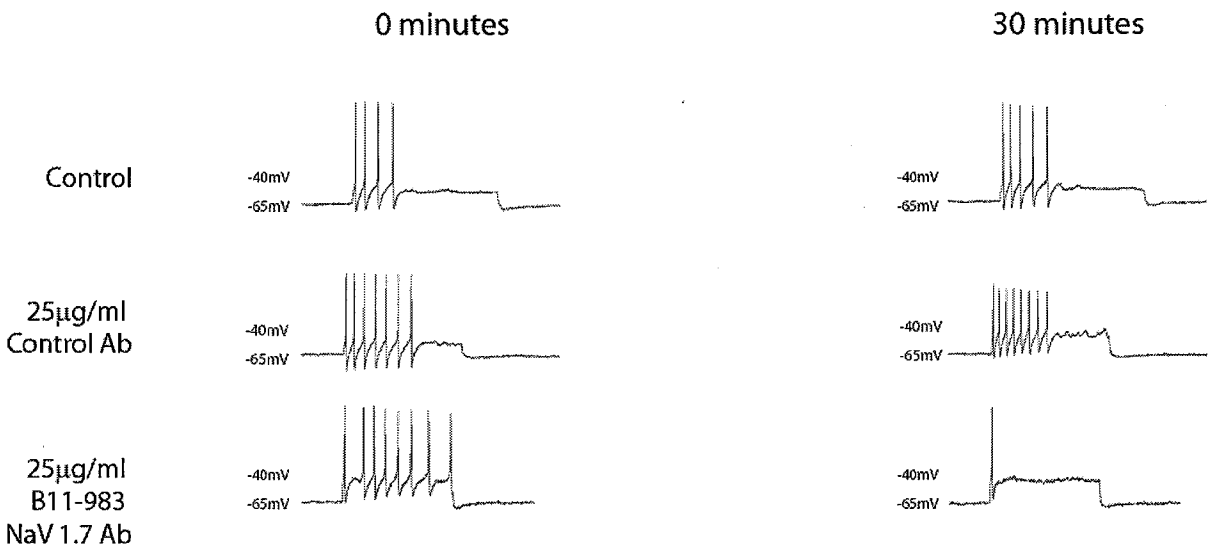

0 minutes
30 minutes
Control
25µg/ml
Control Ab
25µg/ml
B11-983
NaV 1.7 Ab
-40mV
-65mV

Figure 3B

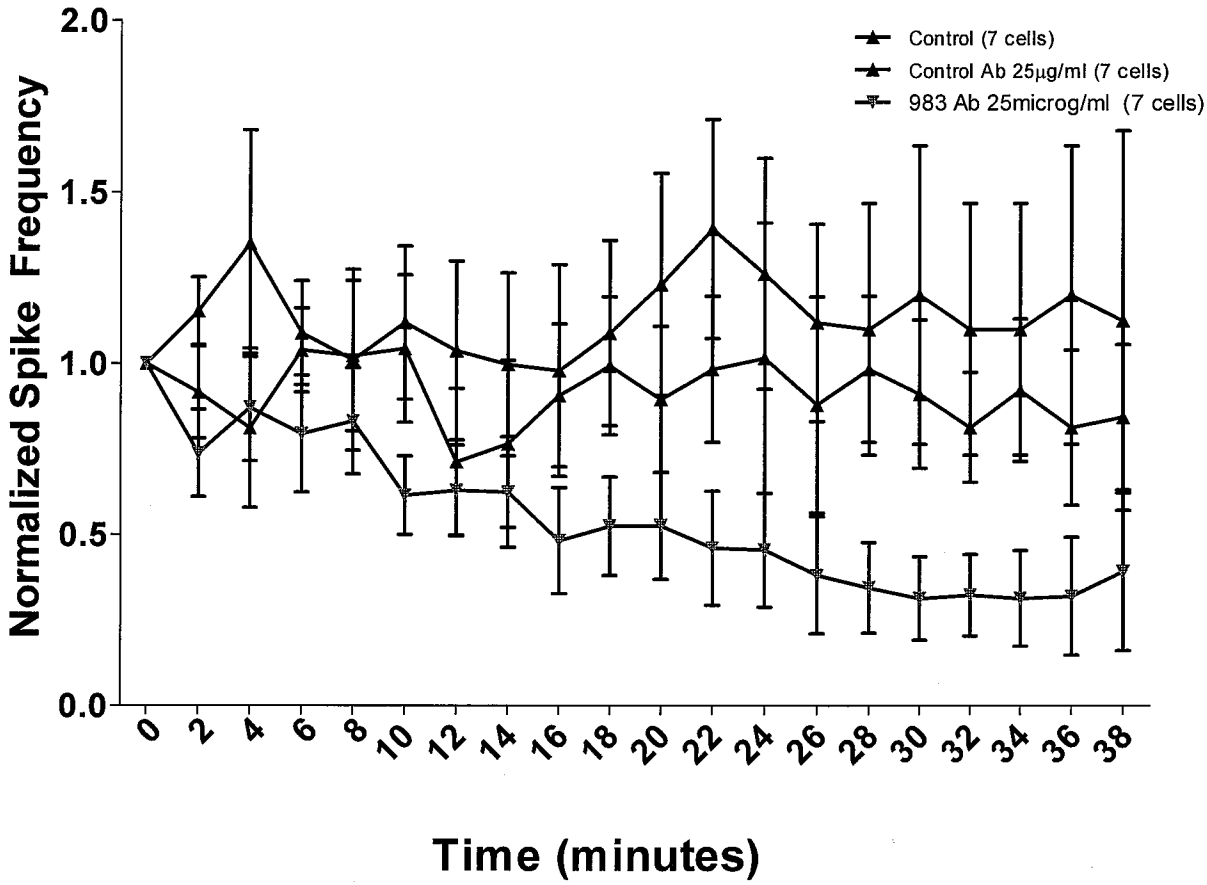

Control (7 cells)
Control Ab 25µg/ml (7 cells)
983 Ab 25microg/ml (7 cells)
Normalized Spike Frequency
2.0
1.5
1.0
0.5
0.0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
Time (minutes)

