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(56) Related Art
Gendeh et al (1997) Biochemistry, September, 36(38):11461-11471
US 6077680 A (Kem et al.) 20 June 2000
WO 2008/088422 A2 (Amgen Inc.) 24 July 2008
Pennington, et al (1999) Biochemistry, November, 38(44):14549-14558
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(54) **Title**: SELECTIVE AND POTENT PEPTIDE INHIBITORS OF $K_v1.3$

(57) **Abstract**: Disclosed are compositions of matter having an amino acid sequence of SEQ ID NO:**4**, or a pharmaceutically acceptable salt thereof, including embodiments comprising a toxin peptide analog related to ShK, HmK, and AETX-K and pharmaceutical compositions or medicaments containing them along with a pharmaceutically acceptable carrier. Some embodiments include a half-life extending moiety. Also disclosed are a method of preventing or mitigating a relapse of a symptom of multiple sclerosis and a method of treating an autoimmune disorder using the compositions.

SELECTIVE AND POTENT PEPTIDE INHIBITORS OF $K_V1.3$

FIELD OF THE INVENTION

[0001] This application claims the benefit of U.S. Provisional Application No. 61/210,594, filed March 20, 2009, which is hereby incorporated by reference in its entirety.

[0002] The instant application contains an ASCII "txt" compliant sequence listing submitted via EFS-WEB on March 19, 2010, which serves as both the computer readable form (CRF) and the paper copy required by 37 C.F.R. Section 1.821(c) and 1.821(e), and is hereby incorporated by reference in its entirety. The name of the "txt" file created on March 18, 2010, is: A-1455-WO-PCT-SeqList031810-482_ST25.txt, and is 348 kb in size.

[0003] Throughout this application various publications are referenced within parentheses or brackets. The disclosures of these publications in their entireties are hereby incorporated by reference in this application in order to more fully describe the state of the art to which this invention pertains.

BACKGROUND OF THE INVENTION

[0004] 1. Field of the Invention

[0005] The present invention is related to the biochemical arts, in particular to therapeutic peptides and conjugates.

[0006] 2. Discussion of the Related Art

[0006a] Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of common general knowledge in the field.

[0007] Ion channels are a diverse group of molecules that permit the exchange of small inorganic ions across membranes. All cells require ion channels for function, but this is especially so for excitable cells such as those present in the nervous system and the heart. The electrical signals orchestrated by ion channels control the thinking brain, the beating heart and the contracting muscle. Ion channels play a role in regulating cell volume, and they control a wide variety of signaling processes.

[0008] The ion channel family includes Na+, K+, and Ca2+ cation and Cl- anion channels. Collectively, ion channels are distinguished as either ligand-gated or voltage-gated. Ligand-gated channels include both extracellular and intracellular ligand-gated channels. The extracellular ligand-gated channels include the nicotinic acetylcholine receptor (nAChR), the serotonin (5-hydroxytryptamine, 5-HT) receptors, the glycine and γ-butyric acid receptors (GABA) and the glutamate-activated channels including kanate, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and N-methyl-D-aspartate receptors (NMDA) receptors. (Harte and Ouzounis (2002), FEBS Lett. 514: 129-34). Intracellular ligand gated channels include those activated by cyclic nucleotides (e.g. cAMP, cGMP), Ca2+ and G-proteins. (Harte and Ouzounis (2002), FEBS Lett. 514: 129-34). The voltage-gated ion channels are categorized by their selectivity for inorganic ion species, including sodium, potassium, calcium and chloride ion channels. (Harte and Ouzounis (2002), FEBS Lett. 514: 129-34).

[0009] A unified nomenclature for classification of voltage-gated channels was recently presented. (Catterall et al. (2000), Pharmacol. Rev. 55: 573-4; Gutman et al. (2000), Pharmacol. Rev. 55, 583-6; Catterall et al. (2000) Pharmacol. Rev. 55: 579-81; Catterall et al. (2000), Pharmacol. Rev. 55: 575-8; Hofmann et al. (2000), Pharmacol. Rev. 55: 587-9; Clapham et al. (2000), Pharmacol Rev. 55: 591-6; Chandy (1991), Nature 352: 26; Goldin et al. (2000), Neuron 28: 365-8; Ertel et al. (2000), Neuron 25: 533-5). The K+ channels constitute the largest and best characterized family of ion channels described to date. Potassium channels are subdivided into three general groups: the 6 transmembrane (6TM) K+ channels, the 2TM-2TM/leak K+ channels and the 2TM/Kir inward rectifying channels. (Tang et al. (2004), Ann. Rev. Physiol. 66, 131-159). These three groups are further subdivided into families based on sequence similarity. The voltage-gated K+ channels, including (Kv1-6, Kv8-9), EAG (POTASSIUM CHANNEL, VOLTAGE-GATED, SUBFAMILY H, MEMBER 1), KQT (Potassium voltage-gated channel subfamily KQT member 1), and Slo (BKCa; POTASSIUM CHANNEL, CALCIUM-ACTIVATED,

LARGE CONDUCTANCE, SUBFAMILY M, ALPHA MEMBER 1), are family members of the 6TM group. The 2TM-2TM group comprises TWIK (POTASSIUM CHANNEL, SUBFAMILY K, MEMBER 1), TREK (POTASSIUM CHANNEL, SUBFAMILY K, MEMBER 2), TASK (POTASSIUM CHANNEL, SUBFAMILY K, MEMBER 3), TRAAK (POTASSIUM CHANNEL, SUBFAMILY K, MEMBER 4), and THIK (POTASSIUM CHANNEL, SUBFAMILY K, MEMBER 13, also known as TANDEM PORE DOMAIN HALOTHANE-INHIBITED POTASSIUM CHANNEL), whereas the 2TM/Kir group consists of Kir1-7. Two additional classes of ion channels include the inward rectifier potassium (IRK) and ATP-gated purinergic (P2X) channels. (Harte and Ouzounis (2002), FEBS Lett. 514: 129-34).

[0010] Toxin peptides produced by a variety of organisms have evolved to target ion channels. Snakes, scorpions, spiders, bees, snails and sea anemone are a few examples of organisms that produce venom that can serve as a rich source of small bioactive toxin peptides or "toxins" that potently and selectively target ion channels and receptors. In most cases, these toxin peptides have evolved as potent antagonists or inhibitors of ion channels, by binding to the channel pore and physically blocking the ion conduction pathway. In some other cases, as with some of the tarantula toxin peptides, the peptide is found to antagonize channel function by binding to a region outside the pore (e.g., the voltage sensor domain).

[0011] Native toxin peptides are usually between about 20 and about 80 amino acids in length, contain 2-5 disulfide linkages and form a very compact structure. Toxin peptides (e.g., from the venom of scorpions, sea anemones and cone snails) have been isolated and characterized for their impact on ion channels. Such peptides appear to have evolved from a relatively small number of structural frameworks that are particularly well suited to addressing the critical issues of potency, stability, and selectivity. (See, e.g., Dauplais et al., On the convergent evolution of animal toxins: conservation of a diad of functional residues in potassium channel-blocking toxins with unrelated structures, J. Biol. Chem. 272(7):4302-09 (1997); Alessandri-Haber et al., Mapping the functional anatomy

of BgK on Kv1.1, Kv1.2, and Kv1.3, J. Biol. Chem. 274(50):35653-61 (1999)). The majority of scorpion and Conus toxin peptides, for example, contain 10-40 amino acids and up to five disulfide bonds, forming extremely compact and constrained structure (microproteins) often resistant to proteolysis. The conotoxin and scorpion toxin peptides can be divided into a number of superfamilies based on their disulfide connections and peptide folds. The solution structure of many of these has been determined by Nuclear Magnetic Resonance (NMR) spectroscopy, illustrating their compact structure and verifying conservation of their family folding patterns. (E.g., Tudor et al., Ionisation behaviour and solution properties of the potassium-channel blocker ShK toxin, Eur. J. Biochem. 251(1-2):133-41(1998); Pennington et al., Role of disulfide bonds in the structure and potassium channel blocking activity of ShK toxin, Biochem. 38(44): 14549-58 (1999); Jaravine et al., Three-dimensional structure of toxin OSK1 from Orthochirus scrobiculosus scorpion venom, Biochem. 36(6):1223-32 (1997); del Rio-Portillo et al.; NMR solution structure of Cn12, a novel peptide from the Mexican scorpion Centruroides noxius with a typical beta-toxin sequence but with alpha-like physiological activity, Eur. J. Biochem. 271(12): 2504-16 (2004); Prochnicka-Chalufour et al., Solution structure of discrepin, a new K+-channel blocking peptide from the alpha-KTx15 subfamily, Biochem. 45(6):1795-1804 (2006)). Conserved disulfide structures can also reflect the individual pharmacological activity of the toxin family. (Nicke et al. (2004), Eur. J. Biochem. 271: 2305-19, Table 1; Adams (1999), Drug Develop. Res.46: 219-34). For example, α-conotoxins have well-defined four cysteine/two disulfide loop structures (Loughnan, 2004) and inhibit nicotinic acetylcholine receptors. In contrast, ω-conotoxins have six cysteine/three disulfide loop consensus structures (Nielsen, 2000) and block calcium channels. Structural subsets of toxins have evolved to inhibit either voltage-gated or calcium-activated potassium channels.

[0012] Due to their potent and relatively selective blockade of specific ion channels, toxin peptides have been used for many years as tools to investigate the pharmacology of ion channels. Other than excitable cells and tissues such as those present in heart, muscle and brain, ion channels are also important to non-

excitable cells such as immune cells. Accordingly, the potential therapeutic utility of toxin peptides has been considered for treating various immune disorders, in particular by inhibition of potassium channels such as Kv1.3 and IKCa1 since these channels indirectly control calcium signaling pathway in lymphocytes. (E.g., Kem et al., ShK toxin compositions and methods of use, US Patent No. 6,077,680; Lebrun et al., Neuropeptides originating in scorpion, US Patent No. 6,689,749; Beeton et al., Targeting effector memory T cells with a selective peptide inhibitor of Kv1.3 channnels for therapy of autoimmune diseases, Molec. Pharmacol. 67(4):1369-81 (2005); Possani Postay et al., VM23 and VM24, two scorpion peptides that block human T-lymphocyte potassium channels (subtype kv1.3) with high selectivity and decrease the in vivo DTH-responses in rats, WO 2008/139243; Mouhat et al., K+ channel types targeted by synthetic OSK1, a toxin from Orthochirus scrobiculosus scorpion venom, Biochem. J. 385:95-104 (2005); Mouhat et al., Pharmacological profiling of Orthochirus scrobiculosus toxin 1 analogs with a trimmed N-terminal domain, Molec. Pharmacol. 69:354- 62 (2006); Mouhat et al., OsK1 derivatives, WO 2006/002850 A2; B.S. Jensen et al. The Ca2+-activated K+ Channel of Intermediate Conductance: A Molecular Target for Novel Treatments?, Current Drug Targets 2:401-422 (2001); Rauer et al., Structure-guided Transformation of Charybdotoxin Yields an Analog That Selectively Targets Ca2+-activated over Voltage-gated K+ Channels, J. Biol. Chem. 275: 1201-1208 (2000); Castle et al., Maurotoxin: A Potent Inhibitor of Intermediate Conductance Ca2+-Activated Potassium Channels, Molecular Pharmacol. 63: 409-418 (2003); Chandy et al., K+ channels as targets for specific Immunomodulation, Trends in Pharmacol. Sciences 25: 280-289 (2004); Lewis & Garcia, Therapeutic Potential of Venom Peptides, Nat. Rev. Drug Discov. 2: 790-802 (2003); Han et al., Structural basis of a potent peptide inhibitor designed for Kv1.3 channel, a therapeutic target of autoimmune disease, J. Biol. Chem. 283(27):19058-65 (2008)].

[0013] Calcium mobilization in lymphocytes is known to be a critical pathway in activation of inflammatory responses [M.W. Winslow et al. (2003) Current Opinion Immunol. 15, 299]. Compared to other cells, T cells show a

unique sensitivity to increased levels of intracellular calcium and ion channels both directly and indirectly control this process. Inositol triphosphate (IP3) is the natural second messenger which activates the calcium signaling pathway. IP3 is produced following ligand-induced activation of the T cell receptor (TCR) and upon binding to its intracellular receptor (a channel) causes unloading of intracellular calcium stores. The endoplasmic reticulum provides one key calcium store. Thapsigargin, an inhibitor of the sarcoplasmic-endoplasmic reticulum calcium ATPase (SERCA), also causes unloading of intracellular stores and activation of the calcium signaling pathway in lymphocytes. Therefore, thapsigargin can be used as a specific stimulus of the calcium signaling pathway in T cells. The unloading of intracellular calcium stores in T cells is known to cause activation of a calcium channel on the cell surface which allows for influx of calcium from outside the cell. This store operated calcium channel (SOCC) on T cells is referred to as "CRAC" (calcium release activated channel) and sustained influx of calcium through this channel is known to be critical for full T cell activation [S. Feske et al. (2005) J. Exp. Med. 202, 651 and N. Venkatesh et al. (2004) PNAS 101, 8969]. For many years it has been appreciated that in order to maintain continued calcium influx into T cells, the cell membrane must remain in a hyperpolarized condition through efflux of potassium ions. In T cells, potassium efflux is accomplished by the voltage-gated potassium channel Kv1.3 and the calcium-activated potassium channel IKCa1 [K.G. Chandy et al. (2004) TIPS 25, 280]. These potassium channels therefore indirectly control the calcium signaling pathway, by allowing for the necessary potassium efflux that allows for a sustained influx of calcium through CRAC.

[0014] Sustained increases in intracellular calcium activate a variety of pathways in T cells, including those leading to activation of NFAT (Nuclear Factor of Activated T cells), NF-kB (NUCLEAR FACTOR OF KAPPA LIGHT CHAIN GENE ENHANCER IN B CELLS) and AP-1 [ACTIVATOR PROTEIN 1; Quintana-A (2005) Pflugers Arch. – Eur. J. Physiol. 450, 1]. These events lead to various T cell responses including alteration of cell size and membrane organization, activation of cell surface effector molecules, cytokine production

and proliferation. Several calcium sensing molecules transmit the calcium signal and orchestrate the cellular response. Calmodulin is one molecule that binds calcium, but many others have been identified (M.J. Berridge et al. (2003) Nat. Rev. Mol. Cell. Biol. 4,517). The calcium-calmodulin dependent phosphatase calcineurin is activated upon sustained increases in intracellular calcium and dephosphorylates cytosolic NFAT. Dephosphorylated NFAT quickly translocates to the nucleus and is widely accepted as a critical transcription factor for T cell activation (F. Macian (2005) Nat. Rev. Immunol. 5, 472 and N. Venkatesh et al. (2004) PNAS 101, 8969). Inhibitors of calcineurin, such as cyclosporin A (Neoral, SandImmune) and FK506 (Tacrolimus) are a main stay for treatment of severe immune disorders such as those resulting in rejection following solid organ transplant (I.M. Gonzalez-Pinto et al. (2005) Transplant. Proc. 37, 1713 and D.R.J. Kuypers (2005) Transplant International 18, 140). Neoral has been approved for the treatment of transplant rejection, severe rheumatoid arthritis (D.E. Yocum et al. (2000) Rheumatol. 39, 156) and severe psoriasis (J. Koo (1998) British J. Dermatol. 139, 88). Preclinical and clinical data has also been provided suggesting calcineurin inhibitors may have utility in treatment of inflammatory bowel disease (IBD; Baumgart DC (2006) Am. J. Gastroenterol. Mar 30; Epub ahead of print), multiple sclerosis (Ann. Neurol. (1990) 27, 591) and asthma (S. Rohatagi et al. (2000) J. Clin. Pharmacol. 40, 1211). Lupus represents another disorder that may benefit from agents blocking activation of helper T cells. Despite the importance of calcineurin in regulating NFAT in T cells, calcineurin is also expressed in other tissues (e.g. kidney) and cyclosporine A & FK506 have a narrow safety margin due to mechanism based toxicity. Renal toxicity and hypertension are common side effects that have limited the promise of cyclosporine & FK506. Due to concerns regarding toxicity, calcineurin inhibitors are used mostly to treat only severe immune disease (Bissonnette-R et al. (2006) J. Am. Acad. Dermatol. 54, 472). Kv1.3 inhibitors offer a safer alternative to calcineurin inhibitors for the treatment of immune disorders. This is because Kv1.3 also operates to control the calcium signaling pathway in T cells, but does so through a distinct mechanism to that of calcineurin inhibitors, and

evidence on Kv1.3 expression and function show that Kv1.3 has a more restricted role in T cell biology relative to calcineurin, which functions also in a variety of non-lymphoid cells and tissues.

[0015] Calcium mobilization in immune cells also activates production of the cytokines interleukin 2 (IL-2) and interferon gamma (designated interchangeably herein as IFNg, IFN-g or IFN-γ) which are critical mediators of inflammation. IL-2 induces a variety of biological responses ranging from expansion and differentiation of CD4+ and CD8+ T cells, to enhancement of proliferation and antibody secretion by B cells, to activation of NK cells [S.L. Gaffen & K.D. Liu (2004) Cytokine 28, 109]. Secretion of IL-2 occurs quickly following T cell activation and T cells represent the predominant source of this cytokine. Shortly following activation, the high affinity IL-2 receptor (IL2-R) is upregulated on T cells endowing them with an ability to proliferate in response to IL-2. T cells, NK cells, B cells and professional antigen presenting cells (APCs) can all secrete IFNg upon activation. T cells represent the principle source of IFNg production in mediating adaptive immune responses, whereas natural killer (NK) cells & APCs are likely an important source during host defense against infection [K. Schroder et al. (2004) J. Leukoc. Biol. 75, 163]. IFNg, originally called macrophage-activating factor, upregulates antigen processing and presentation by monocytes, macrophages and dendritic cells. IFNg mediates a diverse array of biological activities in many cell types [U. Boehm et al. (1997) Annu. Rev. Immunol. 15, 749] including growth & differentiation, enhancement of NK cell activity and regulation of B cell immunoglobulin production and class switching.

[0016] CD40L (TUMOR NECROSIS FACTOR LIGAND SUPERFAMILY, MEMBER 5) is another cytokine expressed on activated T cells following calcium mobilization and upon binding to its receptor on B cells provides critical help allowing for B cell germinal center formation, B cell differentiation and antibody isotype switching. CD40L-mediated activation of CD40 (B CELL-ASSOCIATED MOLECULE CD40; Also known as TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 5) on B cells

can induce profound differentiation and clonal expansion of immunoglobulin (Ig) producing B cells [S. Quezada et al. (2004) Annu. Rev. Immunol. 22, 307]. The CD40 receptor can also be found on dendritic cells and CD40L signaling can mediate dendritic cell activation and differentiation as well. The antigen presenting capacity of B cells and dendritic cells is promoted by CD40L binding, further illustrating the broad role of this cytokine in adaptive immunity. Given the essential role of CD40 signaling to B cell biology, neutralizing antibodies to CD40L have been examined in preclinical and clinical studies for utility in treatment of systemic lupus erythematosis (SLE), - a disorder characterized by deposition of antibody complexes in tissues, inflammation and organ damage [J. Yazdany and J Davis (2004) Lupus 13, 377].

[0017] Small molecule inhibitors of Kv1.3 and IKCa1 potassium channels and the major calcium entry channel in T cells, CRAC, have also been developed to treat immune disorders (A. Schmitz et al. (2005) Molecul. Pharmacol. 68, 1254; K.G. Chandy et al. (2004) TIPS 25, 280; H. Wulff et al. (2001) J. Biol. Chem. 276, 32040; C. Zitt et al. (2004) J. Biol. Chem. 279, 12427), but obtaining small molecules with selectivity toward some of these targets has been difficult.

[0018] The identification of selective and potent peptide Kv1.3 inhibitors with prolonged in vivo activity has been a long standing challenge. Production of toxin peptides is a complex process in venomous organisms, and is an even more complex process synthetically. Due to their conserved disulfide structures and need for efficient oxidative refolding, toxin peptides present challenges to synthesis. Although toxin peptides have been used for years as highly selective pharmacological inhibitors of ion channels, the high cost of synthesis and refolding of the toxin peptides and their short half-life in vivo have impeded the pursuit of these peptides as a therapeutic modality.

[0019] Much of the focus has been given to analogs of the 35-residue ShK peptide, which has a short in vivo half-life of about 30 minutes, and which is a potent inhibitor of both the Kv1.3 ion channel and the Kv1.1 ion channel, a potassium channel expressed in the human nervous system. (E.g., Harvey et al., A three-residue, continuous binding epitope peptidomimetic of ShK toxin as a Kv1.3

inhibitor, Bioorganic & Medicinal Chem. Lett. 15:3193-96 (2005); Lanigan et al., Designed peptide analogues of the potassium channel blocker ShK toxin, Biochem. 40:15528-37 (2001)). Position 22 of ShK has been identified as a key residue which confers Kv1.3 selectivity, and ShK binding to Kv1.3 is sensitive to substitution at Lys9 and Arg11. For example, [Dap22]ShK (SEQ ID NO:317; also known as ShK-Dap22) is a picomolar range inhibitor of Kv1.3 with a reported 35-fold selectivity for murine Kv1.3 over murine Kv1.1. (See, e.g., Kem et al., ShK toxin compositions and methods of use, US Patent No. 6,077,680). [Dap22]ShK is reported to display about 20-fold selectivity for human K(v)1.3 over K(v)1.1, when measured by the whole-cell voltage clamp method but not in equilibrium binding assays (Middleton RE, et al., Substitution of a single residue in Stichodactyla helianthus peptide, ShK-Dap22, reveals a novel pharmacological profile. Biochemistry. 2003 Nov 25 42(46):13698-707). The ShK-Dap22 molecule was reported to have similar potency to native ShK and to provide potent blockade of Kv1.3 with an IC50 of about 23 pM as measured by whole cell patch clamp electrophysiology. (Kalman et al., ShK-Dap22, a potent Kv1.3-specific immunosuppressive polypeptide, J. Biol. Chem. 273(49):32697-707 (1998)).

[0020] Other ShK analogs with phosphotyrosine ("pY"), or other anionically charged chemical entities, or fluorescein modifications at the N-terminus have reportedly resulted in some improved selectivity for mKv1.3 over mKv1.1. An example includes Shk-L5, which involves a phosphotyrosine-AEEA modification at the N-terminus of the ShK peptide. (Beeton et al., Targeting effector memory T cells with a selective peptide inhibitor of Kv1.3 channnels for therapy of autoimmune diseases, Molec. Pharmacol. 67(4):1369-81 (2005); Chandy et al., Analogs of ShK toxin and their uses in selective inhibition of Kv1.3 potassium channels, WO 2006/042151 A2; Pennington et al., Engineering a stable and selective peptide blocker of the Kv1.3 channel in T lymphocytes, Molecular Pharmacology Fast Forward, published January 2, 2009 as doi:10.1124/mol.108.052704 (2009)). AEEA is 2-(2-(2-aminoethoxy)ethoxy)acetic acid (also known as 8-Amino-3,6-Dioxaoctanoic

Acid) and is used as a "linker" group in peptide chemistry in its N-Fmoc-protected form. In ShK-L5, this AEEA hydrophilic bifunctional linker is use as a very short bridge between a phosphotyrosine residue and the ShK peptide. However, from a biochemical perspective, the phosphotyrosine group is not metabolically stable, and the phospho group can be cleaved under physiological conditions. Beeton et al. (2005) indicated that ShK-L5 has an estimated circulating half-life of about 50 min in rats following subcutaneous or intravascular injection, which is comparable to that for the native ShK peptide. (See, e.g., Beeton et al., Selective blockade of T lymphocyte K+ channels ameliorates experimental autoimmune encephalomyelitis, a model for multiple sclerosis, Proc. Natl. Acad. Sci. USA 98(24):13942-47 (2001)). Thus phosphotyrosine-linked derivative of ShK with improved metabolic stability but which also retain high potency have been sought. (Chaurdran, Tet. Letters, 28, 4051-4054 (2007). More recently, M.W. Pennington et al. described ShK-192, which incorporates phosphonophenylalanine-AEEA- at the N-terminus instead of phosphotyrosine-AEEA. (Pennington et al., Engineering a stable and selective peptide blocker of the Kv1.3 channel in T lymphocytes, Molec. Pharmacol. 75(4):762-73 (2009)).