Figure 3C

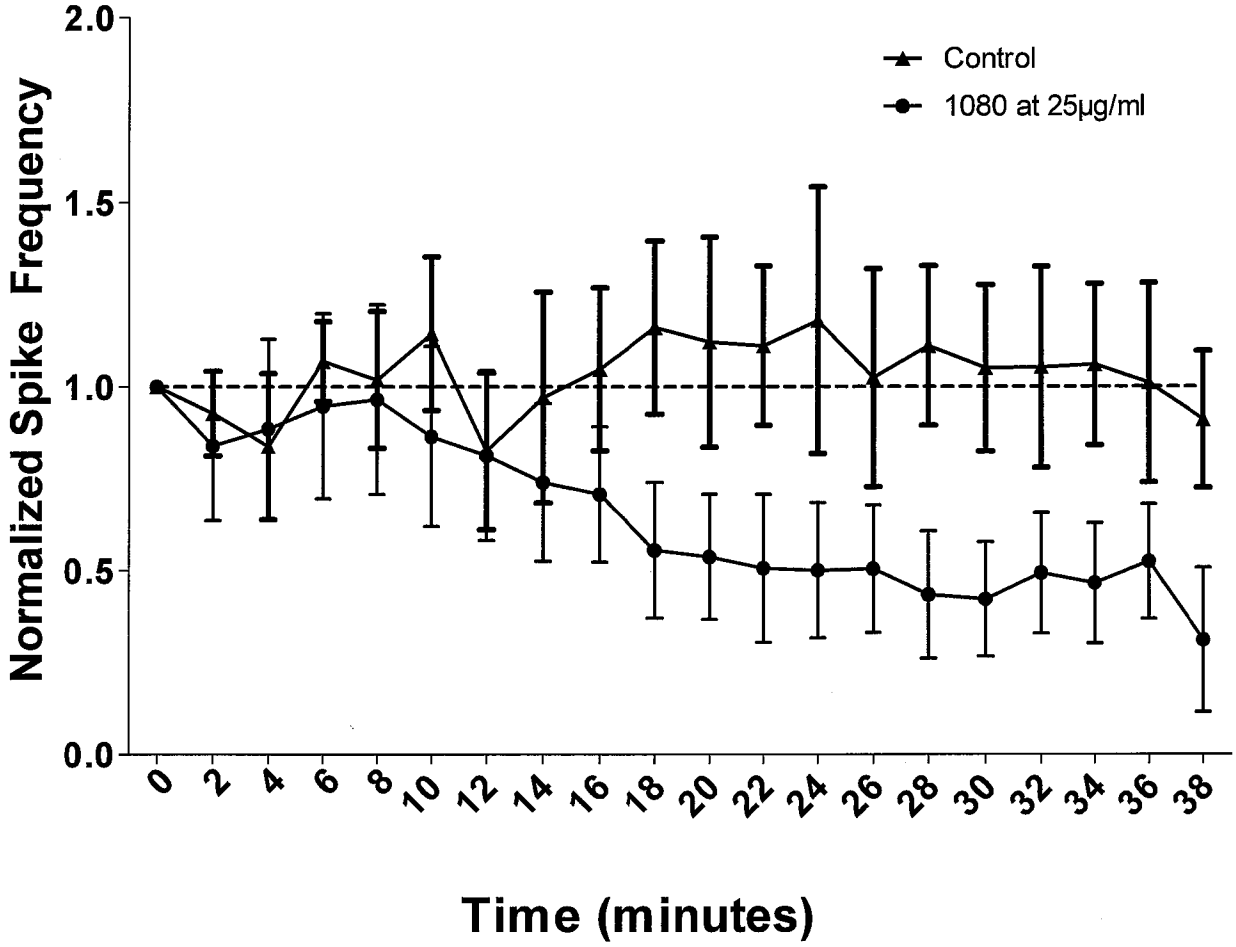
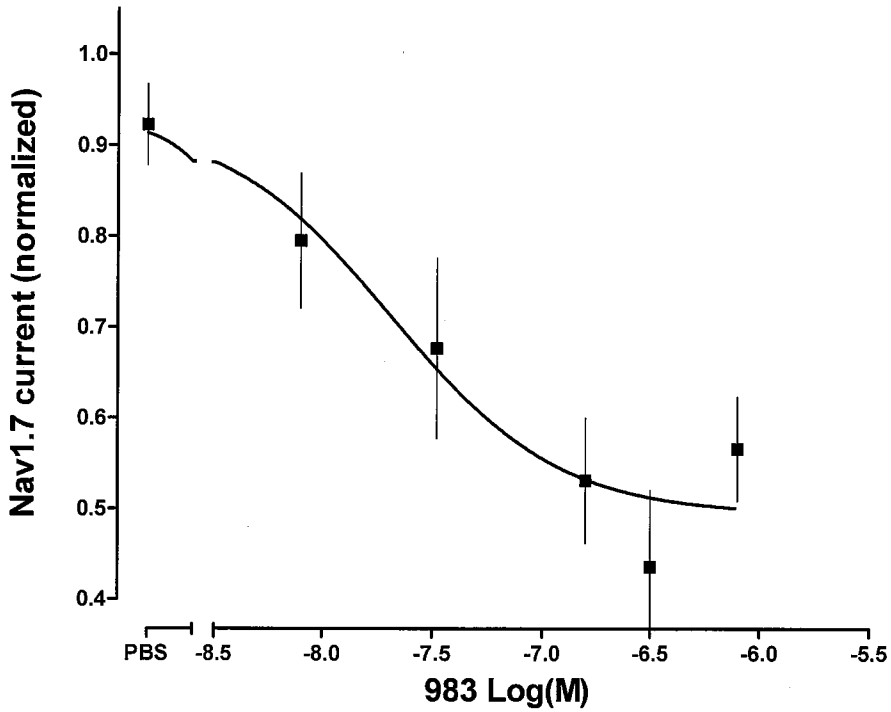

Control
1080 at 25µg/ml
Normalized Spike Frequency
2.0
1.5
1.0
0.5
0.0
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
Time (minutes)

Figure 3D

(a)


Nav1.7 current (normalized)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
PBS
-8.5
-8.0
-7.5
-7.0
-6.5
-6.0
-5.5
983 Log(M)

(b)