[0021] The present invention relates to compositions of matter including ShK peptide analogs with improved Kv1.3 inhibition activity, in vivo stability and/or selectivity, which may also be fused, or otherwise covalently conjugated to a vehicle.

[0021a] It is an object of the present invention to overcome or ameliorate at least one of the disadvantages of the prior art, or to provide a useful alternative.

[0022] SUMMARY OF THE INVENTION

[0023] According to a first aspect, the present invention proides a composition of matter comprising an amino acid sequence of the formula:

[0024] $X_{aa}^{1}X_{aa}^{2}Cys^{3}X_{aa}^{4}Asp^{5}X_{aa}^{6}X_{aa}^{7}X_{aa}^{8}X_{aa}^{9}X_{aa}^{10}X_{aa}^{11}Cys^{12}X_{aa}^{13}X_{aa}^{14}X_{aa}^{15}X_{aa}^{16}Cys^{17}$ $X_{aa}^{18}X_{aa}^{19}X_{aa}^{20}X_{aa}^{21}X_{aa}^{22}X_{aa}^{23}X_{aa}^{24}X_{aa}^{25}X_{aa}^{26}X_{aa}^{27}Cys^{28}X_{aa}^{29}X_{aa}^{30}X_{aa}^{31}Cys^{32}X_{aa}^{33}X_{aa}^{34}Cys^{35}$ $X_{aa}^{36}X_{aa}^{37}X_{aa}^{38}$//SEQ ID NO:4

[0025] or a pharmaceutically acceptable salt thereof,

[0026] wherein:

[0027] $X_{aa}^{1}X_{aa}^{2}$ is absent; or X_{aa}^{1} is absent and X_{aa}^{2} is Glu, Ser, Ala, or Thr; or X_{aa}^{1} is Arg or Ala and X_{aa}^{2} is Glu, Ser, Ala, or Thr;

[0028] X_{aa}^{4} is an alkyl, basic, or acidic amino acid residue;

[0029] X_{aa}^{6} is Thr, Tyr, Ala, or Leu;

[0030] X_{aa}^{7} is Leu, Ile, Ala, or Lys;

[0031] X_{aa}^{8} is Pro, Ala, Arg, Lys, 1-Nal, or Glu;

[0032] X_{aa}^{9} is Lys, Ala, Val or an acidic amino acid residue selected from Glu, Asp, and α-aminoadipic acid;

[0033] X_{aa}^{10} is Ser, Glu, Arg, or Ala;

[0034] X_{aa}^{11} is Arg, Glu; or Ala;

[0035] X_{aa}^{13} is Thr, Ala, Arg, Lys, 1-Nal, or Glu;

[0036] X_{aa}^{14} is Gln, Ala or an acidic amino acid residue selected from Glu, Asp, and α-aminoadipic acid;

[0037] X_{aa}^{15} is an alkyl or aromatic amino acid residue;

[0038] X_{aa}^{16} is a basic, alkyl, or aromatic amino acid residue, other than Ala, Gln, Glu or Arg;

[0039] X_{aa}^{18} is Ala or an acidic or basic amino acid residue;

[0040] X_{aa}^{19} is Thr, Ala or a basic amino acid residue selected from Lys, Arg, His, Orn, D-Orn, Dab, Dap, 1Pip, 2Pal, 3Pal, N-Me-Lys, Nα Methyl-Arg; homoarginine, Cit, Nα-Methyl-Cit, Homocitrulline, Guf, and 4-Amino-Phe, and N-Me-Orn;

[0041] X_{aa}^{20} is Ser, Ala, or a basic amino acid residue selected from Lys, Arg, His, Orn, D-Orn, Dab, Dap, 1Pip, 2Pal, 3Pal, N-Me-Lys, Nα Methyl-Arg; homoarginine, Cit, Nα-Methyl-Cit, Homocitrulline, Guf, and 4-Amino-Phe, and N-Me-Orn;

[0042] X_{aa}^{21} is an alkyl or aromatic amino acid residue, other than Ala or Met;

[0043] X_{aa}^{22} is Lys or Ala;

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[0044] X_{aa}^{23} is Tyr or Ala;

[0045] X_{aa}^{24} is Arg, Lys, or Ala;

[0046] X_{aa}^{25} is Tyr, Leu, or Ala;

[0047] X_{aa}^{26} is Ser, Thr, Asn, Ala, or an aromatic amino acid residue selected from 1-Nal, 2-Nal, Phe, Trp, and Tyr;

[0048] X_{aa}^{27} is Leu, Ala, Asn, or an aromatic amino acid residue selected from 1-Nal, 2-Nal, Phe, Trp, and Tyr;

[0049] X_{aa}^{29} is 1-Nal, 2-Nal, Ala, or a basic amino acid residue selected from Lys, Arg, His, Orn, D-Orn, Dab, Dap, 1Pip, 2Pal, 3Pal, N-Me-Lys, Nα Methyl-Arg; homoarginine, Cit, Nα-Methyl-Cit, Homocitrulline, Guf, and 4-Amino-Phe, and N-Me-Orn;

[0050] X_{aa}^{30} is Ala or an acidic or basic amino acid residue;

[0051] X_{aa}^{31} is Thr, Ala, or an aromatic amino acid residue selected from 1-Nal, 2-Nal, Phe, Trp, and Tyr;

[0052] X_{aa}^{33} is Gly, Ala, Arg, Lys, 1-Nal, or Glu;

[0053] X_{aa}^{34} is Thr, Ser, Ala, Lys, or an aromatic amino acid residue selected from 1-Nal, 2-Nal, Phe, Trp, and Tyr;

[0054] each of X_{aa}^{36}, X_{aa}^{37}, and X_{aa}^{38} is independently absent or is independently a neutral, basic, acidic, or N-alkylated amino acid residue;

[0055] and wherein:

[0056] there is a disulfide bond between residue Cys^{3} and residue Cys^{35},

[0057] there is a disulfide bond between residue Cys^{12} and residue Cys^{28};

[0058] there is a disulfide bond between residue Cys^{17} and residue Cys^{32}; and

[0059] the carboxy-terminal residue is optionally amidated.

[0059a] According to a second aspect, the present invention provides a pharmaceutical composition, comprising the composition of matter of the invention, and a pharmaceutically acceptable carrier.

[0059b] According to a third aspect, the present invention provides a method of preventing or mitigating a relapse of at least one symptom of multiple sclerosis, comprising administering a prophylactically effective amount of a composition of matter of the invention, or a pharmaceutical composition of the invention.

[0059c] According to a fourth aspect, the present invention provides a method of treating an autoimmune disorder, comprising administering a therapeutically effective amount of a composition of matter of the invention, or a pharmaceutical composition of the invention.

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[0059d] According to a fifth aspect, the present invention provides use of a composition of matter of the invention in the preparation of a medicament for:

preventing or mitigating a relapse of at least one symptom of multiple sclerosis; or treating an autoimmune disorder.

[0059e] Unless the context clearly requires otherwise, throughout the description and the claims, the words "comprise", "comprising", and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of "including, but not limited to".

[0060] Encompassed within the present invention are embodiments of the composition in which one or more additional amino acid residues are present at the N-terminal end to the left of amino acid position 1, or at the C-terminal end to the right beyond amino acid position 38, or both. For example, a composition of the invention containing a toxin peptide analog of the invention up to about 100 amino acid residues long, can have one, two, three, four, five, six, seven, eight, nine, ten, twenty, thirty, or more additional amino acid residues present at the N-terminal end to the left of amino acid position 1, or at the C-terminal end to the right beyond amino acid position 38, or at both the N-terminal and C-terminal ends.

[0061] In some embodiments, the inventive composition of matter comprises an amino acid sequence selected from comprising an amino acid sequence selected from SEQ ID NOS: 10, 15, 155, 157, 164, 165, 167 through 172, 179, 194, 196, 203 through 206, 211, 214 through 225, 231, 232, 233, 236, 238, 239, 242 through 254, 260, 263, and 265 through 273 as set forth in Tables 5 and 11-17, respectively.

[0062] In some embodiments, the inventive composition of matter comprises an amino acid sequence selected from SEQ ID NOS: 10, 11, 12, 14, 15, 16, 19 through 29, 31 through 34, 36 through 50, 52, 54, 55, 56, 59, 60, 61, 63, 65 through 100, 130 through 140, 142 through 174, 176 through 254, and 257 through 274 as set forth in Tables 5-17, respectively.

[0063] Another embodiment of the invention is a composition of matter comprising a toxin peptide analog of up to about 100 amino acid residues long comprising an amino acid sequence of SEQ ID NO:13, wherein:

[0064] there is a disulfide bond between residue Cys^{3} and residue Cys^{35} of SEQ ID NO:13;

[0065] there is a disulfide bond between residue Cys^{12} and residue Cys^{28} of SEQ ID NO:13;

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[0066] there is a disulfide bond between residue Cys^{17} and residue Cys^{32} of SEQ ID NO:13; and

[0067] the carboxy-terminal residue is optionally amidated.

[0068] Embodiments of the inventive compositions of matter include toxin peptide analogs as unconjugated "naked" peptides, or covalently linked or conjugated directly or indirectly (i.e., through a linker moiety) to a half life-extending moiety.

[0069] Examples of useful half-life extending moieties include an immunoglobulin (e.g., IgG1, IgG2, IgG3 or IgG4), immunoglobulin Fc domain (e.g., a human immunoglobulin Fc domain, including Fc of IgG1, IgG2, IgG3 or IgG4) or a portion thereof, transthyretin, human serum albumin (HSA), or poly(ethylene glycol) (PEG) of molecular weight of about 1000 Da to about 100000 Da. These and other half-life extending moieties described herein are useful, either individually or in combination. The toxin peptide analogs of the present invention are potent peptide inhibitors of Kv1.3 with improved potency, stability, and/or selectivity over Kv1.1, relative to native ShK or HmK toxin peptides.

[0070] The invention also relates to a pharmaceutical composition comprising the inventive composition of matter and a pharmaceutically acceptable carrier, and to use of the composition of matter in the manufacture of a medicament.

[0071] The inventive composition of matter can be used for practising a method of treating an autoimmune disorder. For example, the inventive composition of matter can be used in treatment of an autoimmune disorder selected from multiple sclerosis, type 1 diabetes, psoriasis, inflammatory bowel disease, contact-mediated dermatitis, rheumatoid arthritis, psoriatic arthritis, asthma, allergy, restinosis, systemic sclerosis, fibrosis, scleroderma, glomerulonephritis, Sjogren syndrome, inflammatory bone resorption, transplant rejection, graft-versus-host disease, and lupus.

[0072] The inventive composition of matter can also be used for practicing a method of preventing or mitigating a relapse of a symptom of multiple sclerosis.

[0073] Although mostly contemplated as therapeutic agents, compositions of this invention can also be useful in screening for therapeutic or diagnostic agents. For example, one can use an Fc-peptide in an assay employing anti-Fc coated plates. The half-life extending moiety, such as Fc, can make insoluble peptides soluble and thus useful in a number of assays.

[0074] Numerous additional embodiments and advantages of the present invention will become apparent upon consideration of the figures and detailed description of the invention. ———

[0075] BRIEF DESCRIPTION OF THE DRAWINGS

[0076] Figure 1A shows the amino acid sequence of the mature ShK toxin peptide (SEQ ID NO:1), which can be encoded for by a nucleic acid sequence containing codons optimized for expression in mammalian cell, bacteria or yeast.

[0077] Figure 1B shows the three disulfide bonds (--S—S--) formed by the six cysteines within the ShK peptide. (Kem et al., U.S. Patent No. 6,077,680).

[0078] Figure 1C shows a space filling stereo model of ShK toxin peptide. Key residues (D5, I7, R11, S20, M21, K22, Y23, F27) important for Kv1.3 binding (based on analoging described herein) are lightly shaded.

[0079] Figure 1D shows a space filling stereo model of the ShK toxin peptide. Amino acid residues that when changed to an analog result in improved Kv1.3 versus Kv1.1 selectivity, are lightly shaded and include I7, S10, Q16, S20, S26, and R29.

[0080] Figure 2A-B shows an alignment of the voltage-gated potassium channel inhibitor Stichodactyla helianthus (ShK) with other closely related members of the sea anemone toxin family. The sequence of the 35 amino acid mature ShK toxin (Swiss-Protein Accession #P29187) isolated from the venom of Stichodactyla helianthus is shown aligned to other closely related members of the sea anemone family. The consensus sequence and predicted disulfide linkages are shown, with highly conserved residues being shaded. The HmK peptide toxin sequence shown (Swiss-Protein Accession #O16846) is of the immature precursor from the Magnificent sea anemone (Radianthus magnifica; Heteractis magnifica). The putative signal peptide and propeptide regions are single & double underlined, respectively. The mature HmK peptide toxin would be predicted to be 35 amino acids in length and span residues 40 through 74. The immature AETX-K toxin precursor (Swiss Protein Accession # Q0EAE5) from the sea anemone Anemonia erythraea is also shown, with the mature peptide extending from residues 49-83. The predicted signal peptide and propeptide regions are single and double underlined, respectively. AeK is the mature peptide toxin, isolated from the venom of the sea anemone Actinia equina (Swiss-Protein Accession #P81897). The sequence of the mature peptide toxins AsKS (Swiss-Protein

Accession #Q9TWG1) and BgK (Swiss-Protein Accession #P29186) isolated from the venom of the sea anemones Anemonia sulcata and Bunodosoma granulifera, respectively, are also shown. Figure 2A shows the amino acid alignment of ShK (SEQ ID NO:1) to other members of the sea anemone family of toxins, HmK (SEQ ID NO:2 (Mature Peptide) within the larger SEQ ID NO:276 (Signal, propeptide and Mature Peptide portions)), AeK (SEQ ID NO:5), AsKs (SEQ ID NO:6), BgK (SEQ ID NO:7), and AETX-K (SEQ ID NO:3 (Mature Peptide) within the larger SEQ ID NO:275 (Signal, propeptide and Mature Peptide portions)). Amino acid residues that are conserved across all the sequences at a given position are listed beneath the aligned sequences. Red shading or blocking indicates conserved cysteine residues which are in bold text. Black shading indicates residues identical to ShK in given position of aligned sequences. Figure 2B shows a disulfide linkage map for members of the family of mature toxin peptides having 3 disulfide linkages (C1-C6, C2-C4, C3-C5), including ShK, BgK, HmK, AeKS, AETX-K, AsK, and DTX1.

[0081] Figure 3 shows a summary of the ShK primary amino acid sequence and the effect of a single amino acid substitutions at each position, based on the analog data described herein. Positions where single substitution analogs tend to reduce Kv1.3 activity ("#") are at residues 5, 7, 11, 20-23, and 27. Positions where single substitution analogs tend to improve Kv1.3 selectivity ("$") are at residues 7, 10, 16, 20, 22, 23, 26, 27, and 29. Positions where single substitution analogs tend to improve Kv1.3 activity ("*") are at residues 2, 4, 10, 15, 18, 30, 31, and 34.

[0082] Figure 4 shows pharmacokinetic data comparing in vivo half life in rats for 20 kD-PEG-ShK (SEQ ID NO:8) at 0.3 and 2 mg/kg with ShK-L5 (SEQ ID NO:17; Beeton et al., Targeting effector memory T cells with a selective peptide inhibitor of Kv1.3 channnels for therapy of autoimmune diseases, Molec. Pharmacol. 67(4):1369-81 (2005); Chandy et al., Analogs of ShK toxin and their uses in selective inhibition of Kv1.3 potassium channels, WO 2006/042151 A2). (See, Example 5 and Example 8).

[0083] Figure 5A shows Coomassie brilliant blue stained Tris-glycine 4-20%, SDS-PAGE of the final pool of 20kDa PEG-[Lys16]Shk (SEQ ID NO:16) product. Lanes 1 – 5 were loaded as follows: SeeBlue® Plus 2 molecular weight protein standards (10 μL; lanes 1 and 4), 2.0 μg product non-reduced (lane 2), blank (lane 3), 2.0 μg product reduced (lane 5).

[0084] Figure 5B shows RP-HPLC chromatograms on final PEG-peptide pools to demonstrate purity of 20kDa PEG-[Lys16]Shk (SEQ ID NO:16) purity >99%.

[0085] Figure 5C shows Coomassie brilliant blue stained Tris-glycine 4-20%, SDS-PAGE of the final pool of 20kDa branched PEG-[Lys16]Shk (SEQ ID NO:315) product. Lanes 1 – 3 were loaded as follows: 2.0 μg product non-reduced (lane 1), SeeBlue® Plus 2 molecular weight protein standards (10 μL; lane 2), 2.0 μg product reduced (lane 3).

[0086] Figure 5D shows RP-HPLC chromatograms on final PEG-peptide pools to demonstrate purity of 20kDa branched PEG-[Lys16]Shk (SEQ ID NO:315) purity >98%.

[0087] Figure 5E shows Coomassie brilliant blue stained Tris-glycine 4-20%, SDS-PAGE of the final pool of 20kDa PEG-[Lys16]Shk-Ala (SEQ ID NO:316) product. Lanes 1 – 3 were loaded as follows: 2.0 μg product non-reduced (lane 1), SeeBlue® Plus 2 molecular weight protein standards (10 μL; lane 2), 2.0 μg product reduced (lane 3).

[0088] Figure 5F shows RP-HPLC chromatograms on final PEG-peptide pools to demonstrate purity of 20kDa PEG-[Lys16]ShK-Ala (SEQ ID NO:316) purity >99%.

[0089] Figure 6A-B demonstrates by PatchXpress® electrophysiology that Nα-20kDa-PEG [Lys16]ShK (SEQ ID NO:16) is more potent in blocking human Kv1.3 current (Figure 6A) than human Kv1.1 current (Figure 6B), as described in Example 5.

[0090] Figure 6C-D shows by PatchXpress® electrophysiology the impact of various concentrations of ShK-L5 (SEQ ID NO:17) on human Kv1.3 current (Figure 6C) or human Kv1.1 current (Figure 6D), as described in Example 5.

[0091] Figure 6E-F demonstrates by PatchXpress® electrophysiology that the monovalent aKLH HC-ShK(1-35 Q16K) Ab (SEQ ID NO:338, 339, 342) is more potent in blocking human Kv1.3 current (Figure 6E) than human Kv1.1 current (Figure 6F), as described in Examples 11 and 12.

[0092] Figure 7 shows AT-EAE data comparing the activity in vivo in rats of Kv1.3-selective inhibitor 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16) and the less Kv1.3-selective 20kDa-PEG-ShK molecule (SEQ ID NO:8) as described in Example 9.

[0093] Figure 8A-D show the AT-EAE data for the individual rats receiving vehicle or doses of 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16) or 20kDa-PEG-ShK molecule (SEQ ID NO:8) as described in Example 9.

[0094] Figure 9A-B shows that in a 12-week pharmacology study in cynomolgus monkeys, weekly dosing of cynomolgus monkeys with Nα-20kDa-PEG-[Lys16]ShK (SEQ ID NO:16) provided sustained suppression of T cell responses, as measured using the ex vivo cyno whole blood PD assay of inflammation that measured production of IL-4 (Figure 9A) and IL-17 (Figure 9B). Arrows indicate the approximate time when weekly doses were delivered. Further details on the study are provided in Example 10 and Table 4F.

[0095] Figure 9C shows predicted versus measured serum concentrations of Nα-20kDa-PEG-[Lys16]ShK (SEQ ID NO:16) in cynomolgus monkeys after weekly subcutaneous (SC) dosing (0.5 mg/kg, n = 6), as described in Example 10. The measured serum trough levels after weekly dosing (open squares), matched closely those predicted based on repeat-dose modeling of the single-dose pharmacokinetic data (solid line).

[0096] Figure 9D shows animal weight gain during the 12-week cyno pharmacology study described in Example 10 and Figure 9A-C; arrows on x-axis indicate SC dosing with Nα-20kDa-PEG-[Lys16]ShK (SEQ ID NO:16).

[0097] Figure 10A-D shows the stability of 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16) in rat, cynomolgus monkey and human plasma, tested by spiking the peptide conjugate into 100% plasma to a final concentration of 200 ng/mL and incubating for various periods of time at 37°C as described in Example 7.

[0098] Figure 11A-B shows representative PK profiles of a single subcutaneous dose (mouse and rat, dose = 2 mg/kg; beagle and cyno, dose = 0.5 mg/kg) of 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16), as described in Example 5 and Example 8.

[0099] Figure 11C shows a representative cyno PK profile of a single subcutaneous dose (0.5 mg/kg) of 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16) that demonstrates drug levels are above 25 nM for one week, as described in Example 5 and Example 8.

[00100] Figure 12A-N shows schematic structures of some embodiments of a composition of the invention that include one or more units of a pharmacologically active toxin peptide analog (squiggle) fused, via an optional peptidyl linker moiety such as but not limited to L5 or L10 described herein, with one or more domains of an immunoglobulin. These schematics show a more typical IgG1, although they are intended to apply as well to IgG2s, which will have 4 disulfide bonds in the hinge and a different arrangement of the disulfide bond linking the heavy and light chain, and IgG3s and IgG4s. Figure 12A represents a monovalent heterodimeric Fc-toxin peptide analog fusion with the toxin peptide analog fused to the C-terminal end of one of the immunoglobulin Fc domain monomers. Figure 12B represents a bivalent homodimeric Fc-toxin peptide analog fusion, with toxin peptide analogs fused to the C-terminal ends of both of the immunoglobulin Fc domain monomers. Figure 12C represents a monovalent heterodimeric toxin peptide analog-Fc fusion with the toxin peptide analog fused to the N-terminal end of one of the immunoglobulin Fc domain monomers. Figure 12D represents a bivalent homodimeric toxin peptide analog-Fc fusion, with toxin peptide analogs fused to the N-terminal ends of both of the immunoglobulin Fc domain monomers. Figure 12E represents a monovalent heterotrimeric Fc-toxin peptide analog/Ab comprising an immunoglobulin heavy chain (HC) + immunoglobulin light chain (LC) + an immunoglobulin Fc monomer with a toxin peptide analog fused to its C-terminal end. Figure 12 F represents a monovalent heterotetrameric (HT) antibody HC-toxin peptide analog fusion, with a toxin peptide analog fused to the C-terminal end of one of the HC

monomers. Figure 12G represents a bivalent HT antibody Ab HC-toxin peptide analog fusion having toxin peptide analogs on the C-terminal ends of both HC monomers. Figure 12H represents a monovalent HT toxin peptide analog-LC Ab, with the toxin peptide analog fused to the N-terminal end of one of the LC monomers. Figure 12I represents a monovalent HT toxin peptide analog-HC Ab, with the toxin peptide analog fused to the N-terminal end of one of the HC monomers. Figure 12J represents a monovalent HT Ab LC-toxin peptide analog fusion (i.e., LC-toxin peptide analog fusion + LC + 2(HC)), with the toxin peptide analog fused to the C-terminal end of one of the LC monomers. Figure 12K represents a bivalent HT Ab LC-toxin peptide analog fusion (i.e., 2(LC-toxin peptide analog fusion) + 2(HC)), with toxin peptide analogs fused to the C-terminal end of both of the LC monomers. Figure 12 L represents a trivalent HT Ab LC-toxin peptide analog/HC-toxin peptide analog (i.e., 2(LC-toxin peptide analog fusion) + HC-toxin peptide analog fusion + HC), with the toxin peptide analogs fused to the C-terminal ends of both of the LC monomers and one of the HC monomers. Figure 12M represents a bivalent antibody with a toxin peptide analog moiety inserted into an internal loop of the immunoglobulin Fc domain of each HC monomer. Figure 12N represents a monovalent antibody with a toxin peptide analog moiety inserted into an internal loop of the immunoglobulin Fc domain of one of the HC monomers. Dimers or trimers will form spontaneously in certain host cells upon expression of a deoxyribonucleic acid (DNA) construct encoding a single chain. In other host cells, the cells can be placed in conditions favoring formation of dimers/trimers or the dimers/trimers can be formed in vitro. If more than one HC monomer, LC monomer, or immunoglobulin Fc domain monomer is part of a single embodiment, the individual monomers can be, if desired, identical or different from each other.

[00101] Figure 13A shows a Coomassie brilliant blue stained Tris-glycine 4-20% SDS-PAGE of the final monovalent Fc-L10-Shk[1-35, Q16K] product. Lanes 1-12 were loaded as follows: lane 1: Novex Mark12 wide range protein standards (10 μl); lane 2: 0.5 μg product non-reduced; lane 3: blank; lane 4: 2.0 μg product, non-reduced; lane 5: blank; lane 6: 10 μg product, non-reduced; lane 7:

Novex Mark12 wide range protein standards (10 μl); lane 8: 0.5 μg product, reduced; lane 9: blank; lane 10: 2.0 μg product, reduced; lane 11: blank; lane 12: 10 μg product, reduced.

[00102] Figure 13B shows size exclusion chromatography on 20 μg of the final monovalent Fc-L10-Shk[1-35, Q16K] product injected onto a Phenomenex BioSep SEC-3000 column (7.8 x 300 mm) in 50 mM NaH_2PO_4, 250 mM NaCl, and pH 6.9 at 1 ml/min observing the absorbance at 280 nm. The deflection observed at 12.5 min is an injection-related artefact.

[00103] Figure 13C shows an LC-MS analysis of the final sample of monovalent Fc-L10-Shk[1-35, Q16K]. The product was chromatographed through a Waters MassPREP micro desalting column using a Waters ACQUITY UPLC system. The column was set at 80°C and the protein eluted using a linear gradient of increasing acetonitrile concentration in 0.1 % formic acid. Part of the column effluent was diverted into a Waters LCT Premier ESI-TOF mass spectrometer for mass analysis. The instrument was run in the positive V mode. The capillary voltage was set at 3,200 V and the cone voltage at 80 V. The mass spectrum was acquired from 800 to 3000 m/z and deconvoluted using the MaxEnt1 software provided by the instrument manufacturer.

[00104] Figure 14A shows a Coomassie brilliant blue stained Tris-glycine 4-20% SDS-PAGE of the final bivalent Fc-L10-Shk[1-35, Q16K] product. Lanes 1-12 were loaded as follows: lane 1: Novex Mark12 wide range protein standards (10 μl); lane 2: 0.5 μg product, non-reduced; lane 3: blank; lane 4: 2.0 μg product, non-reduced; lane 5: blank; lane 6: 10 μg product, non-reduced; lane 7: Novex Mark12 wide range protein standards (10 μl); lane 8: 0.5 μg product, reduced; lane 9: blank; lane 10: 2.0 μg product, reduced; lane 11: blank; lane 12: 10 μg product, reduced.

[00105] Figure 14B shows size exclusion chromatography on 25 μg of the final bivalent Fc-L10-Shk[1-35, Q16K] product injected onto a Phenomenex BioSep SEC-3000 column (7.8 x 300 mm) in 50 mM NaH_2PO_4, 500 mM NaCl, and pH 6.9 at 1 mL/min observing the absorbance at 280 nm. The deflection observed at 12 min is an injection-related artefact.

[00106] Figure 14C shows a MALDI mass spectral analysis of the final sample of bivalent Fc-L10-Shk[1-35, Q16K] analyzed using a Micromass MALDI micro MX mass spectrometer equipped with a nitrogen laser. The sample was run at positive linear mode. The instrument's voltage was set at 12 kV and the high mass detector was set at 5 kV. Each spectrum was produced by accumulating data from about 200 laser shots. External mass calibration was achieved using purified proteins of known molecular masses.

[00107] Figure 15A shows a Coomassie brilliant blue stained Tris-glycine 4-20% SDS-PAGE of the final monovalent Fc-L10-Shk[1-35, Q16K]/anti-KLH Ab product. Lanes 1-12 were loaded as follows: lane 1: Novex Mark12 wide range protein standards (10 μl); lane 2: 0.5 μg product, non-reduced; lane 3: blank; lane 4: 2.0 μg product, non-reduced; lane 5:blank; lane 6: 10 μg product, non-reduced; lane 7: Novex Mark12 wide range protein standards (10 μl); lane 8: 0.5 μg product, reduced; lane 9: blank; lane 10: 2.0 μg product, reduced; lane 11: blank; lane 12: 10 μg product, reduced.

[00108] Figure 15B shows size exclusion chromatography on 50 μg of the final monovalent Fc-L10-Shk[1-35, Q16K]/anti-KLH Ab product injected onto a Phenomenex BioSep SEC-3000 column (7.8 x 300 mm) in 50 mM NaH_2PO_4, 250 mM NaCl, and pH 6.9 at 1 mL/min observing the absorbance at 280 nm.

[00109] Figure 15C shows an LC-MS analysis of the final sample of monovalent Fc-L10-Shk[1-35, Q16K]/anti-KLH Ab. The product was chromatographed through a Waters MassPREP micro desalting column using a Waters ACQUITY UPLC system. The column was set at 80°C and the protein eluted using a linear gradient of increasing acetonitrile concentration in 0.1 % formic acid. Part of the column effluent was diverted into a Waters LCT Premier ESI-TOF mass spectrometer for mass analysis. The instrument was run in the positive V mode. The capillary voltage was set at 3,200 V and the cone voltage at 80 V. The mass spectrum was acquired from 800 to 3000 m/z and deconvoluted using the MaxEnt1 software provided by the instrument manufacturer.

[00110] Figure 16A shows a Coomassie brilliant blue stained Tris-glycine 4-20% SDS-PAGE of the final monovalent anti-KLH HC-L10-Shk[1-35, Q16K]

product. Lanes 1-12 were loaded as follows: lane 1: Novex Mark12 wide range protein standards (10 μl); lane 2: 0.5 μg product, non-reduced; lane 3: blank; lane 4: 2.0 μg product, non-reduced; lane 5:blank; lane 6: 10 μg product, non-reduced; lane 7: Novex Mark12 wide range protein standards (10 μl); lane 8: 0.5 μg product, reduced; lane 9: blank; lane 10: 2.0 μg product, reduced; lane 11: blank; lane 12: 10 μg product, reduced.

[00111] Figure 16B shows size exclusion chromatography on 25 μg of the final monovalent anti-KLH HC-L10-Shk[1-35, Q16K] Ab product injected onto a Phenomenex BioSep SEC-3000 column (7.8 x 300 mm) in 50 mM NaH_2PO_4, 250 mM NaCl, and pH 6.9 at 1 mL/min observing the absorbance at 280 nm. The deflection observed at 11 min is an injection-related artefact.

[00112] Figure 16C shows a MALDI mass spectral analysis of the final sample of monovalent anti-KLH HC-L10-Shk[1-35, Q16K] Ab analyzed using a Micromass MALDI micro MX mass spectrometer equipped with a nitrogen laser. The sample was run at positive linear mode. The instrument's voltage was set at 12 kV and the high mass detector was set at 5 kV. Each spectrum was produced by accumulating data from about 200 laser shots. External mass calibration was achieved using purified proteins of known molecular masses.

[00113] Figure 17A shows a Coomassie brilliant blue stained Tris-glycine 4-20% SDS-PAGE of the final bivalent aKLH HC-L10-Shk[1-35 Q16K] Ab product. Lanes 1-12 were loaded as follows: lane 1: Novex Mark12 wide range protein standards (10 μl); lane 2: 0.5 μg product, non-reduced; lane 3: blank; lane 4: 2.0 μg product, non-reduced; lane 5:blank; lane 6: 10 μg product, non-reduced; lane 7: Novex Mark12 wide range protein standards (10 μl); lane 8: 0.5 μg product, reduced; lane 9: blank; lane 10: 2.0 μg product, reduced; lane 11: blank; lane 12: 10 μg product, reduced.

[00114] Figure 17B shows size exclusion chromatography on 25 μg of the final bivalent anti-KLH HC-L10-Shk[1-35, Q16K] Ab product injected onto a Phenomenex BioSep SEC-3000 column (7.8 x 300 mm) in 50 mM NaH_2PO_4, 500 mM NaCl, and pH 6.9 at 1 mL/min observing the absorbance at 280 nm. The deflection observed at 11.5 min is an injection-related artefact.

[00115] Figure 17C shows a MALDI mass spectral analysis of the final sample of bivalent anti-KLH HC-L10-Shk[1-35, Q16K] Ab analyzed using a Micromass MALDI micro MX mass spectrometer equipped with a nitrogen laser. The sample was run at positive linear mode. The instrument's voltage was set at 12 kV and the high mass detector was set at 5 kV. Each spectrum was produced by accumulating data from about 200 laser shots. External mass calibration was achieved using purified proteins of known molecular masses.

[00116] Figure 18A shows a Coomassie brilliant blue stained Tris-glycine 4-20% SDS-PAGE of the final monovalent aKLH HC-L10-Shk[2-35, Q16K] Ab product. Lanes 1-12 were loaded as follows: lane 1: Novex Mark12 wide range protein standards (10 μl); lane 2: 0.5 μg product, non-reduced; lane 3: blank; lane 4: 2.0 μg product, non-reduced; lane 5:blank; lane 6: 10 μg product, non-reduced; lane 7: Novex Mark12 wide range protein standards (10 μl); lane 8: 0.5 μg product, reduced; lane 9: blank; lane 10: 2.0 μg product, reduced; lane 11: blank; lane 12: 10 μg product, reduced.

[00117] Figure 18B shows size exclusion chromatography on 20 μg of the final monovalent anti-KLH HC-L10-Shk[2-35, Q16K] Ab product injected onto a Phenomenex BioSep SEC-3000 column (7.8 x 300 mm) in 50 mM NaH_2PO_4, 250 mM NaCl, and pH 6.9 at 1 mL/min observing the absorbance at 280 nm. The deflection observed at 11 min is an injection-related artefact.

[00118] Figure 18C shows an LC-MS mass spectral analysis of the final sample of monovalent anti-KLH HC-L10-Shk[2-35, Q16K] Ab. The product was chromatographed through a Waters MassPREP micro desalting column using a Waters ACQUITY UPLC system. The column was set at 80°C and the protein eluted using a linear gradient of increasing acetonitrile concentration in 0.1 % formic acid. Part of the column effluent was diverted into a Waters LCT Premier ESI-TOF mass spectrometer for mass analysis. The instrument was run in the positive V mode. The capillary voltage was set at 3,200 V and the cone voltage at 80 V. The mass spectrum was acquired from 800 to 3000 m/z and deconvoluted using the MaxEnt1 software provided by the instrument manufacturer.

[00119] Figure 19A shows results of pharmacokinetic study in SD rats comparing intravenous administration of CHO-Fc (solid circles; 4 mg/kg) versus bivalent Fc-L10-ShK[2-35] (open squares; 2 mg/kg), described further in Example 12.

[00120] Figure 19B shows results from a pharmacokinetic study on the bivalent dimeric Fc-L10-ShK(2-35) (here designated "FcShK") in SD rats (see Example 12). Serum samples were added to microtiter plates coated with an anti-human Fc antibody to enable affinity capture. Plates were then washed, captured samples were released by SDS and run on a polyacrylamide gel. Samples were then visualized by western blot using an anti-human Fc-specific antibody and secondary-HRP conjugate. At 0.25 hr after IV injection (lane 1), the molecular weight of the band from the serum sample is roughly identical to the original purified material (lanes 5 & 6), suggesting little, if any, degradation. Serum collected at 1 hr (lane 2), 24 hr (lane 3) and 48 hr (lane 4) after injection of the bivalent molecule showed two bands, one being consistent with full-length Fc-L10-ShK(2-35) and the smaller being consistent with Fc alone, suggesting the presence of a monovalent Fc/Fc-L10-ShK(2-35) heterodimer in the slow elimination phase 2-48 hours after IV injection.

[00121] Figure 19C-D shows western blot analysis of serum samples from a pharmacokinetic study on the bivalent Fc-L10-OSK1[K7S] homodimer (Figure 19C; single 2 mg/kg IV dose) and the monovalent Fc/Fc-L10-ShK heterodimer (Figure 19D; single 1mg/kg IV dose) in SD rats. Further details of this study are provided in Example 12. Figure 19C or Figure 19D results are from a single representative animal each. The bivalent Fc-L10-OSK1[K7S] homodimer (Fig. 19C) showed a rapid & extensive distribution phase, but a slow elimination phase from 1-168 hours. During the slow elimination phase two bands were observed, the molecular weight (MW) of the larger being consistent with the full-length Fc-L10-OSK1[K7S] chain and the smaller being consistent with Fc alone. Therefore, despite injection of a bivalent homodimer, a monovalent Fc/Fc-L10-OSK1[K7S] heterodimer appeared to persist in the slow elimination phase. Figure 19D indicates the monovalent Fc/Fc-L10-ShK heterodimer after a single IV dose

remains intact as two chains, and has markedly less distribution compared to bivalent forms, yet retains a slow elimination rate. Lanes labeled 5 ng or 20 ng are the purified monovalent Fc/Fc-L10-ShK heterodimer standard.

[00122] Figure 20 shows results of pharmacokinetic studies (single-subcutaneous dose = 6 mg/kg) performed in SD rats. Open squares represent data for monovalent Fc/Fc-L10-ShK(1-35, Q16K) (heterodimer of SEQ ID NO: 337 and SEQ ID NO:348) closed circles represent data for monovalent anti-KLH antibody-ShK(1-35, Q16K) (tetramer of SEQ ID NO: 338, SEQ ID NO:339, SEQ ID NO:338, and SEQ ID NO:342); and closed triangles represent data for monovalent anti-KLH antibody (loop)-ShK(1-35, Q16K) (tetramer of SEQ ID NO: 338; SEQ ID NO:344; SEQ ID NO:338; and SEQ ID NO:343), described in Example 12 and Table 4H.

[00123] Figure 21 shows results of pharmacokinetic studies (single-subcutaneous dose = 6 mg/kg dose) performed in SD rats for bivalent (open squares) and monovalent (closed circles) anti-KLH antibody-ShK(1-35, Q16K) (respectively, tetramers of [SEQ ID NO: 338, SEQ ID NO:342, SEQ ID NO:338, SEQ ID NO:342] and [SEQ ID NO: 338, SEQ ID NO:339, SEQ ID NO:338, SEQ ID NO:342]), as further described in Example 8, Example 12, and Table 4J.

[00124] Figure 22 shows results of pharmacokinetic studies (single-subcutaneous dose = 6 mg/kg) performed in SD rats for bivalent (open squares) and monovalent (closed circles) anti-KLH antibody (loop)-ShK(1-35, Q16K) (respectively, tetramers of [SEQ ID NO: 338, SEQ ID NO:344, SEQ ID NO:338, SEQ ID NO:344] and [SEQ ID NO: 338, SEQ ID NO:343, SEQ ID NO:338, SEQ ID NO:344]), as further described in Example 8, Example 12, and Table 4L.

[00125] Figure 23 shows the results of pharmacokinetic studies (single, 2 mg/kg subcutaneous dose) in SD rats of monovalent Fc-ShK/Fc heterodimer (open squares), monovalent Fc-ShK/aKLH Ab (heterotrimer or hemibody)(open triangle) and the bivalent ShK-Fc/ShK-Fc homodimer (closed circles). The monovalent heterodimer and heterotrimer provided much greater exposure than the bivalent homodimer. Further details on this study, are provided in Example 12.

[00126] Figure 24 shows a cartoon representation of three monovalent scFc-Shk toxin peptide analog fusions showing the toxin peptide analog insertion (crescent) in the first Fc domain with a 25-amino acid residue peptidyl linker (FcLoop(ShK).L25.Fc; left) or a construct containing the insertion in the second Fc domain with a 20-amino acid residue peptidyl linker (Fc.L20.FcLoop(ShK); center) or a construct containing the toxin peptide analog at the C-terminal of the scFc domain (Fc.L20.Fc.ShK; right). In each of these embodiments, a peptidyl linker (represented as a wavy line) is shown extending from the C-terminal of the first immunoglobulin Fc domain to the N-terminal of the second immunoglobulin Fc domain as part of a single polypeptide chain.

[00127] Figure 25A-C shows sequences of 3 different scFc-Shk constructs. The amino acid sequence used to link the two Fc domains is underlined and the bioactive Shk peptide is in boldface. Additional linker used to fuse Shk to the C-terminus of the scFc in the construct of Figure 25C is also underlined. Figure 25A - Fc.L20.FcLoop(ShK) (SEQ ID NO:411), Figure 25B - FcLoop(ShK).L25.Fc (SEQ ID NO:412) and Figure 25C - Fc.L20.Fc.[Lys16]ShK (SEQ ID NO:410).

[00128] Figure 26A shows Coomassie brilliant blue stained Tris-glycine 4-20% SDS-PAGE gel of purified FcLoop(ShK).L25.Fc (SEQ ID NO:412; "16347") and Fc.L20.FcLoop(ShK) (SEQ ID NO:411; "16369").

[00129] Figure 26B shows RP-HPLC analyses of scFcLoop(Shk) constructs FcLoop(Shk).L25.Fc (#16347; SEQ ID NO:412; upper panel) and Fc.L20.FcLoop(Shk) (#16369; SEQ ID NO:411; lower panel).

[00130] Figure 27A-B shows exemplary nucleic acid and amino acid sequences (SEQ ID NO:277 and SEQ ID NO:278, respectively) of human IgG1 Fc that is optimized for mammalian expression and can be used in this invention.

[00131] Figure 28A-B shows exemplary nucleic acid and amino acid sequences (SEQ ID NO:388 and SEQ ID NO:389, respectively) of human IgG1 Fc that is optimized for bacterial expression and can be used in this invention.

[00132] Figure 29A-B shows by whole cell patch clamp electrophysiology the impact of various concentrations of ShK-192 (SEQ ID NO:438) on human Kv1.3 current (Figure 29A) or human Kv1.1 current (Figure 29B), as described in

Example 1 and Example 5. By whole cell patch clamp electrophysiology, ShK-192 (SEQ ID NO:438) inhibited Kv1.3 currents with an IC_{50} = 0.039±0.005 nM (n=3), whereas it inhibited Kv1.1 currents with an IC_{50} = 3.39±1.61 nM (n=2). The reduced potency on Kv1.1 was confirmed by PatchXpress planar patch clamp electrophysiology where ShK-192 had an IC_{50} = 3.26±0.36 nM (n=8).

[00133] Figure 30 shows representative dose-response curves for cyclosporine A and three lots of 20kDa-PEG-ShK[Lys16] (SEQ ID NO:16) in blocking IL-2 (Figure 30A) and IFNγ (Figure 30B) secretion from T cells induced by thapsigargin stimulation of human whole blood, as described in Examples 2 and 5. The IC_{50} for PEG-ShK[Lys16] in blocking IL-2 and IFNγ production was 0.109±0.081 nM (n=34) and 0.240±0.163 nM (n=34), respectively. Cyclosporin A was about 2000-3000 times less active, with IC_{50} values for IL-2 and IFNγ that were 334.8±172.2 nM (n=64) and 495.2±307.8 nM (n=64), respectively. The curves shown in Figure 30 are normalized to 100 percent of control (POC).

[00134] Figure 31 shows that 20kDa-PEG-ShK[Lys16] (SEQ ID NO: 16) provides potent (average IC_{50} = 0.09 nM) inhibition of IL-17 secretion from T cells in cynomolgus monkey whole blood induced by thapsigargin stimulation, as described in Examples 5 and 8. The response of blood collected from seven separate cynomolgus monkeys (labeled cyno 1 – cyno 7), is shown. Error bars represent the standard error of the mean.

[00135] Figure 32 shows that the molecules 20kDa-PEG-ShK[Lys16] (SEQ ID NO: 16), monovalent aKLH HC-ShK(1-35,Q16K) Ab (SEQ ID NO: 338; 339; 338; 342) and monovalent Fc-L10-ShK(1-35,Q16K) (SEQ ID NO: 337; 348) all provide potent inhibition of antigen (myelin)-mediated proliferation (^{3}H-thymidine incorporation) of the rat T effector memory cell line, PAS, as described in Examples 5, 9, 11 and 12. The IC_{50} values for inhibition by each molecule are shown. Error bars represent the standard error of the mean.

[00136] Figure 33 shows AT-EAE data comparing the activity in vivo of rats treated with vehicle or the Kv1.3-selective inhibitors monovalent aKLH HC-ShK(1-35,Q16K) Ab (SEQ ID NO: 338; 339; 338; 342) and 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16) as described in Examples 5, 9, 11 and 12. The

larger monovalent aKLH HC-ShK(1-35,Q16K) Ab molecule exhibited an ED_{50} of 2.4 nmol/kg (360 μg/kg) for inhibition of encephalomyelitis, which was similar to the 2.47 nmol/ kg (10 μg/kg) ED_{50} of the smaller PEG-ShK[Lys16] molecule. Each molecule was delivered by daily subcutaneous dosing from day -1 through day 7. The figure legend shows the mg/kg (mpk) doses delivered.

[00137] Figure 34A-D show the AT-EAE data for the individual rats receiving vehicle, 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16) or various doses of the monovalent aKLH HC-ShK(1-35,Q16K) Ab molecule (SEQ ID NO: 338; 339; 338; 342) as described in Examples 5, 9, 11 and 12.

[00138] Figure 35 shows AT-EAE data comparing the activity in vivo of rats treated with vehicle or the Kv1.3-selective inhibitor monovalent Fc-L10-ShK(1-35,Q16K) (SEQ ID NO: 337; 348) as described in Example 9, Example 11, and Example 12. The Fc-L10-ShK(1-35,Q16K) molecule exhibited an $ED_{50} \leq$ 2.5 nmol/kg (138 μg/kg) for inhibition of encephalomyelitis. The mg/kg (mpk) doses are shown and involved daily subcutaneous dosing from day -1 through day 7.

[00139] Figure 36A-D show the AT-EAE data for the individual rats receiving vehicle or various doses of the monovalent Fc-L10-ShK(1-35,Q16K) molecule (SEQ ID NO: 337; 348) as described in Example 9, Example 11, and Example 12.

[00140] Figure 37A-B shows levels of serum histamine observed 0.083, 0.25, 1.0 and 96 hours after a single bolus IV injection of 2.0 (Figure 37A, rat #1 - #3) or 5.0 mg/kg (Figure 37B, rat #4) PEG-[Lys16]ShK (SEQ ID NO:16) was given to Sprague-Dawley rats, as described further in Example 10. Background levels of serum histamine measured in benchmark Sprague-Dawley serum reference controls of untreated animals, was 153 ng/ml.

[00141] Figure 38 shows levels of serum histamine observed 0.5, 2, 24 and 48 hours after a single subcutaneous injection of 0.1 (rat #1 - #3), 2.0 (rat #7 - #8) or 5.0 (rat #10 – 12) mg/kg PEG-[Lys16]ShK (SEQ ID NO:16) was given to Sprague-Dawley rats, as described in Example 10. Each panel shows the response at each dose for three separate animals.