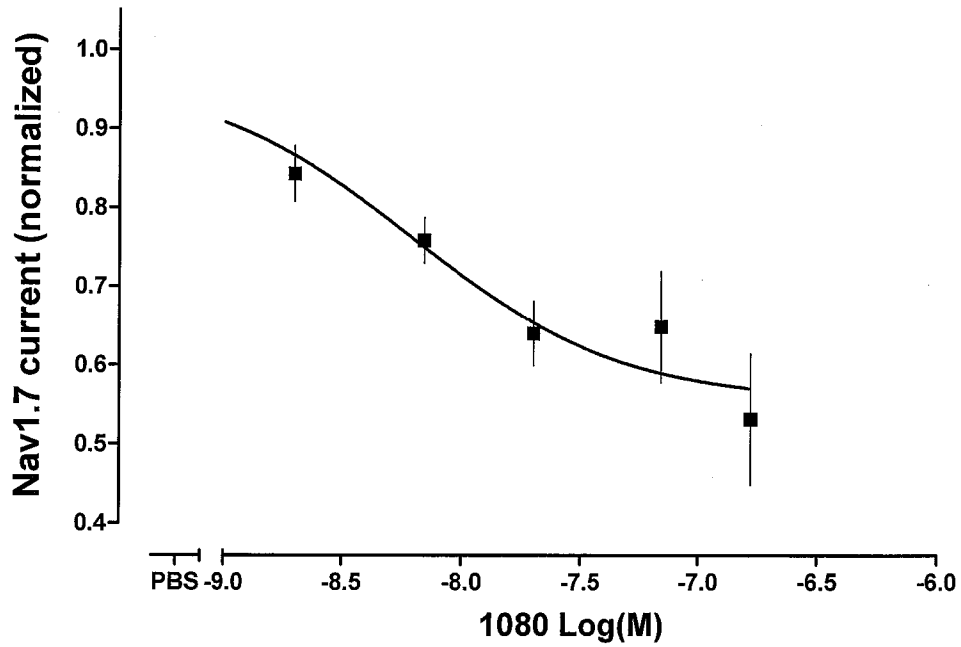

Nav1.7 current (normalized)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
PBS
-9.0
-8.5
-8.0
-7.5
-7.0
-6.5
-6.0
1080 Log(M)

Figure 3e

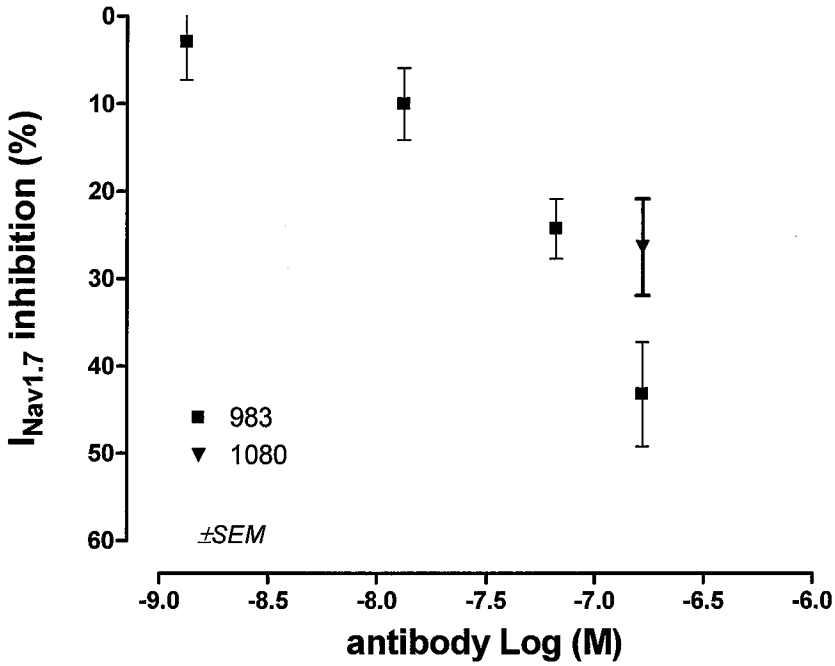

0
10
20
30
40
50
60
$I_{Nav1.7}$ inhibition (%)
983
1080
±SEM
-9.0
-8.5
-8.0
-7.5
-7.0
-6.5
-6.0
antibody Log (M)

Figure 3F

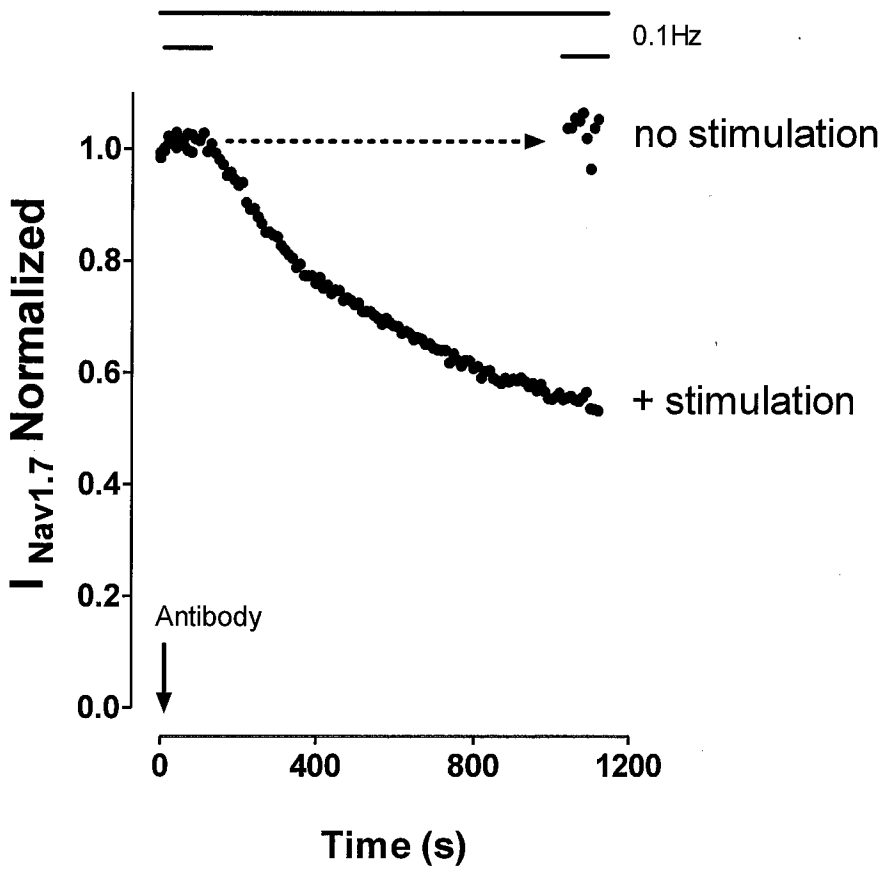

0.1Hz
no stimulation
+ stimulation
Antibody
I Nav1.7 Normalized
1.0
0.8
0.6
0.4
0.2
0.0
0
400
800
1200
Time (s)