[00142] Figure 39 shows levels of histamine released from isolated Sprague-Dawley rat peritoneal mast cells one hour after in vitro incubation with mast cell degranulating peptide (MCDP), compound 48/80, substance P, the calcium ionophore A23187, 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16), monovalent aKLH HC-ShK(1-35, Q16K) Ab, or monovalent Fc-L10-ShK(1-35, Q16K) as described in Example 10.

[00143] Figure 40 shows levels of histamine released from human CD34-derived mast cells one hour after in vitro incubation with mast cell degranulating peptide (MCDP), compound 48/80, substance P, the calcium ionophore A23187, PEG-[Lys16]ShK (SEQ ID NO:16), or monovalent Fc-L10-ShK(1-35, Q16K) as described in Example 10. Total mast cell histamine content in this experiment was 882 ng/ml.

[00144] Figure 41 shows that PEG-[Lys16]ShK (SEQ ID NO:16) induces histamine release from Sprague-Dawley rat (Figure 41A) and Lewis rat (Figure 41B) peritoneal mast cells, but did not induce histamine release from mouse peritoneal mast cells (Figure 41C) or human CD34-derived mast cells (Figure 41D), as described in Example 10. Despite not responding to PEG-[Lys16]ShK (SEQ ID NO:16), mouse peritoneal mast cells and human mast cells did respond to positive controls and other basic secretagogues (e.g. A23187, compound 48/80) run at the same time (not shown). Shown in Figure 41 is the percent histamine release, where level of total histamine is determined as described in Example 10.

[00145] Figure 42 shows a Coomassie brilliant blue stained Tris-glycine 4-20% SDS-PAGE of the final monovalent aKLH 120.6 LC-ShK[1-35, Q16K] Ab product. Lanes 1-12 were loaded as follows: lane 1: Novex Mark12 wide range protein standards (10 μl); lane 2: 0.5 μg product, non-reduced; lane 3: blank; lane 4: 2.0 μg product, non-reduced; lane 5:blank; lane 6: 10 μg product, non-reduced; lane 7: Novex Mark12 wide range protein standards (10 μl); lane 8: 0.5 μg product, reduced; lane 9: blank; lane 10: 2.0 μg product, reduced; lane11: blank; lane 12: 10 μg product, reduced.

[00146] Figure 43 shows size exclusion chromatography on 25 μg of the final monovalent aKLH 120.6 LC-ShK[1-35, Q16K] Ab product injected onto a

Phenomenex BioSep SEC-3000 column (7.8 x 300 mm) in 50 mM NaH_2PO_4, 250 mM NaCl, pH 6.9, at 1 mL/min detecting the absorbance at 280 nm.

[00147] Figure 44A-B shows MALDI-MS mass spectral analysis of the final sample of monovalent aKLH 120.6 LC-ShK[1-35, Q16K] Ab product (non-reduced, Figure 44A; reduced, Figure 44B) using a Micromass MALDI micro MX mass spectrometer equipped with a nitrogen laser. The sample was run at positive linear mode. The instrument's voltage was set at 12 kV and the high mass detector was set at 5 kV. Each spectrum was produced by accumulating data from about 200 laser shots. External mass calibration was achieved using purified proteins of known molecular masses.

[00148] Figure 45 shows a Coomassie brilliant blue stained Tris-glycine 4-20% SDS-PAGE of the final bivalent aKLH 120.6 LC-ShK[1-35, Q16K] Ab product. Lanes 1-12 were loaded as follows: lane 1: Novex Mark12 wide range protein standards (10 μl); lane 2: 0.5 μg product, non-reduced; lane 3: blank; lane 4: 2.0 μg product, non-reduced; lane 5:blank; lane 6: 10 μg product, non-reduced; lane 7: Novex Mark12 wide range protein standards (10 μl); lane 8: 0.5 μg product, reduced; lane 9: blank; lane 10: 2.0 μg product, reduced; lane11: blank; lane 12: 10 μg product, reduced.

[00149] Figure 46 shows size exclusion chromatography on 25 μg of the final bivalent aKLH 120.6 LC-ShK[1-35, Q16K] Ab product injected onto a Phenomenex BioSep SEC-3000 column (7.8 x 300 mm) in 50 mM NaH_2PO_4, 250 mM NaCl, pH 6.9, at 1 mL/min detecting the absorbance at 280 nm.

[00150] Figure 47A-B shows MALDI-MS mass spectral analysis of the final sample of bivalent aKLH 120.6 LC-ShK[1-35, Q16K] Ab product (non-reduced, Figure 47A; reduced, Figure 47B) using a Micromass MALDI micro MX mass spectrometer equipped with a nitrogen laser. The sample was run at positive linear mode. The instrument's voltage was set at 12 kV and the high mass detector was set at 5 kV. Each spectrum was produced by accumulating data from about 200 laser shots. External mass calibration was achieved using purified proteins of known molecular masses.

[00151] Figure 48 shows a Coomassie brilliant blue stained Tris-glycine 4-20% SDS-PAGE of the final trivalent aKLH 120.6 LC-ShK[1-35, Q16K] Ab product. Lanes 1-12 were loaded as follows: lane 1: Novex Mark12 wide range protein standards (10 μl); lane 2: 0.5 μg product, non-reduced; lane 3: blank; lane 4: 2.0 μg product, non-reduced; lane 5:blank; lane 6: 10 μg product, non-reduced; lane 7: Novex Mark12 wide range protein standards (10 μl); lane 8: 0.5 μg product, reduced; lane 9: blank; lane 10: 2.0 μg product, reduced; lane11: blank; lane 12: 10 μg product, reduced.

[00152] Figure 49 shows size exclusion chromatography on 25 μg of the final trivalent aKLH 120.6 LC-ShK[1-35, Q16K] Ab product injected onto a Phenomenex BioSep SEC-3000 column (7.8 x 300 mm) in 50 mM NaH_2PO_4, 250 mM NaCl, pH 6.9, at 1 mL/min detecting the absorbance at 280 nm.

[00153] Figure 50A-B shows MALDI-MS mass spectral analysis of the final sample of trivalent aKLH 120.6 LC-ShK[1-35, Q16K] Ab product (non-reduced, Figure 50A; reduced, Figure 50B) using a Micromass MALDI micro MX mass spectrometer equipped with a nitrogen laser. The sample was run at positive linear mode. The instrument's voltage was set at 12 kV and the high mass detector was set at 5 kV. Each spectrum was produced by accumulating data from about 200 laser shots. External mass calibration was achieved using purified proteins of known molecular masses.

[00154] Figure 51 shows a Coomassie brilliant blue stained Tris-glycine 4-20% SDS-PAGE of the final monovalent aKLH 120.6 IgG2 HC-Shk[1-35, R1A, I4A, Q16K] Ab product. Lanes 1-12 were loaded as follows: lane 1: Novex Mark12 wide range protein standards (10 μl); lane 2: 0.5 μg product, non-reduced; lane 3: blank; lane 4: 2.0 μg product, non-reduced; lane 5:blank; lane 6: 10 μg product, non-reduced; lane 7: Novex Mark12 wide range protein standards (10 μl); lane 8: 0.5 μg product, reduced; lane 9: blank; lane 10: 2.0 μg product, reduced; lane11: blank; lane 12: 10 μg product, reduced.

[00155] Figure 52 shows size exclusion chromatography on 25 μg of the final monovalent aKLH 120.6 IgG2 HC-Shk[1-35, R1A, I4A, Q16K] Ab product injected onto a Phenomenex BioSep SEC-3000 column (7.8 x 300 mm) in 50 mM

NaH_2PO_4, 250 mM NaCl, pH 6.9, at 1 mL/min detecting the absorbance at 280 nm.

[00156] Figure 53 shows reduced LC-MS mass spectral analysis of the heavy chain in the final sample of monovalent aKLH 120.6 IgG2 HC-ShK[1-35, R1A, I4A, Q16K] Ab. The product was chromatographed through a Waters MassPREP micro desalting column using a Waters ACQUITY UPLC system. The column was set at 80°C and the protein eluted using a linear gradient of increasing acetonitrile concentration in 0.1 % formic acid. Part of the column effluent was diverted into a Waters LCT Premier ESI-TOF mass spectrometer for mass analysis. The instrument was run in the positive V mode. The capillary voltage was set at 3,200 V and the cone voltage at 80 V. The mass spectrum was acquired from 800 to 3000 m/z and deconvoluted using the MaxEnt1 software provided by the instrument manufacturer.

[00157] Figure 54A-B shows the results of electrophysiology studies on rat peritoneal mast cells indicating that the cells do not express a recognizable Kv1.3 current or a current sensitive to PEG-[Lys16]ShK as described in Example 10. Figure 54A, shows a representative whole-cell current recorded from holding potential of 0 mV to different potentials between -100 mV and +80 mV in 20 mV increments for 100 milliseconds every 10 seconds. Similar profiles were observed in recordings from three separate cells. Figure 54B shows the current-voltage profile evoked at holding potential of 0 mV and different ramp potentials from -120 mV to +100 mV for 400 milliseconds. An excess amount (100 nM) of the Kv1.3 inhibitors PEG-[Lys16]ShK and charybdotoxin (ChTx), showed no significant effect on the current. For electrophysiology studies on rat peritoneal mast cells, the cells were bathed in 135 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 5 mM Glucose and 10 mM HEPES buffer (pH 7.4). Cells were patched with an internal solution containing 10 mM NaCl, 90 mM KCl, 40 mM KF, 10 mM EGTA and 10 mM HEPES (pH 7.2).

DETAILED DESCRIPTION

[00158] As used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the context clearly indicates otherwise. Thus, for example, reference to "a protein" includes a plurality of proteins; reference to "a cell" includes populations of a plurality of cells.

[00159] "Polypeptide" and "protein" are used interchangeably herein and include a molecular chain of two or more amino acids linked covalently through peptide bonds. The terms do not refer to a specific length of the product. Thus, "peptides," and "oligopeptides," are included within the definition of polypeptide. The terms include post-translational modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like. In addition, protein fragments, analogs, mutated or variant proteins, fusion proteins and the like are included within the meaning of polypeptide. The terms also include molecules in which one or more amino acid analogs or non-canonical or unnatural amino acids are included as can be expressed recombinantly using known protein engineering techniques. In addition, inventive toxin peptide analogs can be derivatized as described herein by well-known organic chemistry techniques.

[00160] "Toxin peptides" include peptides and polypeptides having the same amino acid sequence of a naturally occurring pharmacologically active peptide or polypeptide that can be isolated from a venom, and also include modified peptide analogs of such naturally occurring molecules. (See, e.g., Kalman et al., ShK-Dap22, a potent Kv1.3-specific immunosuppressive polypeptide, J. Biol. Chem. 273(49):32697-707 (1998); Kem et al., US Patent No. 6,077,680; Mouhat et al., OsK1 derivatives, WO 2006/002850 A2; Chandy et al., Analogs of SHK toxin and their uses in selective inhibition of Kv1.3 potassium channels, WO 2006/042151; Sullivan et al., Toxin Peptide therapeutic agents, WO 2006/116156 A2, all of which are incorporated herein by reference in their entirety). Snakes, scorpions, spiders, bees, snails and sea anemone are a few examples of organisms that produce venom that can serve as a rich source of small

bioactive toxin peptides or "toxins" that potently and selectively target ion channels and receptors. An example of a toxin peptide is OSK1 (also known as OsK1), a toxin peptide isolated from Orthochirus scrobiculosus scorpion venom. (e.g., Mouhat et al., K+ channel types targeted by synthetic OSK1, a toxin from Orthochirus scrobiculosus scorpion venom, Biochem. J. 385:95-104 (2005); Mouhat et al., Pharmacological profiling of Orthochirus scrobiculosus toxin 1 analogs with a trimmed N-terminal domain, Molec. Pharmacol. 69:354- 62 (2006); Mouhat et al., OsK1 derivatives, WO 2006/002850 A2). Another example is ShK, isolated from the venom of the sea anemone Stichodactyla helianthus. (E.g., Tudor et al., Ionisation behaviour and solution properties of the potassium-channel blocker ShK toxin, Eur. J. Biochem. 251(1-2):133-41(1998); Pennington et al., Role of disulfide bonds in the structure and potassium channel blocking activity of ShK toxin, Biochem. 38(44): 14549-58 (1999); Kem et al., ShK toxin compositions and methods of use, US Patent No. 6,077,680; Lebrun et al., Neuropeptides originating in scorpion, US Patent No. 6,689,749; Beeton et al., Targeting effector memory T cells with a selective peptide inhibitor of Kv1.3 channnels for therapy of autoimmune diseases, Molec. Pharmacol. 67(4):1369-81 (2005)).

[00161] The toxin peptides are usually between about 20 and about 80 amino acids in length, contain 2-5 disulfide linkages and form a very compact structure. Toxin peptides (e.g., from the venom of scorpions, sea anemones and cone snails) have been isolated and characterized for their impact on ion channels. Such peptides appear to have evolved from a relatively small number of structural frameworks that are particularly well suited to addressing the critical issues of potency and stability. The majority of scorpion and Conus toxin peptides, for example, contain 10-40 amino acids and up to five disulfide bonds, forming extremely compact and constrained structure (microproteins) often resistant to proteolysis. The conotoxin and scorpion toxin peptides can be divided into a number of superfamilies based on their disulfide connections and peptide folds. The solution structure of many toxin peptides has been determined by NMR spectroscopy, illustrating their compact structure and verifying conservation of

family folding patterns. (E.g., Tudor et al., Ionisation behaviour and solution properties of the potassium-channel blocker ShK toxin, Eur. J. Biochem. 251(1-2):133-41(1998); Pennington et al., Role of disulfide bonds in the structure and potassium channel blocking activity of ShK toxin, Biochem. 38(44): 14549-58 (1999); Jaravine et al., Three-dimensional structure of toxin OSK1 from Orthochirus scrobiculosus scorpion venom, Biochem. 36(6):1223-32 (1997); del Rio-Portillo et al.; NMR solution structure of Cn12, a novel peptide from the Mexican scorpion Centruroides noxius with a typical beta-toxin sequence but with alpha-like physiological activity, Eur. J. Biochem. 271(12): 2504-16 (2004); Prochnicka-Chalufour et al., Solution structure of discrepin, a new K+-channel blocking peptide from the alpha-KTx15 subfamily, Biochem. 45(6):1795-1804 (2006)). Other examples are known in the art, or can be found in Sullivan et al., WO06116156 A2 or U.S. Patent Application No. 11/406,454 (titled: Toxin Peptide Therapeutic Agents, published as US 2007/0071764); Mouhat et al., OsK1 derivatives, WO 2006/002850 A2; Sullivan et al., U.S. Patent Application No. 11/978,076 (titled: Conjugated Toxin Peptide Therapeutic Agents, filed 25 October 2007), Lebrun et al., U.S. Patent No. 6,689,749, which are each incorporated by reference in their entireties. Another example, the HmK toxin peptide is from the Magnificent sea anemone (Radianthus magnifica; Heteractis magnifica). Other examples include members of the family of sea anemone toxins HmK, ShK, BgK, AsKs, AeK, and AETX-K, as described above. AETX-K is isolated from the sea anemone Anemonia erythraea, and has the amino acid sequence RACKDYLPKSECTQFRCRTSMKYKYTNCKKTCGTC//SEQ ID NO:3 within the larger amino acid sequence (including putative signal sequence) MKGQMIICLVLIALCMSVVVMAQNLRAEELEKANPKDERVRSFERNQKR ACKDYLPKSECTQFRCRTSMKYKYTNCKKTCGTC// SEQ ID NO: 275. (See, Hasegawa et al., Isolation and DNA cloning of a potassium channel peptide toxin from the sea anemone Anemonia erythraea, Toxicon 48(5):536-42 (2006)). HmK has SEQ ID NO: 2 ((Table 5; mature peptide) within the larger MKSQMIAAVLLIAFCLCVVVTARMELQDVEDMENGFQKRRTCKDLIPVS

ECTDIRCRTSMKYRLNLCRKTCGSC// SEQ ID NO: 276 (including signal and mature peptide portions)).

[00162] The term "peptide analog" refers to a peptide having a sequence that differs from a peptide sequence existing in nature by at least one amino acid residue substitution, internal addition, or internal deletion of at least one amino acid, and/or amino- or carboxy- terminal end truncations or additions, and/or carboxy-terminal amidation. An "internal deletion" refers to absence of an amino acid from a sequence existing in nature at a position other than the N- or C-terminus. Likewise, an "internal addition" refers to presence of an amino acid in a sequence existing in nature at a position other than the N- or C-terminus.

[00163] Embodiments of the inventive composition of matter includes a toxin peptide analog, or a pharmaceutically acceptable salt thereof. "Toxin peptide analogs", such as, but not limited to, an AETX-K peptide analog, an ShK peptide analog, or a HmK peptide analog, contain modifications of a native toxin peptide sequence of interest (e.g., amino acid residue substitutions, internal additions or insertions, internal deletions, and/or amino- or carboxy- terminal end truncations, or additions as previously described above) relative to a native toxin peptide sequence of interest, such as ShK, HmK, or AETX-K. Toxin peptide analogs of the present invention are 33 to about 100 amino acid residues long and, in relation to SEQ ID NO:4, have C^1-C^6, C^2-C^4 and C^3-C^5 disulfide bonding in which, C^1, C^2, C^3, C^4, C^5 and C^6 represent the order of cysteine residues appearing in the primary sequence of the toxin peptide stated conventionally with the N-terminus of the peptide(s) on the left, with the first and sixth cysteines in the amino acid sequence forming a disulfide bond, the second and fourth cysteines forming a disulfide bond, and the third and fifth cysteines forming a disulfide bond. Examples of toxin peptides with such a C^1-C^6, C^2-C^4, C^3-C^5 disulfide bonding pattern include, but are not limited to, ShK, BgK, HmK, AeKS, AsK, AETX-K and DTX1, and analogs of any of the foregoing. As described herein, the toxin peptide analogs of the present invention can also have additional amino acid residues at the N-terminal and/or C-terminal ends, in relation to SEQ ID NO:4.

[00164] By "physiologically acceptable salt" of the composition of matter, for example a salt of the toxin peptide analog, is meant any salt or salts that are known or later discovered to be pharmaceutically acceptable. Some non-limiting examples of pharmaceutically acceptable salts are: acetate; trifluoroacetate; hydrohalides, such as hydrochloride and hydrobromide; sulfate; citrate; maleate; tartrate; glycolate; gluconate; succinate; mesylate; besylate; salts of gallic acid esters (gallic acid is also known as 3,4, 5 trihydroxybenzoic acid) such as PentaGalloylGlucose (PGG) and epigallocatechin gallate (EGCG), salts of cholesteryl sulfate, pamoate, tannate and oxalate salts.

[00165] The term "fusion protein" indicates that the protein includes polypeptide components derived from more than one parental protein or polypeptide. Typically, a fusion protein is expressed from a fusion gene in which a nucleotide sequence encoding a polypeptide sequence from one protein is appended in frame with, and optionally separated by a linker from, a nucleotide sequence encoding a polypeptide sequence from a different protein. The fusion gene can then be expressed by a recombinant host cell as a single protein.

[00166] The terms "-mimetic peptide," "peptide mimetic," and "-agonist peptide" refer to a peptide or protein having biological activity comparable to a naturally occurring protein of interest, for example, but not limited to, a toxin peptide molecule, e.g., naturally occurring ShK, HmK, AETX-K, or OSK1 toxin peptide. These terms further include peptides that indirectly mimic the activity of a naturally occurring peptide molecule, such as by potentiating the effects of the naturally occurring molecule.

[00167] The term "-antagonist peptide," "peptide antagonist," and "inhibitor peptide" refer to a peptide that blocks or in some way interferes with the biological activity of a receptor of interest, or has biological activity comparable to a known antagonist or inhibitor of a receptor of interest, such as, but not limited to, an ion channel (e.g., Kv1.3) or a G-Protein Coupled Receptor (GPCR).

[00168] A "domain" of a protein is any portion of the entire protein, up to and including the complete protein, but typically comprising less than the complete protein. A domain can, but need not, fold independently of the rest of

the protein chain and/or be correlated with a particular biological, biochemical, or structural function or location (e.g., a ligand binding domain, or a cytosolic, transmembrane or extracellular domain).

[00169] As used herein "soluble" when in reference to a protein produced by recombinant DNA technology in a host cell is a protein that exists in aqueous solution; if the protein contains a twin-arginine signal amino acid sequence the soluble protein is exported to the periplasmic space in gram negative bacterial hosts, or is secreted into the culture medium by eukaryotic host cells capable of secretion, or by bacterial host possessing the appropriate genes (e.g., the kil gene). Thus, a soluble protein is a protein which is not found in an inclusion body inside the host cell. Alternatively, depending on the context, a soluble protein is a protein which is not found integrated in cellular membranes. In contrast, an insoluble protein is one which exists in denatured form inside cytoplasmic granules (called an inclusion body) in the host cell, or again depending on the context, an insoluble protein is one which is present in cell membranes, including but not limited to, cytoplasmic membranes, mitochondrial membranes, chloroplast membranes, endoplasmic reticulum membranes, etc.

[00170] A distinction is also drawn between proteins which are “soluble” (i.e., dissolved or capable of being dissolved) in an aqueous solution devoid of significant amounts of ionic detergents (e.g., SDS) or denaturants (e.g., urea, guanidine hydrochloride) and proteins which exist as a suspension of insoluble protein molecules dispersed within the solution. A “soluble” protein will not be removed from a solution containing the protein by centrifugation using conditions sufficient to remove cells present in a liquid medium (e.g., centrifugation at 5,000×g for 4-5 minutes). In some embodiments of the inventive composition, the toxin peptide analog is synthesized by the host cell and segregated in an insoluble form within cellular inclusion bodies, which can then be purified from other cellular components in a cell extract with relative ease, and the toxin peptide analog can in turn be solubilized, refolded and/or further purified.

[00171] A distinction is drawn between a “soluble” protein (i.e., a protein which when expressed in a host cell is produced in a soluble form) and a

"solubilized" protein. An insoluble recombinant protein found inside an inclusion body or found integrated in a cell membrane may be solubilized (i.e., rendered into a soluble form) by treating purified inclusion bodies or cell membranes with denaturants such as guanidine hydrochloride, urea or sodium dodecyl sulfate (SDS). These denaturants must then be removed from the solubilized protein preparation to allow the recovered protein to renature (refold). Although the inventive compositions can be refolded in active form, not all proteins will refold into an active conformation after solubilization in a denaturant and removal of the denaturant. Many proteins precipitate upon removal of the denaturant. SDS may be used to solubilize inclusion bodies and cell membranes and will maintain the proteins in solution at low concentration. However, dialysis will not always remove all of the SDS (SDS can form micelles which do not dialyze out); therefore, SDS-solubilized inclusion body protein and SDS-solubilized cell membrane protein is soluble but not refolded.

[00172] A "secreted" protein refers to those proteins capable of being directed to the ER, secretory vesicles, or the extracellular space as a result of a secretory signal peptide sequence, as well as those proteins released into the extracellular space without necessarily containing a signal sequence. If the secreted protein is released into the extracellular space, the secreted protein can undergo extracellular processing to produce a "mature" protein. Release into the extracellular space can occur by many mechanisms, including exocytosis and proteolytic cleavage. In some other embodiments of the inventive composition, the toxin peptide analog can be synthesized by the host cell as a secreted protein, which can then be further purified from the extracellular space and/or medium.