Figure 3G

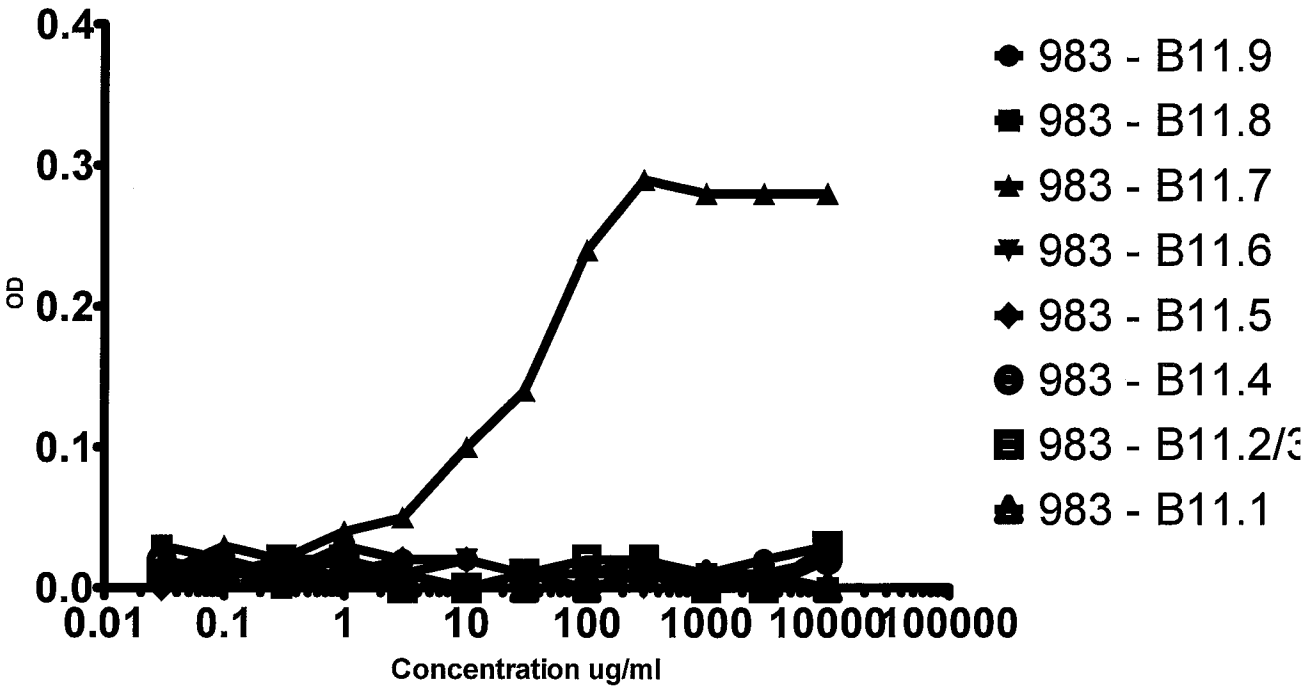

0.4
0.3
0.2
0.1
0.0
OD
0.01
0.1
1
10
100
1000
10000
100000
Concentration ug/ml
983 - B11.9
983 - B11.8
983 - B11.7
983 - B11.6
983 - B11.5
983 - B11.4
983 - B11.2/3
983 - B11.1

Figure 3H

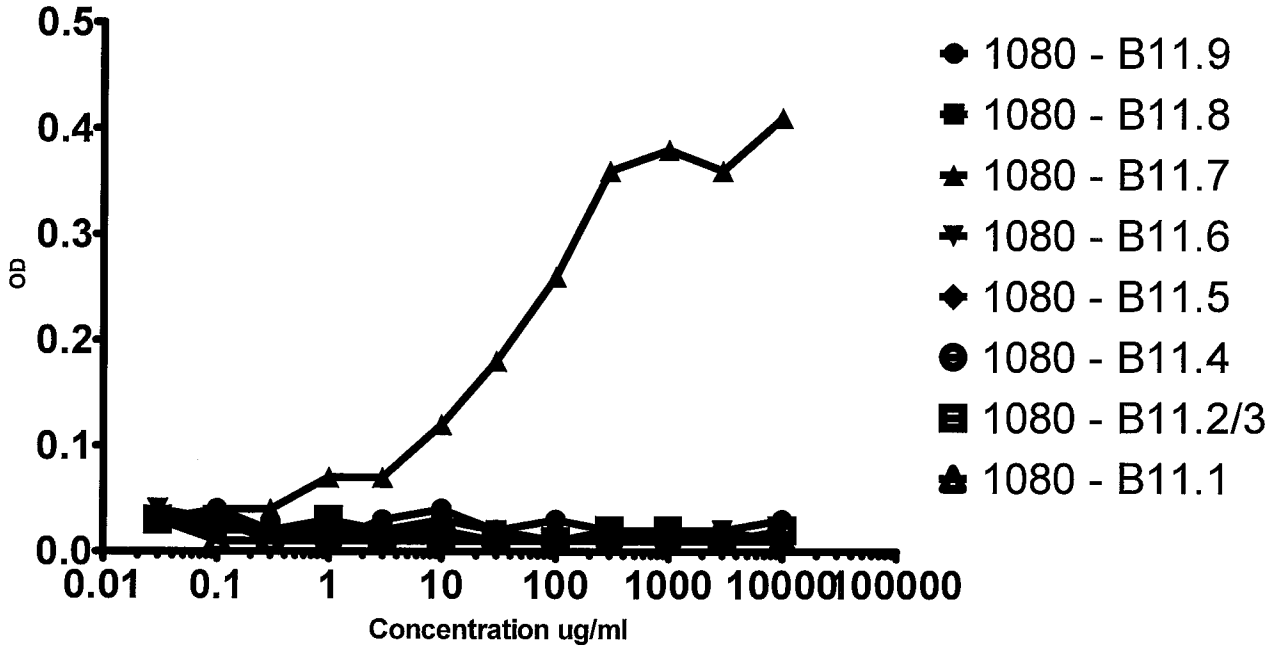

0.5
0.4
0.3
0.2
0.1
0.0
OD
0.01
0.1
1
10
100
1000
10000
100000
Concentration ug/ml
1080 - B11.9
1080 - B11.8
1080 - B11.7
1080 - B11.6
1080 - B11.5
1080 - B11.4
1080 - B11.2/3
1080 - B11.1