[00173] The term "recombinant" indicates that the material (e.g., a nucleic acid or a polypeptide) has been artificially or synthetically (i.e., non-naturally) altered by human intervention. The alteration can be performed on the material within, or removed from, its natural environment or state. For example, a "recombinant nucleic acid" is one that is made by recombining nucleic acids, e.g., during cloning, DNA shuffling or other well known molecular biological procedures. A "recombinant DNA molecule," is comprised of segments of DNA

joined together by means of such molecular biological techniques. The term "recombinant protein" or "recombinant polypeptide" as used herein refers to a protein molecule which is expressed using a recombinant DNA molecule. A "recombinant host cell" is a cell that contains and/or expresses a recombinant nucleic acid.

[00174] A "polynucleotide sequence" or "nucleotide sequence" or "nucleic acid sequence," as used interchangeably herein, is a polymer of nucleotides, including an oligonucleotide, a DNA, and RNA, a nucleic acid, or a character string representing a nucleotide polymer, depending on context. From any specified polynucleotide sequence, either the given nucleic acid or the complementary polynucleotide sequence can be determined. Included are DNA or RNA of genomic or synthetic origin which may be single- or double-stranded, and represent the sense or antisense strand.

[00175] As used herein, the terms "nucleic acid molecule encoding," "DNA sequence encoding," and "DNA encoding" refer to the order or sequence of deoxyribonucleotides along a strand of deoxyribonucleic acid. The order of these deoxyribonucleotides determines the order of ribonucleotides along the mRNA chain, and also determines the order of amino acids along the polypeptide (protein) chain. The DNA sequence thus codes for the RNA sequence and for the amino acid sequence.

[00176] "Expression of a gene" or "expression of a nucleic acid" means transcription of DNA into RNA (optionally including modification of the RNA, e.g., splicing), translation of RNA into a polypeptide (possibly including subsequent post-translational modification of the polypeptide), or both transcription and translation, as indicated by the context.

[00177] The term "gene" is used broadly to refer to any nucleic acid associated with a biological function. Genes typically include coding sequences and/or the regulatory sequences required for expression of such coding sequences. The term "gene" applies to a specific genomic or recombinant sequence, as well as to a cDNA or mRNA encoded by that sequence. A "fusion gene" contains a coding region that encodes a toxin peptide analog. Genes also include non-

expressed nucleic acid segments that, for example, form recognition sequences for other proteins. Non-expressed regulatory sequences including transcriptional control elements to which regulatory proteins, such as transcription factors, bind, resulting in transcription of adjacent or nearby sequences.

[00178] As used herein the term "coding region" or "coding sequence" when used in reference to a structural gene refers to the nucleotide sequences which encode the amino acids found in the nascent polypeptide as a result of translation of an mRNA molecule. The coding region is bounded, in eukaryotes, on the 5' side by the nucleotide triplet "ATG" which encodes the initiator methionine and on the 3' side by one of the three triplets which specify stop codons (i.e., TAA, TAG, TGA).

[00179] Transcriptional control signals in eukaryotes comprise "promoter" and "enhancer" elements. Promoters and enhancers consist of short arrays of DNA sequences that interact specifically with cellular proteins involved in transcription (Maniatis, et al., Science 236:1237 (1987)). Promoter and enhancer elements have been isolated from a variety of eukaryotic sources including genes in yeast, insect and mammalian cells and viruses (analogous control elements, i.e., promoters, are also found in prokaryotes). The selection of a particular promoter and enhancer depends on what cell type is to be used to express the protein of interest. Some eukaryotic promoters and enhancers have a broad host range while others are functional in a limited subset of cell types (for review see Voss, et al., Trends Biochem. Sci., 11:287 (1986) and Maniatis, et al., Science 236:1237 (1987)).

[00180] The term "expression vector" as used herein refers to a recombinant DNA molecule containing a desired coding sequence and appropriate nucleic acid sequences necessary for the expression of the operably linked coding sequence in a particular host cell. Nucleic acid sequences necessary for expression in prokaryotes include a promoter, optionally an operator sequence, a ribosome binding site and possibly other sequences. Eukaryotic cells are known to utilize promoters, enhancers, and termination and polyadenylation signals. A secretory signal peptide sequence can also, optionally, be encoded by the

expression vector, operably linked to the coding sequence for the inventive toxin peptide analog, so that the expressed toxin peptide analog can be secreted by the recombinant host cell, for more facile isolation of the toxin peptide analog from the cell, if desired. Such techniques are well known in the art. (E.g., Goodey, Andrew R.; et al., Peptide and DNA sequences, U.S. Patent No. 5,302,697; Weiner et al., Compositions and methods for protein secretion, U.S. Patent No. 6,022,952 and U.S. Patent No. 6,335,178; Uemura et al., Protein expression vector and utilization thereof, U.S. Patent No. 7,029,909; Ruben et al., 27 human secreted proteins, US 2003/0104400 A1).

[00181] The terms "in operable combination", "in operable order" and "operably linked" as used herein refer to the linkage of nucleic acid sequences in such a manner that a nucleic acid molecule capable of directing the transcription of a given gene and/or the synthesis of a desired protein molecule is produced. The term also refers to the linkage of amino acid sequences in such a manner so that a functional protein is produced.

[00182] Peptides. Recombinant DNA- and/or RNA-mediated protein expression and protein engineering techniques, or any other methods of preparing peptides, are applicable to the making of the inventive toxin peptide analogs and fusion protein conjugates thereof (e.g., fusion proteins containing a toxin peptide analog and an immunoglobulin Fc domain, transthyretin, or human serum albumin). For example, the peptides can be made in transformed host cells. Briefly, a recombinant DNA molecule, or construct, coding for the peptide is prepared. Methods of preparing such DNA molecules are well known in the art. For instance, sequences encoding the peptides can be excised from DNA using suitable restriction enzymes. Any of a large number of available and well-known host cells may be used in the practice of this invention. The selection of a particular host is dependent upon a number of factors recognized by the art. These include, for example, compatibility with the chosen expression vector, toxicity of the peptides encoded by the DNA molecule, rate of transformation, ease of recovery of the peptides, expression characteristics, bio-safety and costs. A balance of these factors must be struck with the understanding that not all hosts

may be equally effective for the expression of a particular DNA sequence. Within these general guidelines, useful microbial host cells in culture include bacteria (such as Escherichia coli sp.), yeast (such as Saccharomyces sp.) and other fungal cells, insect cells, plant cells, mammalian (including human) cells, e.g., CHO cells and HEK293 cells. Modifications can be made at the DNA level, as well. The peptide-encoding DNA sequence may be changed to codons more compatible with the chosen host cell. For E. coli, optimized codons are known in the art. Codons can be substituted to eliminate restriction sites or to include silent restriction sites, which may aid in processing of the DNA in the selected host cell. Next, the transformed host is cultured and purified. Host cells may be cultured under conventional fermentation conditions so that the desired compounds are expressed. Such fermentation conditions are well known in the art. In addition, the DNA optionally further encodes, 5' to the coding region of a fusion protein, a signal peptide sequence (e.g., a secretory signal peptide) operably linked to the expressed toxin peptide analog. For further examples of appropriate recombinant methods and exemplary DNA constructs useful for recombinant expression of the inventive compositions by mammalian cells, including dimeric Fc fusion proteins ("peptibodies") or chimeric immunoglobulin(light chain + heavy chain)-Fc heterotrimers ("hemibodies"), conjugated to pharmacologically active toxin peptide analogs of the invention, see, e.g., Sullivan et al., Toxin Peptide Therapeutic Agents, US2007/0071764 and Sullivan et al., Toxin Peptide Therapeutic Agents, PCT/US2007/022831, published as WO 2008/088422, which are both incorporated herein by reference in their entireties.

[00183] Peptide compositions of the present invention can also be made by synthetic methods. Solid phase synthesis is the preferred technique of making individual peptides since it is the most cost-effective method of making small peptides. For example, well known solid phase synthesis techniques include the use of protecting groups, linkers, and solid phase supports, as well as specific protection and deprotection reaction conditions, linker cleavage conditions, use of scavengers, and other aspects of solid phase peptide synthesis. Suitable techniques are well known in the art. (E.g., Merrifield (1973), Chem.

Polypeptides, pp. 335-61 (Katsoyannis and Panayotis eds.); Merrifield (1963), J. Am. Chem. Soc. 85: 2149; Davis et al. (1985), Biochem. Intl. 10: 394-414; Stewart and Young (1969), Solid Phase Peptide Synthesis; U.S. Pat. No. 3,941,763; Finn et al. (1976), The Proteins (3rd ed.) 2: 105-253; and Erickson et al. (1976), The Proteins (3rd ed.) 2: 257-527; "Protecting Groups in Organic Synthesis," 3rd Edition, T. W. Greene and P. G. M. Wuts, Eds., John Wiley & Sons, Inc., 1999; NovaBiochem Catalog, 2000; "Synthetic Peptides, A User's Guide," G. A. Grant, Ed., W.H. Freeman & Company, New York, N.Y., 1992; "Advanced Chemtech Handbook of Combinatorial & Solid Phase Organic Chemistry," W. D. Bennet, J. W. Christensen, L. K. Hamaker, M. L. Peterson, M. R. Rhodes, and H. H. Saneii, Eds., Advanced Chemtech, 1998; "Principles of Peptide Synthesis, 2nd ed.," M. Bodanszky, Ed., Springer-Verlag, 1993; "The Practice of Peptide Synthesis, 2nd ed.," M. Bodanszky and A. Bodanszky, Eds., Springer-Verlag, 1994; "Protecting Groups," P. J. Kocienski, Ed., Georg Thieme Verlag, Stuttgart, Germany, 1994; "Fmoc Solid Phase Peptide Synthesis, A Practical Approach," W. C. Chan and P. D. White, Eds., Oxford Press, 2000, G. B. Fields et al., Synthetic Peptides: A User's Guide, 1990, 77-183). For further examples of synthetic and purification methods known in the art, which are applicable to making the inventive compositions of matter, see, e.g., Sullivan et al., Toxin Peptide Therapeutic Agents, US2007/0071764 and Sullivan et al., Toxin Peptide Therapeutic Agents, PCT/US2007/022831, published as WO 2008/088422 A2, which are both incorporated herein by reference in their entireties.

[00184] In further describing the toxin peptide analogs herein, a one-letter abbreviation system is frequently applied to designate the identities of the twenty "canonical" amino acid residues generally incorporated into naturally occurring peptides and proteins (Table 1). Such one-letter abbreviations are entirely interchangeable in meaning with three-letter abbreviations, or non-abbreviated amino acid names. Within the one-letter abbreviation system used herein, an upper case letter indicates a L-amino acid, and a lower case letter indicates a

D-amino acid. For example, the abbreviation "R" designates L-arginine and the abbreviation "r" designates D-arginine.

Table 1. One-letter abbreviations for the canonical amino acids. Three-letter abbreviations are in parentheses.

Alanine (Ala)	A
Glutamine (Gln)	Q
Leucine (Leu)	L
Serine (Ser)	S
Arginine (Arg)	R
Glutamic Acid (Glu)	E
Lysine (Lys)	K
Threonine (Thr)	T
Asparagine (Asn)	N
Glycine (Gly)	G
Methionine (Met)	M
Tryptophan (Trp)	W
Aspartic Acid (Asp)	D
Histidine (His)	H
Phenylalanine (Phe)	F
Tyrosine (Tyr)	Y
Cysteine (Cys)	C
Isoleucine (Ile)	I
Proline (Pro)	P
Valine (Val)	V

[00185] An amino acid substitution in an amino acid sequence is typically designated herein with a one-letter abbreviation for the amino acid residue in a particular position, followed by the numerical amino acid position relative to a native sequence of interest, which is then followed by the one-letter symbol for the amino acid residue substituted in. For example, "T30D" symbolizes a

substitution of a threonine residue by an aspartate residue at amino acid position 30, relative to the native sequence of interest.

[00186] Non-canonical amino acid residues can be incorporated into a peptide within the scope of the invention by employing known techniques of protein engineering that use recombinantly expressing cells. (See, e.g., Link et al., Non-canonical amino acids in protein engineering, Current Opinion in Biotechnology, 14(6):603-609 (2003)). The term "non-canonical amino acid residue" refers to amino acid residues in D- or L-form that are not among the 20 canonical amino acids generally incorporated into naturally occurring proteins, for example, β-amino acids, homoamino acids, cyclic amino acids and amino acids with derivatized side chains. Examples include (in the L-form or D-form) β-alanine, β-aminopropionic acid, piperidinic acid, aminocaprioic acid, aminoheptanoic acid, aminopimelic acid, desmosine, diaminopimelic acid, N^{α}-ethylglycine, N^{α}-ethylaspargine, hydroxylysine, allo-hydroxylysine, isodesmosine, allo-isoleucine, ω-methylarginine, N^{α}-methylglycine, N^{α}-methylisoleucine, N^{α}-methylvaline, , γ-carboxyglutamate, ε-N,N,N-trimethyllysine, ε-N-acetyllysine, O-phosphoserine, N^{α}-acetylserine, N^{α}-formylmethionine, 3-methylhistidine, 5-hydroxylysine, and other similar amino acids, and those listed in Table 2 below, and derivatized forms of any of these as described herein. Table 2 contains some exemplary non-canonical amino acid residues that are useful in accordance with the present invention and associated abbreviations as typically used herein, although the skilled practitioner will understand that different abbreviations and nomenclatures may be applicable to the same substance and appear interchangeably herein.

Table 2. Useful non-canonical amino acids for amino acid addition, insertion, or substitution into peptide sequences in accordance with the present invention. In the event an abbreviation listed in Table 2 differs from another abbreviation for the same substance disclosed elsewhere herein, both abbreviations are understood to be applicable. The amino acids listed in Table 2 can be in the L-form or D-form.

Amino Acid	**Abbreviation(s)**
Acetamidomethyl	Acm
Acetylarginine	acetylarg
α-aminoadipic acid	Aad
aminobutyric acid	Abu
6-aminohexanoic acid	Ahx; εAhx
3-amino-6-hydroxy-2-piperidone	Ahp
2-aminoindane-2-carboxylic acid	Aic
α-amino-isobutyric acid	Aib
3-amino-2-naphthoic acid	Anc
2-aminotetraline-2-carboxylic acid	Atc
aminophenylalanine	Aminophe; Amino-Phe
4-amino-phenylalanine	4AmP
4-amidino-phenylalanine	4AmPhe
2-amino-2-(1-carbamimidoylpiperidin-4-yl)acetic acid	4AmPig
Arg ψ(CH_2NH) -reduced amide bond	rArg
β-homoarginine	bhArg
β-homolysine	bhomoK
β-homo Tic	BhTic
β-homophenylalanine	BhPhe
β-homoproline	BhPro
β-homotryptophan	BhTrp
4,4'-biphenylalanine	Bip
β, β-diphenyl-alanine	BiPhA
β-phenylalanine	BPhe
p-carboxyl-phenylalanine	Cpa
citrulline	Cit
cyclohexylalanine	Cha
cyclohexylglycine	Chg
cyclopentylglycine	Cpg
2-amino-3-guanidinopropanoic acid	3G-Dpr

α, γ-diaminobutyric acid	Dab
2,4-diaminobutyric acid	Dbu
diaminopropionic acid	Dap
α, β-diaminopropionoic acid (or 2,3-diaminopropionic acid	Dpr
3,3-diphenylalanine	Dip
4-guanidino phenylalanine	Guf
4-guanidino proline	4GuaPr
homoarginine	hArg; hR
homocitrulline	hCit
homoglutamine	hQ
homolysine	hLys; hK; homoLys
homophenylalanine	hPhe; homoPhe
4-hydroxyproline (or hydroxyproline)	Hyp
2-indanylglycine (or indanylglycine)	IgI
indoline-2-carboxylic acid	Idc
Iodotyrosine	I-Tyr
Lys ψ(CH_2NH)-reduced amide bond	rLys
methionine oxide	Met[O]
methionine sulfone	Met[O]$_2$
N^{α}-methylarginine	NMeR
Nα-[$(CH_2)_3NHCH(NH)NH_2$] substituted glycine	N-Arg
N^{α}-methylcitrulline	NMeCit
N^{α}-methylglutamine	NMeQ
N^{α}-methylhomocitrulline	N $^{\alpha}$-MeHoCit
N^{α}-methylhomolysine	NMeHoK
N^{α}-methylleucine	N$^{\alpha}$-MeL; NMeL; NMeLeu; NMe-Leu
N^{α}-methyllysine	NMe-Lys
*N*ε-methyl-lysine	N-eMe-K
*N*ε-ethyl-lysine	N-eEt-K
*N*ε-isopropyl-lysine	N-eIPr-K
N^{α}-methylnorleucine	NMeNle; NMe-Nle
N^{α}-methylornithine	N $^{\alpha}$-MeOrn; NMeOrn

N^{α}-methylphenylalanine	NMe-Phe
4-methyl-phenylalanine	MePhe
α-methylphenyalanine	AMeF
N^{α}-methylthreonine	NMe-Thr; NMeThr
N^{α}-methylvaline	NMeVal; NMe-Val
Nε-(O-(aminoethyl)-O'-(2-propanoyl)-undecaethyleneglycol)-Lysine	K(NPeg11)
Nε-(O-(aminoethyl)-O'-(2-propanoyl)-(ethyleneglycol)27-Lysine	K(NPeg27)
3-(1-naphthyl)alanine	1-Nal; 1Nal
3-(2-naphthyl)alanine	2-Nal; 2Nal
nipecotic acid	Nip
nitrophenylalanine	nitrophe
norleucine	Nle
norvaline	Nva or Nvl
O-methyltyrosine	Ome-Tyr
octahydroindole-2-carboxylic acid	Oic
ornithine	Orn
Orn ψ(CH2NH)-reduced amide bond	rOrn
4-piperidinylalanine	4PipA
4-pyridinylalanine	4Pal
3-pyridinylalanine	3Pal
2-pyridinylalanine	2Pal
para-aminophenylalanine	4AmP; 4-Amino-Phe
para-iodophenylalanine (or 4-iodophenylalanine)	pI-Phe
phenylglycine	Phg
4-phenyl-phenylalanine (or biphenylalanine)	4Bip
4,4'-biphenyl alanine	Bip
pipecolic acid	Pip
4-amino-1-piperidine-4-carboxylic acid	4Pip
sarcosine	Sar
1,2,3,4-tetrahydroisoquinoline	Tic
1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid	Tiq

1,2,3,4-tetrahydroisoquinoline-7-hydroxy-3-carboxylic acid	Hydroxyl-Tic
1,2,3,4-tetrahydronorharman-3-carboxylic acid	Tpi
thiazolidine-4-carboxylic acid	Thz
3-thienylalanine	Thi

[00187] Nomenclature and Symbolism for Amino Acids and Peptides by the UPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN) have been published in the following documents: Biochem. J., 1984, 219, 345-373; Eur. J. Biochem., 1984, 138, 9-37; 1985, 152, 1; 1993, 213, 2; Internat. J. Pept. Prot. Res., 1984, 24, following p 84; J. Biol. Chem., 1985, 260, 14-42; Pure Appl. Chem., 1984, 56, 595-624; Amino Acids and Peptides, 1985, 16, 387-410; Biochemical Nomenclature and Related Documents, 2nd edition, Portland Press, 1992, pages 39-69.

[00188] The one or more useful modifications to peptide domains of the inventive compositions can include amino acid additions or insertions, amino acid deletions, peptide truncations, amino acid substitutions, and/or chemical derivatization of amino acid residues, accomplished by known chemical techniques. For example, the thusly modified amino acid sequence includes at least one amino acid residue inserted or substituted therein, relative to the amino acid sequence of the native sequence of interest, in which the inserted or substituted amino acid residue has a side chain comprising a nucleophilic or electrophilic reactive functional group by which the peptide is conjugated to a linker and/or half-life extending moiety. In accordance with the invention, useful examples of such a nucleophilic or electrophilic reactive functional group include, but are not limited to, a thiol, a primary amine, a seleno, a hydrazide, an aldehyde, a carboxylic acid, a ketone, an aminooxy, a masked (protected) aldehyde, or a masked (protected) keto functional group. Examples of amino acid residues having a side chain comprising a nucleophilic reactive functional group include, but are not limited to, a lysine residue, a homolysine, an α,β-diaminopropionic acid residue, an α,γ-diaminobutyric acid residue, an ornithine residue, a cysteine,

a homocysteine, a glutamic acid residue, an aspartic acid residue, or a selenocysteine residue.

[00189] Amino acid residues are commonly categorized according to different chemical and/or physical characteristics. The term "acidic amino acid residue" refers to amino acid residues in D- or L-form having side chains comprising acidic groups. Exemplary acidic residues include aspartatic acid and glutamatic acid residues. The term "alkyl amino acid residue" refers to amino acid residues in D- or L-form having C_{1-6}alkyl side chains which may be linear, branched, or cyclized, including to the amino acid amine as in proline, wherein the C_{1-6}alkyl is substituted by 0, 1, 2 or 3 substituents selected from C_{1-4}haloalkyl, halo, cyano, nitro, $-C(=O)R^b$, $-C(=O)OR^a$, $-C(=O)NR^aR^a$, $-C(=NR^a)NR^aR^a$, $-NR^aC(=NR^a)NR^aR^a$, $-OR^a$, $-OC(=O)R^b$, $-OC(=O)NR^aR^a$, $-OC_{2-6}$alkylNR^{a}R^a, $-OC_{2-6}$alkylOR^a, $-SR^a$, $-S(=O)R^b$, $-S(=O)_2R^b$, $-S(=O)_2NR^aR^a$, $-NR^aR^a$, $-N(R^a)C(=O)R^b$, $-N(R^a)C(=O)OR^b$, $-N(R^a)C(=O)NR^aR^a$, $-N(R^a)C(=NR^a)NR^aR^a$, $-N(R^a)S(=O)_2R^b$, $-N(R^a)S(=O)_2NR^aR^a$, $-NR^aC_{2-6}$alkylNR^{a}R^a and $-NR^aC_{2-6}$alkylOR^a; wherein R^a is independently, at each instance, H or R^b; and R^b is independently, at each instance C_{1-6}alkyl substituted by 0, 1, 2 or 3 substituents selected from halo, C_{1-4}alk, C_{1-3}haloalk, $-OC_{1-4}$alk, $-NH_2$, $-NHC_{1-4}$alk, and $-N(C_{1-4}alk)C_{1-4}$alk; or any protonated form thereof, including alanine, valine, leucine, isoleucine, proline, serine, threonine, lysine, arginine, histidine, aspartate, glutamate, asparagine, glutamine, cysteine, methionine, hydroxyproline, but which residues do not contain an aryl or aromatic group. The term "aromatic amino acid residue" refers to amino acid residues in D- or L-form having side chains comprising aromatic groups. Exemplary aromatic residues include tryptophan, tyrosine, 3-(1-naphthyl)alanine, or phenylalanine residues. The term "basic amino acid residue" refers to amino acid residues in D- or L-form having side chains comprising basic groups. Exemplary basic amino acid residues include histidine, lysine, homolysine, ornithine, arginine, N-methyl-arginine, ω-aminoarginine, ω-methyl-arginine, 1-methyl-histidine, 3-methyl-histidine, and homoarginine (hR) residues. The term "hydrophilic amino acid residue" refers to amino acid residues in D- or L-form having side chains comprising polar groups.

Exemplary hydrophilic residues include cysteine, serine, threonine, histidine, lysine, asparagine, aspartate, glutamate, glutamine, and citrulline (Cit) residues. The terms "lipophilic amino acid residue" refers to amino acid residues in D- or L-form having sidechains comprising uncharged, aliphatic or aromatic groups. Exemplary lipophilic sidechains include phenylalanine, isoleucine, leucine, methionine, valine, tryptophan, and tyrosine. Alanine (A) is amphiphilic—it is capable of acting as a hydrophilic or lipophilic residue. Alanine, therefore, is included within the definition of both "lipophilic residue" and "hydrophilic residue." The term "nonfunctional amino acid residue" refers to amino acid residues in D- or L-form having side chains that lack acidic, basic, or aromatic groups. Exemplary neutral amino acid residues include methionine, glycine, alanine, valine, isoleucine, leucine, and norleucine (Nle) residues.