Figure 4

Light DNA CA167_00983 (SEQ ID NO: 61)	GCCCAAGTGC TGACCCAGAC TGCATCCCCC GTGTCTGCGG CTGTTGGAGG CACAGTCACC ATCAATTGCC AGTCCAGTCA GAGTGTTTAT AAGAACAACG ACTTAGCCTG GTATCAGCAG AAACCAGGGC AGCCTCCCAA GCTCCTGATC TATTATGCAT CCACTCTGGC ATCTGGGGTC TCATCGCGGT TCAAAGGCAG TGGATCTGGG ACAGAGTTCA CTCTCACCAT CAGCGACGCG CAGTGTGACG ATGCTGCCAC TTACTACTGT CTAGGTAGTT ATGATTGTAG TAGTGCTGAT TGTAATGCTT TCGGCGGAGG GACCAAGGTG GTCGTCAAA
Heavy DNA CA167_00983 (SEQ ID NO: 62)	CAGTCGGTGG AGGAGTCCGG GGGTCGCCTG GTCACGCCTG GGACACCCCT GACACTCACC TGCACAGTCT CTGGATTCTC CCTCAGTAAC TATGCAATGA GTTGGGTCCG CCAGGCTCCA GGGAAGGGGC TGGAATGGAT CGGAATCATT GGTAAAAGTG GTAGTACGGC CTACGCGAGC TGGGCGAAAG GCCGATTCAC CATCTCCAGA ACCTCGACCA CGGTGGATCT GGAAATCACC AGTCCGACAA CCGAGGACAC GGCCACCTAT TTCTGTGTCA GATTTGTGCT CTTGTGGGGC CCGGGGACCC TCGTCACCGT CTCG
Light Protein CA167_00983 (SEQ ID NO: 63)	AQVLTQTASP VSAAVGGTVT INCQSSQSVY KNNDLAWYQQ KPGQPPKLLI YYASTLASGV SSRFKGSGSG TEFTLTISDA QCDDAATYYC LGSYDCSSAD CNAFGGGTKV VVK
Heavy Protein CA167_00983 (SEQ ID NO: 64)	QSVEESGGRL VTPGTPLTLT CTVSGFSLSN YAMSWVRQAP GKGLEWIGII GKSGSTAYAS WAKGRFTISR TSTTVDLEIT SPTTEDTATY FCVRFVLLWG PGTLVTVS
Light DNA CA167_00984 (SEQ ID NO: 65)	GCGCAAGTGC TGACCCAGAC TCCATCCTCC GTGTCTGCAG CTGTGGGAGG CACAGTCACC ATCAATTGCC AGTCCAGTCA GAGTGTTAAT AACAACAACT TCTTATCCTG GTATCAGCAG AAACCAGGGC AGCCTCCCAA GCAACTGATC TACAGGGCTT CCACTCTGGC ATCTGGGGTC CCATCGCGGT TCAAAGGCAG TGGATCTGGG ACACAGTTCA CTCTCACCAT CAGCGACGTG CAGTGTGACG ATGCTGCCAC TTACTTCTGT GCAGGCGGTT ATAGTGGTAA TATTTATGCT TTCGGCGGAG GGACCGAGGT GGTGGTCGAA
Heavy DNA CA167_00984 (SEQ ID NO: 66)	CAGTCGGTGG AGGAGTCCGG GGGTCGCCTG GTCACGCCTG GGACACCCCT GACACTCACC TGCACAGTCT CTGAATTCTC CCTCAGTGAC TATATAATAA ACTGGGTCCG CCAGGCTCCA GGGAAGGGGC TGGAATGGAT CGGGATCATG GGTACTAGTG GTACCGCATA CTACGCGAGC TGGGCGAAAG GCCGATTCAC CATCTCCAAA ACCTCGTCGA CCACGGTGGA TCTGAGAATG ACCAGTCTGA CAACCGAGGA CACGGCCACC TATTTCTGTG CCAGAGGGGG TGTTGCTACT TCTAATTTCT GGGGCCAAGG CACCCTGGTC ACCGTCTCG
Light Protein CA167_00984 (SEQ ID NO: 67)	AQVLTQTPSS VSAAVGGTVT INCQSSQSVN NNNFLSWYQQ KPGQPPKQLI YRASTLASGV PSRFKGSGSG TQFTLTISDV QCDDAATYFC AGGYSGNIYA FGGGTEVVVE
Heavy Protein CA167_00984 (SEQ ID NO: 68)	QSVEESGGRL VTPGTPLTLT CTVSEFSLSD YIINWVRQAP GKGLEWIGIM GTSGTAYYAS WAKGRFTISK TSSTTVDLRM TSLTTEDTAT YFCARGGVAT SNFWGQGTLV TVS

Figure 4 continued

Light DNA CA167_00985 (SEQ ID NO: 69)	GCCCAAGTGC TGACCCAGAC TGCATCCCCT GTGTCTGCAG CTGTGGGAGG CACAGTCACC ATCAATTGTC AGTCCAGTCA GAGCGTTTAT GGTAACAATT GGTTAGGCTG GTATCAGCAG AAACCAGGGC AGCCTCCCAA GCTCCTGATC TATTCTGCAT CTACTCTGGC ATCTGGGGTC CCATCGCGGT TCAGTGGCAG TGGATCTGGG ACACAGTTCA CTCTCACCAT CAGCGACCTG GAGTGTGACG ATGGTGCCAC TTACTATTGT GTAGGCGGGT ATAGTGGTAA TATTCATGTT TTCGGCGGAG GGACCAAGGT GGTGGTCGAA
Heavy DNA CA167_00985 (SEQ ID NO: 70)	CAGTCGGTGG AGGAGTCCGG GGGTCGCCTG GTCACGCCTG GGACACCCCT GACACTCACC TGCACAGTCT CTGGATTCTC CCTCAACGAC TACGACATGA GCTGGGTCCG CCAGGCTCCA GGGAAGGGGC TGGAATGGAT CACAACCATT TATGTTAGTG GTAACACATA CTACGCGACC TGGGCGAAAG GCCGATTCAC CATCTCCAAA ACCTCGACCA CGGTGGATCT GAAAATGACC AGTCCGACAG CCGAGGACAC GGCCACCTAT TTCTGTGCCA GAGCGGTTCC TGGTAGTGGT AAGGGGTTGT GGGGCCCGGG CACCCTCGTC ACCGTCTCG
Light Protein CA167_00985 (SEQ ID NO: 71)	AQVLTQTASP VSAAVGGTVT INCQSSQSVY GNNWLGWYQQ KPGQPPKLLI YSASTLASGV PSRFSGSGSG TQFTLTISDL ECDDGATYYC VGGYSGNIHV FGGGTKVVVE
Heavy Protein CA167_00985 (SEQ ID NO: 72)	QSVEESGGRL VTPGTPLTLT CTVSGFSLND YDMSWVRQAP GKGLEWITTI YVSGNTYYAT WAKGRFTISK TSTTVDLKMT SPTAEDTATY FCARAVPGSG KGLWGPGTLV TVS

Figure 5

Light DNA CA167_01080 (SEQ ID NO: 73)	GCCCAAGTGC TGACCCAGAC TGCATCGCCC GTGTCTGCAG CTGTGGGAAA CACAGTCACC ATCACTTGCC AGTCCAGTCA GAGTGTTTGG AAGAATAACG ACTTATCCTG GTATCAGCAG AAACTAGGGC AGCCTCCCAA GCTCCTGATC TATTATGCAT CCACTCTGGC ATCTGGGGTC TCATCGCGGT TCAAAGCCAG TGGATCTGGG ACACAGTTCA CTCTCACCAT CAGCGACGTG CAATGTGACG ATGCTGGCAC TTACTACTGT GTAGGCAGTT ATGATTGTAG TAGTGCTGAT TGTAATGCTT TCGGCGGAGG GACCAAGGTG GTCGTCAAA
Heavy DNA CA167_01080 (SEQ ID NO: 74)	CAGTCGCTGG AGGAGTCCGG GGGTCGCCTG GTCACGCCGG AGACACCCCT GACACTCACC TGCACAGCCT CTGGAATCGA CCTCAGTAAG TGGCCAATGA CCTGGGTCCG CCAGGCTCCA GGGAAGGGAC TGGAGTGGAT CGGAATTATT GGTAGGAGTG GTAGCACGAA TTACGCGAGC TGGGCGAAAG GCCGATTCAC CATCTCCAAA ACCTCGACCA CGGTGGATCT GAAAATGACC AGTCCGACAA CCGAGGACAC GGCCACTTAT TTCTGTGCCA GAGGTGGTAG TTATTATGAT TTGTGGGGCC AGGGGACCCT GGTCACCGTC TCG
Light Protein CA167_01080 (SEQ ID NO: 75)	AQVLTQTASP VSAAVGNTVT ITCQSSQSVW KNNDLSWYQQ KLGQPPKLLI YYASTLASGV SSRFKASGSG TQFTLTISDV QCDDAGTYYC VGSYDCSSAD CNAFGGGTKV VVK
Heavy Protein CA167_01080 (SEQ ID NO: 76)	QSLEESGGRL VTPETPLTLT CTASGIDLSK WPMTWVRQAP GKGLEWIGII GRSGSTNYAS WAKGRFTISK TSTTVDLKMT SPTTEDTATY FCARGGSYYD LWGQGTLVTV S
Light DNA CA167_01081 (SEQ ID NO: 77)	GCCGCCGTGC TGACCCAGAC TCCATCTCCC GTGTCTGCAG CTGTGGGAGG CACAGTCAGC ATCAGTTGCC AGTCCAGTCA GAGTGTTGAT AATAACAACT ACTTATCCTG GTATCAGCAG AAACCAGGGC AGCCTCCCAA GCTCCTGATC TATGATGCAT CCGATCTGGC ATCTGGGGTC CCATCGCGGT TCAAAGGCAG TGGATCTGGG ACACAGTTCA CTCTCACCAT CAGCGACGTG CAGTGTGACG ATGCTGCCAC TTACTACTGT GCAGGCGGTT ATATAACTAG TAGTGATATT TTTTATGATT TCGGCGGAGG GACCAAGGTG GTGGTCAAA
Heavy DNA CA167_01081 (SEQ ID NO: 78)	CAGTCGGTGG AGGAGTCCGG GGGTCGCCTG GTCACGCCTG GGACACCCCT GACACTCACC TGCACAGTCT CTGGATTCTC CCTCAGTACC TATGCAATGA GCTGGGTCCG CCAGGCTCCA GGGAAGGGGC TGGAATGGAT CGGAATCGTT GGAAAGAGTG GTATTATAAA GTACGCGAGC TGGGCGAAAG GCCGGTTCAC CATCTCCAAA ACCTCGACCA CGGTGGATCT GAAAATGACC AGTCTGACAA CCGAGGACAC GGCCATTTAT TTCTGTGCCA GACTATGGAG CTTGTGGGGC CAAGGGACCC TCGTCACCGT CTCG
Light Protein CA167_01081 (SEQ ID NO: 79)	AAVLTQTPSP VSAAVGGTVS ISCQSSQSVD NNNYLSWYQQ KPGQPPKLLI YDASDLASGV PSRFKGSGSG TQFTLTISDV QCDDAATYYC AGGYITSSDI FYDFGGGTKV VVK
Heavy Protein CA167_01081 (SEQ ID NO: 80)	QSVEESGGRL VTPGTPLTLT CTVSGFSLST YAMSWVRQAP GKGLEWIGIV GKSGIIKYAS WAKGRFTISK TSTTVDLKMT SLTTEDTAIY FCARLWSLWG QGTLVTVS