[00190] Additional useful embodiments of toxin peptide analogs can result from conservative modifications of the amino acid sequences of the toxin polypeptides disclosed herein. Conservative modifications will produce half-life extending moiety-conjugated peptides having functional, physical, and chemical characteristics similar to those of the conjugated (e.g., PEG-conjugated) peptide from which such modifications are made. Such conservatively modified forms of the conjugated toxin peptide analogs disclosed herein are also contemplated as being an embodiment of the present invention.

[00191] In contrast, substantial modifications in the functional and/or chemical characteristics of peptides may be accomplished by selecting substitutions in the amino acid sequence that differ significantly in their effect on maintaining (a) the structure of the molecular backbone in the region of the substitution, for example, as an α-helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the size of the molecule.

[00192] For example, a "conservative amino acid substitution" may involve a substitution of a native amino acid residue with a nonnative residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position. Furthermore, any native residue in the polypeptide may also be substituted with alanine, as has been previously described for "alanine scanning

mutagenesis" (see, for example, MacLennan et al., Acta Physiol. Scand. Suppl., 643:55-67 (1998); Sasaki et al., 1998, Adv. Biophys. 35:1-24 (1998), which discuss alanine scanning mutagenesis).

[00193] Desired amino acid substitutions (whether conservative or non-conservative) can be determined by those skilled in the art at the time such substitutions are desired. For example, amino acid substitutions can be used to identify important residues of the peptide sequence, or to increase or decrease the affinity of the peptide or vehicle-conjugated peptide molecules described herein.

[00194] Naturally occurring residues may be divided into classes based on common side chain properties:

1) hydrophobic: norleucine (Nor or Nle), Met, Ala, Val, Leu, Ile;
2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
3) acidic: Asp, Glu;
4) basic: His, Lys, Arg;
5) residues that influence chain orientation: Gly, Pro; and
6) aromatic: Trp, Tyr, Phe.

[00195] Conservative amino acid substitutions may involve exchange of a member of one of these classes with another member of the same class. Conservative amino acid substitutions may encompass non-naturally occurring amino acid residues, which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include peptidomimetics and other reversed or inverted forms of amino acid moieties.

[00196] Non-conservative substitutions may involve the exchange of a member of one of these classes for a member from another class. Such substituted residues may be introduced into regions of the toxin peptide analog.

[00197] In making such changes, according to certain embodiments, the hydropathic index of amino acids may be considered. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics. They are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3);

proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

[00198] The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is understood in the art (see, *for example*, Kyte *et al.*, 1982, *J. Mol. Biol.* 157:105-131). It is known that certain amino acids may be substituted for other amino acids having a similar hydropathic index or score and still retain a similar biological activity. In making changes based upon the hydropathic index, in certain embodiments, the substitution of amino acids whose hydropathic indices are within ±2 is included. In certain embodiments, those that are within ±1 are included, and in certain embodiments, those within ±0.5 are included.

[00199] It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity, particularly where the biologically functional protein or peptide thereby created is intended for use in immunological embodiments, as disclosed herein. In certain embodiments, the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, *i.e.*, with a biological property of the protein.

[00200] The following hydrophilicity values have been assigned to these amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 ± 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5) and tryptophan (-3.4). In making changes based upon similar hydrophilicity values, in certain embodiments, the substitution of amino acids whose hydrophilicity values are within ±2 is included, in certain embodiments, those that are within ±1 are included, and in certain embodiments, those within ±0.5 are included. One may also identify epitopes from primary amino acid sequences on the basis of hydrophilicity. These regions are also referred to as "epitopic core regions."

[00201] Examples of conservative substitutions include the substitution of one non-polar (hydrophobic) amino acid residue such as isoleucine, valine, leucine norleucine, alanine, or methionine for another, the substitution of one polar (hydrophilic) amino acid residue for another such as between arginine and lysine, between glutamine and asparagine, between glycine and serine, the substitution of one basic amino acid residue such as lysine, arginine or histidine for another, or the substitution of one acidic residue, such as aspartic acid or glutamic acid for another. The phrase "conservative amino acid substitution" also includes the use of a chemically derivatized residue in place of a non-derivatized residue, provided that such polypeptide displays the requisite bioactivity. Other exemplary amino acid substitutions that can be useful in accordance with the present invention are set forth in Table 3 below.

Table 3. Some Useful Amino Acid Substitutions.

Original Residues	Exemplary Substitutions
Ala	Val, Leu, Ile
Arg	Lys, Gln, Asn
Asn	Gln
Asp	Glu
Cys	Ser, Ala
Gln	Asn
Glu	Asp
Gly	Pro, Ala
His	Asn, Gln, Lys, Arg
Ile	Leu, Val, Met, Ala, Phe, Norleucine
Leu	Norleucine, Ile, Val, Met, Ala, Phe
Lys	Arg, 1,4-Diamino-

	butyric Acid, Gln, Asn
Met	Leu, Phe, Ile
Phe	Leu, Val, Ile, Ala, Tyr
Pro	Ala
Ser	Thr, Ala, Cys
Thr	Ser
Trp	Tyr, Phe
Tyr	Trp, Phe, Thr, Ser
Val	Ile, Met, Leu, Phe, Ala, Norleucine

[00202] In exemplary embodiments of the inventive composition, in relation to SEQ ID NO: 4:

[00203] The alkyl, basic, or acidic amino acid residue of X_{aa}^4 is selected from Ser, Thr, Ala, Gly, Leu, Ile, Val, Met, Cit, Homocitrulline, Oic, Pro, Hyp, Tic, D-Tic, D-Pro, Guf, and 4-Amino-Phe,Thz, Aib, Sar, Pip, Bip, Phe, Tyr, Lys, His, Trp, Arg, N^{α} Methyl-Arg; homoarginine, 1-Nal, 2-Nal, Orn, D-Orn, Asn, Gln, Glu, Asp, α-aminoadipic acid, and *para*-carboxyl-phenylalanine ; or more particularly X_{aa}^4 is selected from Ala, Ile, Lys, Orn, Glu and Asp;

[00204] and/or

[00205] The acidic amino acid residue of X_{aa}^9 and X_{aa}^{14} is each independently selected from Glu, Asp, and α-aminoadipic acid;

[00206] and/or

[00207] the alkyl or aromatic amino acid residue of X_{aa}^{15} is selected from Ala, 1-Nal, 2-Nal, Phe, Tyr, Val, Ile, and Leu, or more particularly the amino acid residue of X_{aa}^{15} is selected from Phe, Ala, and Ile;

[00208] and/or

[00209] the basic, alkyl, or aromatic amino acid residue of X_{aa}^{16} is selected from Lys, Orn, Dab, Dap 1-Nal, 2-Nal, Tyr, Phe, Pip, 2Pal, 3Pal, N-Me-Lys, N-

Me-Orn, alpha-methyl-lysine, Lys(N^{ε}-Me), Lys(N^{ε}-Me)$_2$, Lys(N^{ε}-Me)$_3$, para-Methyl-Phe, AMeF (alpha-methyl-phenylalanine), and homoPhe;

[00210] and/or

[00211] the basic or acidic amino acid residue of X_{aa}^{18} and X_{aa}^{30} is each independently selected from Lys, Arg, Orn, Glu, Asp, His, Trp, and 2-phenylacetic acid (Pac);

[00212] and/or

[00213] the basic amino acid residue of X_{aa}^{19}, X_{aa}^{20} and X_{aa}^{29} is each independently selected from Lys, Arg, His, Orn, D-Orn, Dab, Dap, 1Pip, 2Pal, 3Pal, N-Me-Lys, Nα Methyl-Arg; homoarginine, Cit, Nα-Methyl-Cit, Homocitrulline, Guf, and 4-Amino-Phe, and N-Me-Orn; and/or

[00214] the alkyl or aromatic amino acid residue of X_{aa}^{21} is selected from Nle, Nva, Abu, Phe,Tyr, Asn, Gln, Met[O], Val, Ile, Leu, Met[O_2], Cha, Chg, Asn, Trp, para-Methyl-Phe, alpha-methyl-Phe, and homoPhe;

[00215] and/or

[00216] the aromatic amino acid residue of X_{aa}^{26}, X_{aa}^{27}, X_{aa}^{31}, and X_{aa}^{34} is each independently selected from 1-Nal, 2-Nal, Phe, Trp, and Tyr.

[00217] and/or

[00218] the amino acid residue of X_{aa}^{36}, X_{aa}^{37}, and X_{aa}^{38}, if present, is each independently selected from Ala, Leu, Lys, Glu, Asp, Phe, Arg, Phe, Asp-amide, Aib-amide, Tyr, Ser-amide, Thr-amide, Glu, Glu-amide, beta-Ala, and N-Me-Ala.

[00219] In some embodiments the carboxy-terminal residue is amidated. For example, some C-terminally amidated embodiments are set forth in Table 11, Table 12, Table 14, Table 16, and Table 17.

[00220] Some embodiments of the composition of matter include C-terminal extensions beyond position X_{aa}^{35}. Some examples are set forth in Table 15 and Table 16.

[00221] Certain embodiments of the inventive composition of matter that have particular utility in improving the potency, stability, selectivity, and/or ease of synthesis of the toxin peptide analogs involve substitutions as summarized in Table 4 below, relative to SEQ ID NO: 4 and the native ShK sequence.

Table 4. Toxin Peptide Analogs with Improved Therapeutic Potential

SEQ ID NO:4 Residue #	Native ShK Amino Acid	Improved Analog	Description of Improvement
2	Ser	**Glu**	Potency
4	Ile	**Glu**, **Lys**, Ala	**Potency**
7	Ile	**Lys**	Selectivity
10	Ser	**Glu**, **Arg**, Ala	Potency, selectivity
15	Phe	Ala	Potency
16	Gln	**Lys**, Nal	**Selectivity**
18	Lys	Arg, Ala	**Potency**
20	Ser	**Lys**, **Arg**	**Selectivity**
21	Met	Gln, Nva	Limit oxidation
22	Lys	**Ala**	Selectivity
23	Tyr	Ala	Selectivity
26	Ser	Nal	Selectivity
27	Phe	Nal	Selectivity
29	Arg	Lys, Nal	**Potency, selectivity**
30	Lys	**Glu**, Arg	**Potency**
31	Thr	Nal	Potency
34	Thr	Nal	Potency
C-terminus	carboxy	Amide, Ala added	Potency, selectivity, & synthesis

[00222] In some embodiments, the inventive composition of matter comprises a toxin peptide analog comprising an amino acid sequence selected from SEQ ID NOS: 10, 11, 12, 14, 15, 16, 19 through 29, 31 through 34, 36 through 50, 52, 54, 55, 56, 59, 60, 61, 63, 65 through 100, 130 through 140, 142 through 174, 176 through 254, and 257 through 274.

[00223] As stated herein above, in accordance with the present invention, the peptide portions of the inventive composition of matter can also be chemically derivatized at one or more amino acid residues by known organic chemistry techniques. "Chemical derivative" or "chemically derivatized" refers to a subject peptide having one or more residues chemically derivatized by reaction of a functional side group. Such derivatized molecules include, for example, those molecules in which free amino groups have been derivatized to form amine

hydrochlorides, p-toluene sulfonyl groups, carbobenzoxy groups, t-butyloxycarbonyl groups, chloroacetyl groups or formyl groups. Free carboxyl groups may be derivatized to form salts, methyl and ethyl esters or other types of esters or hydrazides. Free hydroxyl groups may be derivatized to form O-acyl or O-alkyl derivatives. The imidazole nitrogen of histidine may be derivatized to form N-im-benzylhistidine. Also included as chemical derivatives are those peptides which contain one or more naturally occurring amino acid derivatives of the twenty canonical amino acids, whether in L- or D- form. For example, 4-hydroxyproline may be substituted for proline; 5-hydroxylysine maybe substituted for lysine; 3-methylhistidine may be substituted for histidine; homoserine may be substituted for serine; and ornithine may be substituted for lysine.

[00224] Useful derivatizations include, in some embodiments, those in which the amino terminal of the peptide is chemically blocked so that conjugation with the vehicle will be prevented from taking place at an N-terminal free amino group. There may also be other beneficial effects of such a modification, for example a reduction in the toxin peptide analog's susceptibility to enzymatic proteolysis. The N-terminus can be acylated or modified to a substituted amine, or derivatized with another functional group, such as an aromatic moiety (e.g., an indole acid, benzyl (Bzl or Bn), dibenzyl (DiBzl or Bn_2), or benzyloxycarbonyl (Cbz or Z)), *N,N*-dimethylglycine or creatine. For example, in some embodiments, an acyl moiety, such as, but not limited to, a formyl, acetyl (Ac), propanoyl, butanyl, heptanyl, hexanoyl, octanoyl, or nonanoyl, can be covalently linked to the N-terminal end of the peptide, which can prevent undesired side reactions during conjugation of the vehicle to the peptide. Other exemplary N-terminal derivative groups include $-NRR^1$ (other than $-NH_2$), $-NRC(O)R^1$, $-NRC(O)OR^1$, $-NRS(O)_2R^1$, $-NHC(O)NHR^1$, succinimide, or benzyloxycarbonyl-NH- (Cbz-NH-), wherein R and R^1 are each independently hydrogen or lower alkyl and wherein the phenyl ring may be substituted with 1 to 3 substituents selected from C_1-C_4 alkyl, C_1-C_4 alkoxy, chloro, and bromo.

[00225] In some embodiments, one or more peptidyl [-C(O)NR-] linkages (bonds) between amino acid residues can be replaced by a non-peptidyl linkage.

Exemplary non-peptidyl linkages are -CH_2-carbamate [-CH_2-OC(O)NR-], phosphonate , -CH_2-sulfonamide [-CH_2-$S(O)_2$NR-], urea [-NHC(O)NH-], -CH_2-secondary amine, and alkylated peptide [-C(O)NR^6- wherein R^6 is lower alkyl].

[00226] In some embodiments, one or more individual amino acid residues can be derivatized. Various derivatizing agents are known to react specifically with selected sidechains or terminal residues, as described in detail below by way of example.

[00227] Lysinyl residues and amino terminal residues may be reacted with succinic or other carboxylic acid anhydrides, which reverse the charge of the lysinyl residues. Other suitable reagents for derivatizing alpha-amino-containing residues include imidoesters such as methyl picolinimidate; pyridoxal phosphate; pyridoxal; chloroborohydride; trinitrobenzenesulfonic acid; O-methylisourea; 2,4 pentanedione; and transaminase-catalyzed reaction with glyoxylate.

[00228] Arginyl residues may be modified by reaction with any one or combination of several conventional reagents, including phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginyl residues requires that the reaction be performed in alkaline conditions because of the high pKa of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine epsilon-amino group.

[00229] Specific modification of tyrosyl residues has been studied extensively, with particular interest in introducing spectral labels into tyrosyl residues by reaction with aromatic diazonium compounds or tetranitromethane. Most commonly, N-acetylimidizole and tetranitromethane are used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively.

[00230] Carboxyl sidechain groups (aspartyl or glutamyl) may be selectively modified by reaction with carbodiimides (R'-N=C=N-R') such as 1-cyclohexyl-3-(2-morpholinyl-(4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues may be converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

[00231] Glutaminyl and asparaginyl residues may be deamidated to the corresponding glutamyl and aspartyl residues. Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues falls within the scope of this invention.

[00232] Cysteinyl residues can be replaced by amino acid residues or other moieties either to eliminate disulfide bonding or, conversely, to stabilize cross-linking. (See, e.g., Bhatnagar et al., J. Med. Chem., 39:3814-3819 (1996)).

[00233] Derivatization with bifunctional agents is useful for cross-linking the peptides or their functional derivatives to a water-insoluble support matrix, if desired, or to other macromolecular vehicles. Commonly used cross-linking agents include, e.g., 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane. Derivatizing agents such as methyl-3-[(p-azidophenyl)dithio]propioimidate yield photoactivatable intermediates that are capable of forming crosslinks in the presence of light. Alternatively, reactive water-insoluble matrices such as cyanogen bromide-activated carbohydrates and the reactive substrates, e.g., as described in U.S. Pat. Nos. 3,969,287; 3,691,016; 4,195,128; 4,247,642; 4,229,537; and 4,330,440, are employed for protein immobilization.

[00234] Other possible modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, oxidation of the sulfur atom in Cys, methylation of the alpha-amino groups of lysine, arginine, and histidine side chains. Creighton, Proteins: Structure and Molecule Properties (W. H. Freeman & Co., San Francisco), 79-86 (1983).

[00235] The above examples of derivatizations are not intended to be an exhaustive treatment, but merely illustrative.

[00236] The production of the composition of matter can also involve suitable protein purification techniques, when applicable. In some embodiments of the composition of matter of the invention, the molecule can be prepared to

include a suitable isotopic label (e.g., ^{125}I, ^{14}C, ^{13}C, ^{35}S, ^{3}H, ^{2}H, ^{13}N, ^{15}N, ^{18}O, ^{17}O, *etc.*), for ease of quantification or detection.

[00237] Half-life extending moieties. Optionally, for modulation of the pharmacokinetic profile of the molecule to fit the therapeutic need, the composition of the present invention can include one or more half-life extending moieties of various masses and configurations, which half-life extending moiety, or moieties, can be covalently fused, attached, linked or conjugated to the toxin peptide analog. A "half-life extending moiety" refers to a molecule that prevents or mitigates in vivo degradation by proteolysis or other activity-diminishing chemical modification, increases in vivo half-life or other pharmacokinetic properties such as but not limited to increasing the rate of absorption, reduces toxicity, reduces immunogenicity, improves solubility, increases biological activity and/or target selectivity of the toxin peptide analog with respect to a target of interest, and/or increases manufacturability, compared to an unconjugated form of the toxin peptide analog. In accordance with the invention, the half-life extending moiety is one that is pharmaceutically acceptable.

[00238] The half-life extending moiety can be selected such that the inventive composition achieves a sufficient hydrodynamic size to prevent clearance by renal filtration in vivo. For example, a half-life extending moiety can be selected that is a polymeric macromolecule, which is substantially straight chain, branched-chain (br), or dendritic in form. Alternatively, a half-life extending moiety can be selected such that, in vivo, the inventive composition of matter will bind to a serum protein to form a complex, such that the complex thus formed avoids substantial renal clearance. The half-life extending moiety can be, for example, a lipid; a cholesterol group (such as a steroid); a carbohydrate or oligosaccharide; or any natural or synthetic protein, polypeptide or peptide that binds to a salvage receptor.

[00239] Exemplary half-life extending moieties that can be used, in accordance with the present invention, include an immunoglobulin Fc domain, or a portion thereof, or a biologically suitable polymer or copolymer, for example, a polyalkylene glycol compound, such as a polyethylene glycol (PEG) or a

polypropylene glycol. Other appropriate polyalkylene glycol compounds include, but are not limited to, charged or neutral polymers of the following types: dextran, polylysine, colominic acids or other carbohydrate based polymers, polymers of amino acids, and biotin derivatives. In some monomeric fusion protein embodiments an immunoglobulin (including light and heavy chains) or a portion thereof, can be used as a half-life-extending moiety, preferably an immunoglobulin of human origin, and including any of the immunoglobulins, such as, but not limited to, IgG1, IgG2, IgG3 or IgG4.

[00240] Other examples of the half-life extending moiety, in accordance with the invention, include a copolymer of ethylene glycol, a copolymer of propylene glycol, a carboxymethylcellulose, a polyvinyl pyrrolidone, a poly-1,3-dioxolane, a poly-1,3,6-trioxane, an ethylene/maleic anhydride copolymer, a polyaminoacid (e.g., polylysine or polyornithine), a dextran n-vinyl pyrrolidone, a poly n-vinyl pyrrolidone, a propylene glycol homopolymer, a propylene oxide polymer, an ethylene oxide polymer, a polyoxyethylated polyol, a polyvinyl alcohol, a linear or branched glycosylated chain, a polyacetal, a long chain fatty acid, a long chain hydrophobic aliphatic group, or a polysialic acid (e.g., PolyXen™ technology; Gregoriadis et al., Improving the therapeutic efficacy of peptides and proteins: a role for polysialic acids, Intl. J. Pharmaceutics, 300:125-30 (2005), incorporated herein by reference in its entirety).

[00241] In other embodiments of the composition of matter, the half-life extending moiety is an anionically charged chemical entity, covalently linked to the N-terminus of the toxin peptide analog, which anionically charged chemical entities include, but are not limited to, phosphotyrosine, phosphoserine, p-phosphono(difluoro-methyl)-phenylalanine (Pfp), p-phosphono-methyl-phenylalanine (Pmp), p-phosphatidyl-phenylalanine (Ppa), or p-phosphono-methylketo-phenylalanine (Pkp), which can be covalently linked to the N-terminal of the toxin peptide analog, optionally indirectly, via an AEEA linker or other linker as described herein. (See, Chandy et al., Analogs of ShK toxin and their uses in selective inhibition of Kv1.3 potassium channels, WO 2006/042151 A2; Beeton et al., Targeting effector memory T cells with a selective peptide inhibitor

of Kv1.3 channels for therapy of autoimmune diseases, Molec. Pharmacol. 67(4):1369-81 (2005); Pennington et al., Engineering a stable and selective peptide blocker of the Kv1.3 channel in T lymphocytes, Molecular Pharmacology Fast Forward, published January 2, 2009 as doi:10.1124/mol.108.052704 (2009), all of which references are incorporated herein by reference in their entireties). AEEA is 2-(2-(2-aminoethoxy)ethoxy)acetic acid (also known as 8-Amino-3,6-Dioxaoctanoic Acid). (See, e.g., Beeton et al., Targeting effector memory T cells with a selective peptide inhibitor of Kv1.3 channels for therapy of autoimmune diseases, Molec. Pharmacol. 67(4):1369-81 (2005)).

[00242] Other embodiments of the half-life extending moiety, in accordance with the invention, include peptide ligands or small (organic) molecule ligands that have binding affinity for a long half-life serum protein under physiological conditions of temperature, pH, and ionic strength. Examples include an albumin-binding peptide or small molecule ligand, a transthyretin-binding peptide or small molecule ligand, a thyroxine-binding globulin-binding peptide or small molecule ligand, an antibody-binding peptide or small molecule ligand, or another peptide or small molecule that has an affinity for a long half-life serum protein. (See, e.g., Blaney et al., Method and compositions for increasing the serum half-life of pharmacologically active agents by binding to transthyretin-selective ligands, US Patent. No. 5,714,142; Sato et al., Serum albumin binding moieties, US 2003/0069395 A1; Jones et al., Pharmaceutical active conjugates, US Patent No. 6,342,225). A "long half-life serum protein" is one of the hundreds of different proteins dissolved in mammalian blood plasma, including so-called "carrier proteins" (such as albumin, transferrin and haptoglobin), fibrinogen and other blood coagulation factors, complement components, immunoglobulins, enzyme inhibitors, precursors of substances such as angiotensin and bradykinin and many other types of proteins. The invention encompasses the use of any single species of pharmaceutically acceptable half-life extending moiety, such as, but not limited to, those described herein, or the use of a combination of two or more different half-life extending moieties, such as PEG and immunoglobulin Fc domain or a portion thereof (see, e.g., Feige et al., Modified peptides as

therapeutic agents, US Patent No. 6,660,843), such as a CH2 domain of Fc, albumin (e.g., human serum albumin (HSA); see, e.g., Rosen et al., Albumin fusion proteins, US Patent No. 6,926,898 and US 2005/0054051; Bridon et al., Protection of endogenous therapeutic peptides from peptidase activity through conjugation to blood components, US 6,887,470), a transthyretin (TTR; see, e.g., Walker et al., Use of transthyretin peptide/protein fusions to increase the serum half-life of pharmacologically active peptides/proteins, US 2003/0195154 A1; 2003/0191056 A1), or a thyroxine-binding globulin (TBG), or a combination such as immunoglobulin(light chain+heavy chain) and Fc domain (the heterotrimeric combination a so-called "hemibody"), for example as described in Sullivan et al., Toxin Peptide Therapeutic Agents, PCT/US2007/022831, published as WO 2008/088422, which is incorporated herein by reference in its entirety.