Figure 6

Light DNA CA167_01082 (SEQ ID NO:81)	GACATTGTGA TGACCCAGAC TCCAGCCTCC GTGTCTGAAC CTGTGGGAGG CACAGTCACC ATCAAGTGCC AGGCCAGTCA GAGCATTAGC AACTGGTTAG CCTGGTATCA GCAGAAACCA GGGCAGCCTC CCAAGCTCCT GATCTACAGG GCATCCACTC TGGCATCTGG GGTCTCATCG CGGTTCAAAG GCAGTGGATC TGGGACAGAG TTCACTCTCA CCATCAGCGA CCTGGAGTGT GCCGATGCTG CCACTTACTA CTGTCAAAGC GATTATGGTA TAGATACTTA TGGAAGTGCT TTCGGCGGAG GGACCAAGGT GGTGGTCAAA
Heavy DNA CA167_01082 (SEQ ID NO: 82)	CAGTCGCTGG AGGAGTCCCG GGGTCGCCTG GTCACGCCTG GGACACCCCT GACACTCACC TGCACAGTCT CTGGAATCGA CCTCAGTAGT TATGCAATGA CCTGGGTCCG CCAGGCTCCA GGGAAGGGGC TGGAATGGAT CGGAATGGTT CGTCGTAGTG GTACCACATA CTACGCGAGC TGGGCGAAAG GCCGATTCAC CATCTCCAAA ACCTCGACCA CGGTGGATCT GAAAATCATC AGTCCGACAA CCGAGGACAC GGCCACCTAT TTCTGTGCCA GATGTGATAA TAGTGCTGGT GACTGGAGTT ACGGCATGGA CCTCTGGGGC CCGGGGACCC TGGTCACCGT CTCG
Light Protein CA167_01082 (SEQ ID NO: 83)	DIVMTQTPAS VSEPVGGTVT IKCQASQSIS NWLAWYQQKP GQPPKLLIYR ASTLASGVSS RFKGSGSGTE FTLTISDLEC ADAATYYCQS DYGIDTYGSA FGGGTKVVVK
Heavy Protein CA167_01082 (SEQ ID NO: 84)	QSLEESRGRL VTPGTPLTLT CTVSGIDLSS YAMTWVRQAP GKGLEWIGMV RRSGTTYYAS WAKGRFTISK TSTTVDLKII SPTTEDTATY FCARCDNSAG DWSYGMDLWG PGTLVTVS
Light DNA CA167_01083 (SEQ ID NO: 85)	GCCCAAGTGC TGACCCAGAC TGCATCGCCC GTGTCTGCAG CTGTGGGAAG CACAGTCACC ATCAATTGCC AGGCCAGTCA GAGTGTTTAT CAGAACAACT ACTTAGCCTG GTTTCAGCAG AAACCAGGGC AGCCTCCCAA GCGCCTGATC TATTCTGCAT CCACTCTGGC ATCTGGGGTC TCATCGCGGT TCAAAGGCAG TGGATCTGGG ACACAGTTCA CTCTCACCAT CAGCGACGTG CAGTGTGACG ATGCTGCCAC TTATTACTGT CTGGGCGCCT ATGATTGTAG TGGTGTTGAT TGTAGTGCTT TCGGCGGAGG GACCAAGGTG GTCGTCAAA
Heavy DNA CA167_01083 (SEQ ID NO: 86)	CAGTCGGTGG AGGAGTCCGG GGGTCGCCTG GTCACGCCTG GGACACCCCT GACACTCACC TGCACCGTCT CTGGATTCTC CCTCAGTACC AATGCAATGA TCTGGGTCCG CCAGGCTCCA GGGAAGGGGC TGGAATATAT CGGTGTGATT GCTGGTAGTG GTAGCACATC TTACGCGAGC TGGGCGAAAG GCCGATTCAC CATCTCCAAA ACCTCGACCA CGGTGGATCT GAAAATCACC AGTCCGACAA CCGAGGACAC GGCCACCTAT TTCTGTGCCA GAGGGGGTTG GGTTAGTGGT CCGGAGAGCT TGTGGGGCCA AGGCACCCTC GTCACCGTCT CG
Light Protein CA167_01083 (SEQ ID NO: 87)	AQVLTQTASP VSAAVGSTVT INCQASQSVY QNNYLAWFQQ KPGQPPKRLI YSASTLASGV SSRFKGSGSG TQFTLTISDV QCDDAATYYC LGAYDCSGVD CSAFGGGTKV VVK
Heavy Protein CA167_01083 (SEQ ID NO: 88)	QSVEESGGRL VTPGTPLTLT CTVSGFSLST NAMIWVRQAP GKGLEYIGVI AGSGSTSYAS WAKGRFTISK TSTTVDLKIT SPTTEDTATY FCARGGWVSG PESLWGQGTL VTVS

Figure 6 continued

Light DNA CA167_01084 (SEQ ID NO: 89)	GCCCAAGTGC	TGACCCAGAC	TCCATCTTCC	ACGTCTGCAG	CTGTGGGAGG	CACAGTCACC	ATCAGTTGCC	AGTCCAGTCC	GAGTGTTTAT
	GGTAATAACT	GGTTAGGCTG	GTATCAGAAG	AAACCAGGGC	AGCCTCCCAA	GCTCCTGATC	TATTCTGCAT	CCACTCTGGC	ATCTGGGGTC
	TCATCGCGGT	TTAAAGGCAG	TGGATCTGGG	ACACAGTTCA	CTCTCACCAT	CAGCGACCTG	GAGTGTGACG	ATGCTGCCAC	TTACTACTGT
	GCAGGCGGTT	ATAGTGGTAA	TATTCATGTT	TTCGGCGGAG	GGACCAAGGT	GGTGGTCAAA			
Heavy DNA CA167_01084 (SEQ ID NO: 90)	CAGTCGGTGG	AGGAGTCCGG	GGGTCGCCTG	GTCACGCCTG	GGACACCCCT	GACACTCACC	TGCACAGTCT	CTGGATTCTC	CCTCAATAAC
	TACGACATGA	CCTGGGTCCG	CCAGGCTCCA	GGGAAGGGGC	TGGAATGGAT	CGGAAGTATT	TTTGTTAGTG	GTAATATATA	CTACGCGAGC
	TGGGCGAAAG	GCCGATTCAC	CATCTCCAAA	ACCTCGACCA	CGGTGGATCT	GAAAATGACC	AGTCCGACAA	CCGAGGACAC	GGCCACCTAT
	TTCTGTGCCA	GAGCAATTCT	TGGTAGTAGT	AAGGGGTTGT	GGGGCCCAGG	CACCCTGGTC	ACCGTCTCG		
Light Protein CA167_01084 (SEQ ID NO: 91)	AQVLTQTPSS	TSAAVGGTVT	ISCQSSPSVY	GNNWLGWYQK	KPGQPPKLLI	YSASTLASGV	SSRFKGSGSG	TQFTLTISDL	ECDDAATYYC
	AGGYSGNIHV	FGGGTKVVVK							
Heavy Protein CA167_01084 (SEQ ID NO: 92)	QSVEESGGRL	VTPGTPLTLT	CTVSGFSLNN	YDMTWVRQAP	GKGLEWIGSI	FVSGNIYYAS	WAKGRFTISK	TSTTVDLKMT	SPTTEDTATY
	FCARAILGSS	KGLWGPGTLV	TVS						