[00243] Conjugation of the toxin peptide analogs(s) to the half-life extending moiety, or moieties, can be via the N-terminal and/or C-terminal of the toxin peptide, or can be intercalary as to its primary amino acid sequence, F1 being linked closer to the toxin peptide analog's N-terminus.

[00244] Particularly useful half-life extending moieties include immunoglobulins (e.g., human immunoglobulin, including IgG1, IgG2, IgG3 or IgG4). The term "immunoglobulin" encompasses full antibodies comprising two dimerized heavy chains (HC), each covalently linked to a light chain (LC); a single undimerized immunoglobulin heavy chain and covalently linked light chain (HC + LC); or a chimeric immunoglobulin (light chain + heavy chain)-Fc heterotrimer (a so-called "hemibody").

[00245] An "antibody", or interchangeably "Ab", is a tetrameric glycoprotein. In a naturally-occurring antibody, each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" chain of about 220 amino acids (about 25 kDa) and one "heavy" chain of about 440 amino acids (about 50-70 kDa). The amino-terminal portion of each chain includes a "variable" ("V") region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. The variable

region differs among different antibodies. The constant region is the same among different antibodies. Within the variable region of each heavy or light chain, there are three hypervariable subregions that help determine the antibody's specificity for antigen. The variable domain residues between the hypervariable regions are called the framework residues and generally are somewhat homologous among different antibodies. Immunoglobulins can be assigned to different classes depending on the amino acid sequence of the constant domain of their heavy chains. Human light chains are classified as kappa (κ) and lambda (λ) light chains. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See generally, Fundamental Immunology, Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)). Within the scope of the invention, an "antibody" also encompasses a recombinantly made antibody, and antibodies that are lacking glycosylation.

[00246] The term "light chain" or "immunoglobulin light chain" includes a full-length light chain and fragments thereof having sufficient variable region sequence to confer binding specificity. A full-length light chain includes a variable region domain, V_L, and a constant region domain, C_L. The variable region domain of the light chain is at the amino-terminus of the polypeptide. Light chains include kappa chains and lambda chains.

[00247] The term "heavy chain" or "immunoglobulin heavy chain" includes a full-length heavy chain and fragments thereof having sufficient variable region sequence to confer binding specificity. A full-length heavy chain includes a variable region domain, V_H, and three constant region domains, C_H1, C_H2, and C_H3. The V_H domain is at the amino-terminus of the polypeptide, and the C_H domains are at the carboxyl-terminus, with the C_H3 being closest to the carboxy-terminus of the polypeptide. Heavy chains are classified as mu (μ), delta (Δ), gamma (γ), alpha (α), and epsilon (ε), and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. In separate embodiments of the invention, heavy chains may be of any isotype, including IgG (including IgG1, IgG2, IgG3 and IgG4 subtypes), IgA (including IgA1 and IgA2 subtypes), IgM and IgE.

Several of these may be further divided into subclasses or isotypes, e.g. IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. Different IgG isotypes may have different effector functions (mediated by the Fc region), such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). In ADCC, the Fc region of an antibody binds to Fc receptors (FcγRs) on the surface of immune effector cells such as natural killers and macrophages, leading to the phagocytosis or lysis of the targeted cells. In CDC, the antibodies kill the targeted cells by triggering the complement cascade at the cell surface.

[00248] An "Fc region", or used interchangeably herein, "Fc domain" or "immunoglobulin Fc domain", contains two heavy chain fragments, which in a full antibody comprise the C_H1 and C_H2 domains of the antibody. The two heavy chain fragments are held together by two or more disulfide bonds and by hydrophobic interactions of the C_H3 domains.

[00249] The term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (*e.g.*, IgG_1, IgG_2, IgG_3, or IgG_4) that is responsible for increasing the in vivo serum half-life of the IgG molecule.

[00250] "Allotypes" are variations in antibody sequence, often in the constant region, that can be immunogenic and are encoded by specific alleles in humans. Allotypes have been identified for five of the human IGHC genes, the IGHG1, IGHG2, IGHG3, IGHA2 and IGHE genes, and are designated as G1m, G2m, G3m, A2m, and Em allotypes, respectively. At least 18 Gm allotypes are known: nG1m(1), nG1m(2), G1m (1, 2, 3, 17) or G1m (a, x, f, z), G2m (23) or G2m (n), G3m (5, 6, 10, 11, 13, 14, 15, 16, 21, 24, 26, 27, 28) or G3m (b1, c3, b5, b0, b3, b4, s, t, g1, c5, u, v, g5). There are two A2m allotypes A2m(1) and A2m(2).

[00251] For a detailed description of the structure and generation of antibodies, see Roth, D.B., and Craig, N.L., *Cell*, 94:411-414 (1998), herein incorporated by reference in its entirety. Briefly, the process for generating DNA encoding the heavy and light chain immunoglobulin sequences occurs primarily in developing B-cells. Prior to the rearranging and joining of various immunoglobulin gene segments, the V, D, J and constant (C) gene segments are

found generally in relatively close proximity on a single chromosome. During B-cell-differentiation, one of each of the appropriate family members of the V, D, J (or only V and J in the case of light chain genes) gene segments are recombined to form functionally rearranged variable regions of the heavy and light immunoglobulin genes. This gene segment rearrangement process appears to be sequential. First, heavy chain D-to-J joints are made, followed by heavy chain V-to-DJ joints and light chain V-to-J joints. In addition to the rearrangement of V, D and J segments, further diversity is generated in the primary repertoire of immunoglobulin heavy and light chains by way of variable recombination at the locations where the V and J segments in the light chain are joined and where the D and J segments of the heavy chain are joined. Such variation in the light chain typically occurs within the last codon of the V gene segment and the first codon of the J segment. Similar imprecision in joining occurs on the heavy chain chromosome between the D and J_H segments and may extend over as many as 10 nucleotides. Furthermore, several nucleotides may be inserted between the D and J_H and between the V_H and D gene segments which are not encoded by genomic DNA. The addition of these nucleotides is known as N-region diversity. The net effect of such rearrangements in the variable region gene segments and the variable recombination which may occur during such joining is the production of a primary antibody repertoire.

[00252] The term "hypervariable" region refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a complementarity determining region or CDR [i.e., residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain as described by Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)]. Even a single CDR may recognize and bind antigen, although with a lower affinity than the entire antigen binding site containing all of the CDRs.

[00253] An alternative definition of residues from a hypervariable "loop" is described by Chothia et al., *J. Mol.Biol. 196*: 901-917 (1987) as residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain .

[00254] "Framework" or "FR" residues are those variable region residues other than the hypervariable region residues.

[00255] "Antibody fragments" comprise a portion of an intact full length antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies (Zapata et al., Protein Eng.,8(10):1057-1062 (1995)); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

[00256] Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment which contains the constant region. The Fab fragment contains all of the variable domain, as well as the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. The Fc fragment displays carbohydrates and is responsible for many antibody effector functions (such as binding complement and cell receptors), that distinguish one class of antibody from another.

[00257] Pepsin treatment yields an $F(ab')_2$ fragment that has two "Single-chain Fv" or "scFv" antibody fragments comprising the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. Fab fragments differ from Fab' fragments by the inclusion of a few additional residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Preferably, the Fv polypeptide further comprises a polypeptide linker between the VH and VL domains that enables the Fv to form the desired structure for antigen binding. For a review of scFv see Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. l 13, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

[00258] A "Fab fragment" is comprised of one light chain and the C_H1 and variable regions of one heavy chain. The heavy chain of a Fab molecule cannot form a disulfide bond with another heavy chain molecule.

[00259] A "Fab' fragment" contains one light chain and a portion of one heavy chain that contains the V_H domain and the C_H1 domain and also the region between the C_H1 and C_H2 domains, such that an interchain disulfide bond can be formed between the two heavy chains of two Fab' fragments to form an $F(ab')_2$ molecule.

[00260] A "$F(ab')_2$ fragment" contains two light chains and two heavy chains containing a portion of the constant region between the C_H1 and C_H2 domains, such that an interchain disulfide bond is formed between the two heavy chains. A $F(ab')_2$ fragment thus is composed of two Fab' fragments that are held together by a disulfide bond between the two heavy chains.

[00261] "Fv" is the minimum antibody fragment that contains a complete antigen recognition and binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen binding site on the surface of the VH VL dimer. A single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[00262] "Single-chain antibodies" are Fv molecules in which the heavy and light chain variable regions have been connected by a flexible linker to form a single polypeptide chain, which forms an antigen-binding region. Single chain antibodies are discussed in detail in International Patent Application Publication No. WO 88/01649 and United States Patent No. 4,946,778 and No. 5,260,203, the disclosures of which are incorporated by reference in their entireties.

[00263] "Single-chain Fv" or "scFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain, and optionally comprising a polypeptide linker between the V_H and V_L domains that enables the Fv to form the desired structure for antigen

binding (Bird et al., *Science* 242:423-426, 1988, and Huston et al., *Proc. Natl. Acad. Sci. USA* 85:5879-5883, 1988). An "Fd" fragment consists of the V_H and C_H1 domains.

[00264] The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) in the same polypeptide chain (VH VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993).

[00265] A "domain antibody" is an immunologically functional immunoglobulin fragment containing only the variable region of a heavy chain or the variable region of a light chain. In some instances, two or more V_H regions are covalently joined with a peptide linker to create a bivalent domain antibody. The two V_H regions of a bivalent domain antibody may target the same or different antigens.

[00266] The terms "DNP" or "dinitrophenol" are used interchangeably herein and denote the antigen 2,4-dinitrophenol. "Anti-DNP" or "αDNP" or "aDNP" are used interchangeably herein to refer to an antigen binding protein, e.g., an antibody or antibody fragment, that specifically binds DNP.

[00267] The terms "KLH" or "keyhole limpet hemocyanin" are used interchangeably herein and denote the Imject® Mariculture Keyhole Limpet hemocyanin (mcKLH; Pierce Biotechnology, Rockford, IL). According to the manufacturer, mcKLH is harvested from select populations of the mollusk Megathura crenulata (keyhole limpet) that are grown in mariculture, rather than being extracted from wild populations; KLH has a high molecular mass (4.5×10^5 - 1.3×10^7 Daltons of mixed aggregates of 350 and 390 kDa subunits) and elicits a stronger immune response than BSA or ovalbumin. "Anti-KLH" or "αKLH" or "aKLH" are used interchangeably herein to refer to an antigen binding protein, e.g., an antibody or antibody fragment, that specifically binds KLH.

[00268] The term "epitope" is the portion of a molecule that is bound by an antigen binding protein (for example, an antibody). The term includes any determinant capable of specifically binding to an antigen binding protein, such as an antibody or to a T-cell receptor. An epitope can be contiguous or non-contiguous (e.g., in a single-chain polypeptide, amino acid residues that are not contiguous to one another in the polypeptide sequence but that within the context of the molecule are bound by the antigen binding protein). In certain embodiments, epitopes may be mimetic in that they comprise a three dimensional structure that is similar to an epitope used to generate the antigen binding protein, yet comprise none or only some of the amino acid residues found in that epitope used to generate the antigen binding protein. Most often, epitopes reside on proteins, but in some instances may reside on other kinds of molecules, such as nucleic acids. Epitope determinants may include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl or sulfonyl groups, and may have specific three dimensional structural characteristics, and/or specific charge characteristics. Generally, antibodies specific for a particular target antigen will preferentially recognize an epitope on the target antigen in a complex mixture of proteins and/or macromolecules.

[00269] The term "identity" refers to a relationship between the sequences of two or more polypeptide molecules or two or more nucleic acid molecules, as determined by aligning and comparing the sequences. "Percent identity" means the percent of identical residues between the amino acids or nucleotides in the compared molecules and is calculated based on the size of the smallest of the molecules being compared. For these calculations, gaps in alignments (if any) must be addressed by a particular mathematical model or computer program (i.e., an "algorithm"). Methods that can be used to calculate the identity of the aligned nucleic acids or polypeptides include those described in Computational Molecular Biology, (Lesk, A. M., ed.), 1988, New York: Oxford University Press; Biocomputing Informatics and Genome Projects, (Smith, D. W., ed.), 1993, New York: Academic Press; Computer Analysis of Sequence Data, Part I, (Griffin, A. M., and Griffin, H. G., eds.), 1994, New Jersey: Humana Press; von Heinje, G.,

1987, Sequence Analysis in Molecular Biology, New York: Academic Press; Sequence Analysis Primer, (Gribskov, M. and Devereux, J., eds.), 1991, New York: M. Stockton Press; and Carillo et al., 1988, SIAM J. Applied Math. 48:1073. For example, sequence identity can be determined by standard methods that are commonly used to compare the similarity in position of the amino acids of two polypeptides. Using a computer program such as BLAST or FASTA, two polypeptide or two polynucleotide sequences are aligned for optimal matching of their respective residues (either along the full length of one or both sequences, or along a pre-determined portion of one or both sequences). The programs provide a default opening penalty and a default gap penalty, and a scoring matrix such as PAM 250 [a standard scoring matrix; see Dayhoff et al., in *Atlas of Protein Sequence and Structure*, vol. 5, supp. 3 (1978)] can be used in conjunction with the computer program. For example, the percent identity can then be calculated as: the total number of identical matches multiplied by 100 and then divided by the sum of the length of the longer sequence within the matched span and the number of gaps introduced into the longer sequences in order to align the two sequences. In calculating percent identity, the sequences being compared are aligned in a way that gives the largest match between the sequences.

[00270] The GCG program package is a computer program that can be used to determine percent identity, which package includes GAP (Devereux et al., 1984, Nucl. Acid Res. 12:387; Genetics Computer Group, University of Wisconsin, Madison, WI). The computer algorithm GAP is used to align the two polypeptides or two polynucleotides for which the percent sequence identity is to be determined. The sequences are aligned for optimal matching of their respective amino acid or nucleotide (the "matched span", as determined by the algorithm). A gap opening penalty (which is calculated as 3x the average diagonal, wherein the "average diagonal" is the average of the diagonal of the comparison matrix being used; the "diagonal" is the score or number assigned to each perfect amino acid match by the particular comparison matrix) and a gap extension penalty (which is usually 1/10 times the gap opening penalty), as well as a comparison matrix such as PAM 250 or BLOSUM 62 are used in conjunction

with the algorithm. In certain embodiments, a standard comparison matrix (see, Dayhoff et al., 1978, Atlas of Protein Sequence and Structure 5:345-352 for the PAM 250 comparison matrix; Henikoff et al., 1992, Proc. Natl. Acad. Sci. U.S.A. 89:10915-10919 for the BLOSUM 62 comparison matrix) is also used by the algorithm.

[00271] Recommended parameters for determining percent identity for polypeptides or nucleotide sequences using the GAP program include the following:

[00272] Algorithm: Needleman et al., 1970, J. Mol. Biol. 48:443-453;

[00273] Comparison matrix: BLOSUM 62 from Henikoff et al., 1992, supra;

[00274] Gap Penalty: 12 (but with no penalty for end gaps)

[00275] Gap Length Penalty: 4

[00276] Threshold of Similarity: 0

[00277] Certain alignment schemes for aligning two amino acid sequences may result in matching of only a short region of the two sequences, and this small aligned region may have very high sequence identity even though there is no significant relationship between the two full-length sequences. Accordingly, the selected alignment method (GAP program) can be adjusted if so desired to result in an alignment that spans at least 50 contiguous amino acids of the target polypeptide.

[00278] The term "modification" when used in connection withimmmunoglobulins, including antibodies and antibody fragments, of the invention, include, but are not limited to, one or more amino acid changes (including substitutions, insertions or deletions); chemical modifications; covalent modification by conjugation to therapeutic or diagnostic agents; labeling (e.g., with radionuclides or various enzymes); covalent polymer attachment such as PEGylation (derivatization with polyethylene glycol) and insertion or substitution by chemical synthesis of non-natural amino acids.

[00279] The term "derivative" when used in connection with an immunoglobulin (including antibodies and antibody fragments) within the scope

of the invention refers to immunoglobulin proteins that are covalently modified by conjugation to therapeutic or diagnostic agents, labeling (e.g., with radionuclides or various enzymes), covalent polymer attachment such as PEGylation (derivatization with polyethylene glycol) and insertion or substitution by chemical synthesis of non-natural amino acids. Derivatives of the invention will retain the binding properties of underivatized molecules of the invention.

[00280] In some embodiments of the invention, the half-life extending moiety is an immunoglobulin Fc domain (e.g., a human immunoglobulin Fc domain, including Fc of allotype IgG1, IgG2, IgG3 or IgG4) or a portion thereof (e.g., CH2 domain of the Fc domain), human serum albumin (HSA), or poly(ethylene glycol) (PEG), in particular PEG of molecular weight of about 1000 Da to about 100000 Da.

[00281] Monovalent dimeric or bivalent dimeric Fc-toxin peptide analog fusions are useful embodiments of the inventive composition of matter. A "monovalent dimeric" Fc-toxin peptide analog fusion, or interchangeably, "monovalent dimer", or interchangeably, "monovalent heterodimer", is a Fc-toxin peptide analog fusion that includes a toxin peptide analog conjugated with only one of the dimerized Fc domains (e.g., as represented schematically in Figure 2B of Sullivan et al., Toxin Peptide Therapeutic Agents, US2007/0071764 and Sullivan et al., Toxin Peptide Therapeutic Agents, PCT/US2007/022831, published as WO 2008/088422, which are both incorporated herein by reference in their entireties). A "bivalent dimeric" Fc-toxin peptide analog fusion, or interchangeably, "bivalent dimer" or "bivalent homodimer", is a Fc-toxin peptide analog fusion having both of the dimerized Fc domains each conjugated separately with a toxin peptide analog (e.g., as represented schematically in Figure 2C of Sullivan et al., Toxin Peptide Therapeutic Agents, US2007/0071764 and Sullivan et al., Toxin Peptide Therapeutic Agents, PCT/US2007/022831, published as WO 2008/088422).

[00282] Immunoglobulin Fc domains include Fc variants, which are suitable half-life extending moieties within the scope of this invention. A native Fc can be extensively modified to form an Fc variant in accordance with this

invention, provided binding to the salvage receptor is maintained; see, for example WO 97/34631, WO 96/32478, and WO 04/110 472. In such Fc variants, one can remove one or more sites of a native Fc that provide structural features or functional activity not required by the fusion molecules of this invention. One can remove these sites by, for example, substituting or deleting residues, inserting residues into the site, or truncating portions containing the site. The inserted or substituted residues can also be altered amino acids, such as peptidomimetics or D-amino acids. Fc variants can be desirable for a number of reasons, several of which are described below. Exemplary Fc variants include molecules and sequences in which:

1. Sites involved in disulfide bond formation are removed. Such removal can avoid reaction with other cysteine-containing proteins present in the host cell used to produce the molecules of the invention. For this purpose, the cysteine-containing segment at the N-terminus can be truncated or cysteine residues can be deleted or substituted with other amino acids (e.g., alanyl, seryl). In particular, one can truncate the N-terminal 20-amino acid segment of SEQ ID NO: 278 or delete or substitute the cysteine residues at positions 7 and 10 of SEQ ID NO: 278. Even when cysteine residues are removed, the single chain Fc domains can still form a dimeric Fc domain that is held together non-covalently.
2. A native Fc is modified to make it more compatible with a selected host cell. For example, one can remove the PA dipeptide sequence near the N-terminus of a typical native Fc, which can be recognized by a digestive enzyme in E. coli such as proline iminopeptidase. One can also add an N-terminal methionine residue, especially when the molecule is expressed recombinantly in a bacterial cell such as E. coli. The Fc domain of SEQ ID NO: 278 (Figure 27A-B) is one such Fc variant.
3. A portion of the N-terminus of a native Fc is removed to prevent N-terminal heterogeneity when expressed in a selected host cell. For this purpose, one can delete any of the first 20 amino acid residues at the N-terminus, particularly those at positions 1, 2, 3, 4 and 5.

4. One or more glycosylation sites are removed. Residues that are typically glycosylated (e.g., asparagine) can confer cytolytic response. Such residues can be deleted or substituted with unglycosylated residues (e.g., alanine).
5. Sites involved in interaction with complement, such as the C1q binding site, are removed. For example, one can delete or substitute the EKK tripeptide sequence of human IgG1. Complement recruitment may not be advantageous for the molecules of this invention and so can be avoided with such an Fc variant.
6. Sites are removed that affect binding to Fc receptors other than a salvage receptor. A native Fc can have sites for interaction with certain white blood cells that are not required for the fusion molecules of the present invention and so can be removed.
7. The ADCC site is removed to decrease or eliminate ADCC effector function, or alternatively, modified for enhanced ADCC effector function by non-fucosylation or de-fucosylation. ADCC sites are known in the art; see, for example, Molec. Immunol. 29 (5): 633-9 (1992) with regard to ADCC sites in IgG1. These sites, as well, are not required for the fusion molecules of the present invention and so can be removed, or enhanced for ADCC effector function, as may be desired. (See, Iida et al., Two mechanisms of the enhanced antibody-dependent cellular cytotoxicity (ADCC) efficacy of non-fucosylated therapeutic antibodies in human blood, BMC Cancer 9:58 doi:10.1186/1471-2407-9-58 (2009)).
8. When the native Fc is derived from a non-human antibody, the native Fc can be humanized. Typically, to humanize a native Fc, one will substitute selected residues in the non-human native Fc with residues that are normally found in human native Fc. Techniques for antibody humanization are well known in the art.
9. One or more toxin peptide analog sequences can be inserted into an internal conjugation site, or sites, within a loop region of an immunoglobulin Fc domain, as disclosed in U.S. Patent No.7,442,778 B2.

The term "loop" region or "Fc-loop" region refers to a primary sequence of amino acid residues which connects two regions comprising secondary structure, such as an α-helix or a β-sheet, in the immediate N-terminal and C-terminal directions of primary structure from the loop region. Examples include, but are not limited to, CH2 or CH3 loop regions. One of skill in the art understands that a loop region, while not itself comprising secondary structure, may influence or contribute to secondary or higher order protein structure. The term "internal" conjugation site means that the toxin peptide analog moiety, or moieties, is non-terminal, i.e., not through the α-amino site or the α-carboxy site of the Fc domain, although there optionally can also be additional moieties conjugated terminally at the N-terminal and/or C-terminal of the Fc domain. (See, e.g., Example 11, Example 12, Table 4H and Table 4L herein).

10. A linker of suitable length and neutral charge, such as "L25" (GGGGSGGGGSGGGGSGGGGSGGGGS; SEQ ID NO:293) or "L20" (GGGGSGGGGSGGGGSGGGGS; SEQ ID NO:86), can be covalently fused between the C-terminal of one monomer of an Fc domain and the N-terminal of a second Fc domain monomer, with a toxin peptide analog fused to the N-terminal of the first Fc domain monomer or the C-terminal of the second Fc domain monomer, or within a loop region of the first and/or second Fc domain monomer. Such a molecule can be recombinantly expressed in bacterial or mammalian cells to produce a variant "monovalent dimeric" Fc-toxin peptide analog fusion with the typical disulfide bond formation between the Fc monomers. (See, e.g., Example 13 herein).