Figure 7

Light DNA CA167_01085 (SEQ ID NO: 93)	GCCTATGATA TGACCCAGAC TCCAGCCTCT GTGGAGGTAG CTGTGGGAGG CACAGTCACC ATCAAGTGCC AGGCCAGTCA GAGCATTTAC AGCTATTTAG CCTGGTATCA GCAGAAACCA GGGCAGCCTC CCAAGCTCCT GATTTATTCT GCATCCTATC TAGCATCTGG GGTCCCATCG CGGTTCAGCG GCAGTGGATC TGGGACAGAG TTCACTCTCA CCATCAGCGA CCTGGAGTGT GCCGATGCTG CCACTTATTA CTGTCAACAC GGGTACATTA GTGGTAATGT TGATAATGCT TTCGGCGGAG GGACCAAGGT GGTCGTCAAA
Heavy DNA CA167_01085 (SEQ ID NO: 94)	CAGTCGGTGG AGGAGTCCGG GGGTCGCCTG GTCACGCCTG GGACACCCCT GACACTCACC TGCACAGTCT CTGGATTCTC CCTCAGCATC TACGACATGA GCTGGGTCCG CCAGGCTCCA GGGAAGGGGC TGGAATGGAT CGGATCCATT TATGTTAGTG GTAATATATA CTACGCGAGC TGGGCGAAAG GCCGATTCAC CATCTCCAAA ACCTCGACCA CGGTGGATCT GAAAATGACC AGTCCGACAA CCGAGGACAC GGCCACCTAT TTCTGTGCCA GAGCGGTTCC TGGTAGTAGT AAGGGGTTGT GGGGCCAGGG GACCCTCGTC ACCGTCTCG
Light Protein CA167_01085 (SEQ ID NO: 95)	AYDMTQTPAS VEVAVGGTVT IKCQASQSIY SYLAWYQQKP GQPPKLLIYS ASYLASGVPS RFSGSGSGTE FTLTISDLEC ADAATYYCQH GYISGNVDNA FGGGTKVVVK
Heavy Protein CA167_01085 (SEQ ID NO: 96)	QSVEESGGRL VTPGTPLTLT CTVSGFSLSI YDMSWVRQAP GKGLEWIGSI YVSGNIYYAS WAKGRFTISK TSTTVDLKMT SPTTEDTATY FCARAVPGSS KGLWGQGTLV TVS
Light DNA CA167_01086 (SEQ ID NO: 97)	GCGCAAGTGC TGACCCAGAC TCCATCCCCT GTGTCTGCAG CTGTGGGAGG CAAAGTCACC ATCAATTGCC AGTCCAGTCA GAGTATTTAT ACTAACTACT TATCCTGGTA TCAGCAGAAA CCAGGACAGC CTCCCAGGCT CCTGATCTAT TCTGCATCCA CTCTGGCATC TGGGGTCCCA TCGCGGTTCA AAGGCAGTGG ATCTGGGACA CAGTTCACTC TCACAATCAG CGAAGTACAG TGTGACGATG CTGCCACTTA CTACTGTCAA GCCTATTTTA CTGGTGAGAT TTTTCCTTTC GGCGGAGGGA CCAAGGTGGT CGTCAAA
Heavy DNA CA167_01086 (SEQ ID NO: 98)	CAGGAGCAAC TGAAGGAGTC CGGGGGAGGC CTGGTAACGC CTGGAGGAAC CCTGACACTC ACCTGCACCG TCTCTGGATT CTCCCTCGAT AACTACCACA TGGGCTGGGT CCGCCAGGCT CCAGGGAAGG GGCTCAATTA CATCGGATTC ATTACTCGTG GTGGTACCAC ATACTACGCG AGCTGGGCGA AGGGCCGATT CACCATCTCC AAAACCTCGA CCACGGTGGA TCTGATGATC ACCAGTCCGA CAACCGGGGA CACGGCCACC TATTTCTGTG CCAGAGGAAG TGGCGCTAGC GGCTTTTACT TGTGGGGCCC AGGCACCCTG GTCACCGTCT CG
Light Protein CA167_01086 (SEQ ID NO: 99)	AQVLTQTPSP VSAAVGGKVT INCQSSQSIY TNYLSWYQQK PGQPPRLLIY SASTLASGVP SRFKGSGSGT QFTLTISEVQ CDDAATYYCQ AYFTGEIFPF GGGTKVVVK
Heavy Protein CA167_01086 (SEQ ID NO: 100)	QEQLKESGGG LVTPGGTLTL TCTVSGFSLD NYHMGWVRQA PGKGLNYIGF ITRGGTTYYA SWAKGRFTIS KTSTTVDLMI TSPTTGDTAT YFCARGSGAS GFYLWGPGTL VTVS

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/066274

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28
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 practical, search terms used)

EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2007/109324 A2 (XENON PHARMACEUTICALS INC [CA]; FRASER ROBERT A [CA]; SHERRINGTON ROBI) 27 September 2007 (2007-09-27) * abstract ----- -/--	1-15

[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 document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 January 2011	10/02/2011

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	Kalsner, Inge

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/066274

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BLACK JOEL A ET AL: "Multiple sodium channel isoforms and mitogen-activated protein kinases are present in painful human neuromas", ANNALS OF NEUROLOGY, JOHN WILEY AND SONS, BOSTON, US, vol. 64, no. 6, 1 December 2008 (2008-12-01), pages 644-653, XP009143330, ISSN: 0364-5134, DOI: DOI:10.1002/ANA.21527 [retrieved on 2008-12-23] the whole document	1-15
A	MEIRI ET AL: "Detection of cell surface sodium channels by monoclonal antibodies - could the channels become exposed to the external surface and 'down regulated' by binding to antibodies?", BRAIN RESEARCH, ELSEVIER, AMSTERDAM, NL, vol. 368, no. 1, 12 March 1986 (1986-03-12), pages 188-192, XP024274584, ISSN: 0006-8993, DOI: DOI:10.1016/0006-8993(86)91061-9 [retrieved on 1986-03-12] the whole document	1-15
A	COX JAMES J ET AL: "An SCN9A channelopathy causes congenital inability to experience pain", NATURE (LONDON),, vol. 444, no. 7121, 1 December 2006 (2006-12-01), pages 894-898, XP009119589, DOI: DOI:10.1038/NATURE05413 the whole document	1-15
A	DIB-HAJJ SULAYMAN D ET AL: "Voltage-gated sodium channels: therapeutic targets for pain.", PAIN MEDICINE (MALDEN, MASS.) OCT 2009 LNKD- PUBMED:19818036, vol. 10, no. 7, October 2009 (2009-10), pages 1260-1269, XP002617523, ISSN: 1526-4637 the whole document	1-15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2010/066274

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

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

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purpose of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in 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/EP2010/066274

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007109324 A2	27-09-2007	NONE	