[00283] Other examples of Fc variants include the following: In SEQ ID NO: 278, the leucine at position 15 can be substituted with glutamate; the glutamate at position 99, with alanine; and the lysines at positions 101 and 103, with alanines. In addition, phenyalanine residues can replace one or more tyrosine residues. For purposes of the invention, a variant Fc domain can also be part of a

monomeric immunoglobulin heavy chain, an antibody, or a heterotrimeric hemibody (LC+HC+Fc).

[00284] An alternative half-life extending moiety would be a protein, polypeptide, peptide, antibody, antibody fragment, or small molecule (e.g., a peptidomimetic compound) capable of binding to a salvage receptor. For example, one could use as a half-life extending moiety a polypeptide as described in U.S. Pat. No. 5,739,277, issued April 14, 1998 to Presta et al. Peptides could also be selected by phage display for binding to the FcRn salvage receptor. Such salvage receptor-binding compounds are also included within the meaning of "half-life extending moiety" and are within the scope of this invention. Such half-life extending moieties should be selected for increased half-life (e.g., by avoiding sequences recognized by proteases) and decreased immunogenicity (e.g., by favoring non-immunogenic sequences, as discovered in antibody humanization).

[00285] As noted above, polymer half-life extending moieties can also be used. Various means for attaching chemical moieties useful as half-life extending moieties are currently available, see, e.g., Patent Cooperation Treaty ("PCT") International Publication No. WO 96/11953, entitled "N-Terminally Chemically Modified Protein Compositions and Methods," herein incorporated by reference in its entirety. This PCT publication discloses, among other things, the selective attachment of water-soluble polymers to the N-terminus of proteins.

[00286] In some embodiments of the inventive compositions, the polymer half-life extending moiety is polyethylene glycol (PEG), covalently linked at the N-terminal, C-terminal or at one or more intercalary side chains of toxin peptide analog. Some embodiments of the inventive composition of matter further include one or more PEG moieties conjugated to a non-PEG half-life extending moiety or to the toxin peptide analog, or to any combination of any of these. For example, an Fc domain or portion thereof in the inventive composition can be made mono-PEGylated, di-PEGylated, or otherwise multi-PEGylated, by the process of reductive alkylation.

[00287] Covalent conjugation of proteins and peptides with poly(ethylene glycol) (PEG) has been widely recognized as an approach to significantly extend

the in vivo circulating half-lives of therapeutic proteins. PEGylation achieves this effect predominately by retarding renal clearance, since the PEG moiety adds considerable hydrodynamic radius to the protein. (Zalipsky, S., et al., Use of functionalized poly(ethylene glycol)s for modification of polypeptides., in poly(ethylene glycol) chemistry: Biotechnical and biomedical applications., J.M. Harris, Ed., Plenum Press: New York., 347-370 (1992)). Additional benefits often conferred by PEGylation of proteins and peptides include increased solubility, resistance to proteolytic degradation, and reduced immunogenicity of the therapeutic polypeptide. The merits of protein PEGylation are evidenced by the commercialization of several PEGylated proteins including PEG-Adenosine deaminase (Adagen™/Enzon Corp.), PEG-L-asparaginase (Oncaspar™/Enzon Corp.), PEG-Interferon α-2b (PEG-Intron™/Schering/Enzon), PEG-Interferon α-2a (PEGASYS™/Roche) and PEG-G-CSF (Neulasta™/Amgen) as well as many others in clinical trials.

[00288] By "PEGylated peptide" or "PEGylated protein" is meant a peptide having a polyethylene glycol (PEG) moiety covalently bound to an amino acid residue of the peptide itself or to a peptidyl or non-peptidyl linker that is covalently bound to a residue of the peptide.

[00289] By "polyethylene glycol" or "PEG" is meant a polyalkylene glycol compound or a derivative thereof, with or without coupling agents or derivatization with coupling or activating moieties (e.g., with aldehyde, hydroxysuccinimidyl, hydrazide, thiol, triflate, tresylate, azirdine, oxirane, orthopyridyl disulphide, vinylsulfone, iodoacetamide or a maleimide moiety). In accordance with the present invention, useful PEG includes substantially linear, straight chain PEG, branched PEG (brPEG), or dendritic PEG. (See, e.g., Merrill, US Patent No. 5,171,264; Harris et al., Multiarmed, monofunctional, polymer for coupling to molecules and surfaces, US Patent No. 5,932,462; Shen, N-maleimidyl polymer derivatives, US Patent No. 6,602,498).

[00290] Briefly, the PEG groups are generally attached to the peptide portion of the composition of the invention via acylation or reductive alkylation (or reductive amination) through a reactive group on the PEG moiety (e.g., an

aldehyde, amino, thiol, or ester group) to a reactive group on the inventive compound (e.g., an aldehyde, amino, or ester group).

[00291] A useful strategy for the PEGylation of synthetic peptides consists of combining, through forming a conjugate linkage in solution, a peptide and a PEG moiety, each bearing a special functionality that is mutually reactive toward the other. The peptides can be easily prepared with conventional solid phase synthesis (see, for example, Figures 5 and 6 and the accompanying text herein). The peptides are "preactivated" with an appropriate functional group at a specific site. The precursors are purified and fully characterized prior to reacting with the PEG moiety. Ligation of the peptide with PEG usually takes place in aqueous phase and can be easily monitored by reverse phase analytical HPLC. The PEGylated peptides can be easily purified by preparative HPLC and characterized by analytical HPLC, amino acid analysis and laser desorption mass spectrometry.

[00292] PEG is a well-known, water soluble polymer that is commercially available or can be prepared by ring-opening polymerization of ethylene glycol according to methods well known in the art (Sandler and Karo, Polymer Synthesis, Academic Press, New York, Vol. 3, pages 138-161). In the present application, the term "PEG" is used broadly to encompass any polyethylene glycol molecule, in mono-, bi-, or poly- functional form, without regard to size or to modification at an end of the PEG, and can be represented by the formula:

$$X\text{-}O(CH_2CH_2O)_{n-1}CH_2CH_2OH, \qquad (I)$$

where n is 20 to 2300 and X is H or a terminal modification, e.g., a C_{1-4} alkyl.

In some useful embodiments, a PEG used in the invention terminates on one end with hydroxy or methoxy, i.e., X is H or CH_3 ("methoxy PEG"). It is noted that the other end of the PEG, which is shown in formula (I) terminating in OH, covalently attaches to an activating moiety via an ether oxygen bond, an amine linkage, or amide linkage. When used in a chemical structure, the term "PEG" includes the formula (I) above without the hydrogen of the hydroxyl group

shown, leaving the oxygen available to react with a free carbon atom of a linker to form an ether bond. More specifically, in order to conjugate PEG to a peptide, the peptide must be reacted with PEG in an "activated" form. Activated PEG can be represented by the formula:

(PEG)-(A) (II)

[00293] where PEG (defined supra) covalently attaches to a carbon atom of the activation moiety (A) to form an ether bond, an amine linkage, or amide linkage, and (A) contains a reactive group which can react with an amino, azido, alkyne, imino, maleimido, N-succinimidyl, carboxyl, aminooxy, seleno, or thiol group on an amino acid residue of a peptide or a linker moiety covalently attached to the peptide, e.g., the toxin peptide analog.

[00294] Techniques for the preparation of activated PEG and its conjugation to biologically active peptides are well known in the art. (E.g., see U.S. Pat. Nos. 5,643,575, 5,919,455, 5,932,462, and 5,990,237; Kinstler et al., N-terminally chemically modified protein compositions and methods, US Patent Nos. 5,985,265, and 5,824,784; Thompson et al., PEGylation of polypeptides, EP 0575545 B1; Petit, Site specific protein modification, US Patent Nos. 6,451,986, and 6,548,644; S. Herman et al., Poly(ethylene glycol) with reactive endgroups: I. Modification of proteins, J. Bioactive Compatible Polymers, 10:145-187 (1995); Y. Lu et al., Pegylated peptides III: Solid-phase synthesis with PEGylating reagents of varying molecular weight: synthesis of multiply PEGylated peptides, Reactive Polymers, 22:221-229 (1994); A.M. Felix et al., PEGylated Peptides IV: Enhanced biological activity of site-directed PEGylated GRF analogs, Int. J. Peptide Protein Res., 46:253-264 (1995); A.M. Felix, Site-specific poly(ethylene glycol)ylation of peptides, ACS Symposium Series 680(poly(ethylene glycol)): 218-238 (1997); Y. Ikeda et al., Polyethylene glycol derivatives, their modified peptides, methods for producing them and use of the modified peptides, EP 0473084 B1; G.E. Means et al., Selected techniques for the modification of

protein side chains, in: Chemical modification of proteins, Holden Day, Inc., 219 (1971)).

[00295] Activated PEG, such as PEG-aldehydes or PEG-aldehyde hydrates, can be chemically synthesized by known means or obtained from commercial sources, e.g., Shearwater Polymers, (Huntsville, Al) or Enzon, Inc. (Piscataway, N.J.).

[00296] An example of a useful activated PEG for purposes of the present invention is a PEG-aldehyde compound (e.g., a methoxy PEG-aldehyde), such as PEG-propionaldehyde, which is commercially available from Shearwater Polymers (Huntsville, Al). PEG-propionaldehyde is represented by the formula PEG-CH_2CH_2CHO. (See, e.g., U.S. Pat. No. 5,252,714). Also included within the meaning of "PEG aldehyde compound" are PEG aldehyde hydrates, e.g., PEG acetaldehyde hydrate and PEG bis aldehyde hydrate, which latter yields a bifunctionally activated structure. (See., e.g., Bentley et al., Poly(ethylene glycol) aldehyde hydrates and related polymers and applications in modifying amines, US Patent No. 5,990,237) (See., e.g., Bentley et al., Poly(ethylene glycol) aldehyde hydrates and related polymers and applications in modifying amines, US Patent No. 5,990,237). An activated multi-branched PEG-aldehyde compound can be used (PEG derivatives comprising multiple arms to give divalent, trivalent, tetravalent, octavalent constructs). Using a 4-arm PEG derivative four (4) toxin peptide analogs are attached to each PEG molecule. For example, in accordance with the present invention, the toxin peptide analog can be conjugated to a polyethylene glycol (PEG) at 1, 2, 3 or 4 amino functionalized sites of the PEG.

[00297] In being conjugated in accordance with the inventive method, the polyethylene glycol (PEG), as described herein, is covalently bound by reductive amination directly to at least one solvent-exposed free amine moiety of an amino acid residue of the toxin peptide analog itself. In some embodiments of the inventive method, the toxin peptide analog is conjugated to a PEG at one or more primary or secondary amines on the toxin peptide analog, or to two PEG groups at a single primary amine site on the toxin peptide analog (e.g., this can occur when the reductive amination reaction involves the presence of excess PEG-aldehyde

compound). We have observed that when PEGylation by reductive amination is at a primary amine on the peptide, it is not uncommon to have amounts (1 to 100% range) of reaction product that have two or more PEGs present per molecule, and if the desired PEGylation product is one with only one PEG per molecule, then this "over-PEGylation" may be undesirable. When PEGylated product with a single PEG per PEGylation product molecule is desired, an embodiment of the inventive method can be employed that involves PEGylation using secondary amines of the pharmacologically active peptide, because only one PEG group per molecule will be transferred in the reductive amination reaction.

[00298] Amino acid residues that can provide a primary amine moiety include residues of lysine, homolysine, ornithine, α, β-diaminopropionic acid (Dap), α, β-diaminopropionoic acid (Dpr), and α, γ-diaminobutyric acid (Dab), aminobutyric acid (Abu), and α-amino-isobutyric acid (Aib). The polypeptide N-terminus also provides a useful α-amino group for PEGylation. Amino acid residues that can provide a secondary amine moiety include ε-N-alkyl lysine, α-N-alkyl lysine, δ-N-alkyl ornithine, α-N-alkyl ornithine, or an N-terminal proline, where the alkyl is C_1 to C_6.

[00299] Another useful activated PEG for generating the PEGylated toxin peptide analogs of the present invention is a PEG-maleimide compound, such as, but not limited to, a methoxy PEG-maleimide, such as maleimido monomethoxy PEG, are particularly useful for generating the PEG-conjugated peptides of the invention. (E.g., Shen, *N*-maleimidyl polymer derivatives, US Patent No. 6,602,498; C. Delgado et al., The uses and properties of PEG-linked proteins., Crit. Rev. Therap. Drug Carrier Systems, 9:249-304 (1992); S. Zalipsky et al., Use of functionalized poly(ethylene glycol)s for modification of polypeptides, in: Poly(ethylene glycol) chemistry: Biotechnical and biomedical applications (J.M. Harris, Editor, Plenum Press: New York, 347-370 (1992); S. Herman et al., Poly(ethylene glycol) with reactive endgroups: I. Modification of proteins, J. Bioactive Compatible Polymers, 10:145-187 (1995); P.J. Shadle et al., Conjugation of polymer to colony stimulating factor-1, U.S. Patent No. 4,847,325; G. Shaw et al., Cysteine added variants IL-3 and chemical modifications thereof,

U.S. Patent No. 5,166,322 and EP 0469074 B1; G. Shaw et al., Cysteine added variants of EPO and chemical modifications thereof, EP 0668353 A1; G. Shaw et al., Cysteine added variants G-CSF and chemical modifications thereof, EP 0668354 A1; N.V. Katre et al., Interleukin-2 muteins and polymer conjugation thereof, U.S. Patent No. 5,206,344; R.J. Goodson and N.V. Katre, Site-directed pegylation of recombinant interleukin-2 at its glycosylation site, Biotechnology, 8:343-346 (1990)).

[00300] A poly(ethylene glycol) vinyl sulfone is another useful activated PEG for generating the PEG-conjugated toxin peptide analogs of the present invention by conjugation at thiolated amino acid residues, e.g., at C residues. (E.g., M. Morpurgo et al., Preparation and characterization of poly(ethylene glycol) vinyl sulfone, Bioconj. Chem., 7:363-368 (1996); see also Harris, Functionalization of polyethylene glycol for formation of active sulfone-terminated PEG derivatives for binding to proteins and biologically compatible materials, U.S. Patent Nos. 5,446,090; 5,739,208; 5,900,461; 6,610,281 and 6,894,025; and Harris, Water soluble active sulfones of poly(ethylene glycol), WO 95/13312 A1).

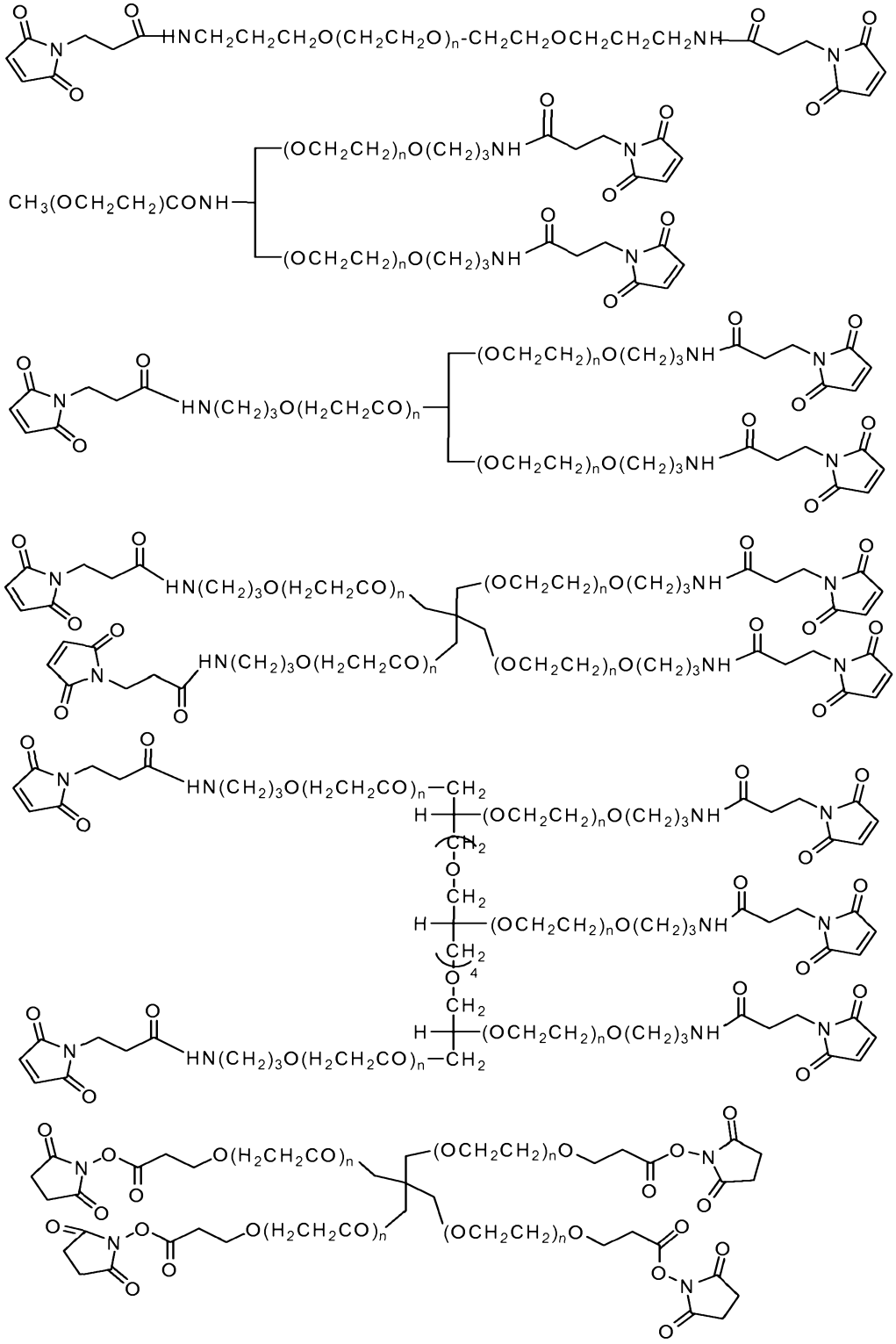
[00301] Another activated form of PEG that is useful in accordance with the present invention, is a PEG-N-hydroxysuccinimide ester compound, for example, methoxy PEG-N-hydroxysuccinimidyl (NHS) ester.

[00302] Heterobifunctionally activated forms of PEG are also useful. (See, e.g., Thompson et al., PEGylation reagents and biologically active compounds formed therewith, U.S. Patent No. 6,552,170).

[00303] In still other embodiments of the inventive method of producing a composition of matter, the toxin peptide analog is reacted by known chemical techniques with an activated PEG compound, such as but not limited to, a thiol-activated PEG compound, a diol-activated PEG compound, a PEG-hydrazide compound, a PEG-oxyamine compound, or a PEG-bromoacetyl compound. (See, e.g., S. Herman, Poly(ethylene glycol) with Reactive Endgroups: I. Modification of Proteins, J. Bioactive and Compatible Polymers, 10:145-187 (1995); S. Zalipsky, Chemistry of Polyethylene Glycol Conjugates with Biologically Active

Molecules, Advanced Drug Delivery Reviews, 16:157-182 (1995); R. Greenwald et al., Poly(ethylene glycol) conjugated drugs and prodrugs: a comprehensive review, Critical Reviews in Therapeutic Drug Carrier Systems, 17:101-161 (2000)).

[00304] An even more preferred activated PEG for generating the PEG-conjugated toxin peptide analogs of the present invention is a multivalent PEG having more than one activated residues. Preferred multivalent PEG moieties include, but are not limited to, those shown below:



[00305] In still other embodiments of making the composition of matter, the inventive toxin peptide analog is reacted by known chemical techniques with an activated multi-branched PEG compound (PEG derivatives comprising multiple arms to give divalent, trivalent, tetravalent, octavalent constructs), such as but not limited to, pentaerythritol tetra-polyethyleneglycol ether. Functionalization and activated derivatives , such as, but not limited to, N-succinimidyloxycarbonyl)propyl, p-nitrophenyloxycarbonyl, (—CO_2-p-$C_6H_4NO_2$), 3-(N-maleimido)propanamido, 2-sulfanylethyl, and 3-aminopropyl. Using a 4-arm PEG derivative, four toxin peptide analogs are attached to each PEG molecule. For example, in accordance with the present invention, the toxin peptide analog can be conjugated to a polyethylene glycol (PEG) at:

(a) 1, 2, 3 or 4 amino functionalized sites of the PEG;

(b) 1, 2, 3 or 4 thiol functionalized sites of the PEG;

(c) 1, 2, 3 or 4 maleimido functionalized sites of the PEG;

(d) 1, 2, 3 or 4 N-succinimidyl functionalized sites of the PEG;

(e) 1, 2, 3 or 4 carboxyl functionalized sites of the PEG; or

(f) 1, 2, 3 or 4 *p*-nitrophenyloxycarbonyl functionalized sites of the PEG.

[00306] The smallest practical size of PEG is about 500 Daltons (Da), below which PEG becomes toxic. Above about 500 Da, any molecular mass for a PEG can be used as practically desired, e.g., from about 1,000 Daltons (Da) to 100,000 Da (n is 20 to 2300). The number of PEG monomers (n) is approximated from the average molecular mass using a MW = 44 Da for each monomer. It is preferred that the combined molecular mass of PEG on an activated linker is suitable for pharmaceutical use. Thus, the combined molecular mass of the PEG molecule should not exceed about 100,000 Da.

[00307] In some embodiments, the combined or total average molecular mass of PEG used in a PEG-conjugated toxin peptide analog of the present invention is from about 3,000 Da to 60,000 Da (total n is from 70 to 1,400), more preferably from about 10,000 Da to 40,000 Da (total n is about 230 to about 910). The most preferred combined mass for PEG is from about 20,000 Da to 30,000 Da (total n is about 450 to about 680).

[00308] It will be appreciated that "multimers" of the composition of matter can be made, since the half-life extending moiety employed for conjugation to the toxin peptide analog (with or without an intervening linker moiety) can be multivalent (e.g., bivalent, trivalent, tetravalent or a higher order valency) as to the number of amino acid residues at which the half-life extending moiety can be conjugated. In some embodiments the peptide portion of the inventive composition of matter can be multivalent (e.g., bivalent, trivalent, tetravalent or a higher order valency), and, thus, some "multimers" of the inventive composition of matter may have more that one half life extending moiety. Consequently, it is possible by the inventive method of producing a composition of matter to produce a variety of conjugated half-life extending moiety:peptide structures. By way of example, a univalent half-life extending moiety and a univalent peptide will produce a 1:1 conjugate; a bivalent peptide and a univalent half-life extending moiety may form conjugates wherein the peptide conjugates bear two half-life extending moiety moieties, whereas a bivalent half-life extending moiety and a univalent peptide may produce species where two peptide entities are linked to a single half-life extending moiety; use of higher-valence half-life extending moiety can lead to the formation of clusters of peptide entities bound to a single half-life extending moiety, whereas higher-valence peptides may become encrusted with a plurality of half-life extending moiety moieties. By way of further example, if the site of conjugation of a multivalent half-life extending moiety to the toxin peptide analog is a cysteine or other aminothiol the methods disclosed by D'Amico et al. may be employed (D'Amico et al., Method of conjugating aminothiol containing molecules to vehicles, published as US 2006/0199812, which application is incorporated herein by reference in its entirety).

[00309] The peptide moieties may have more than one reactive group which will react with the activated half-life extending moiety and the possibility of forming complex structures must always be considered; when it is desired to form simple structures such as 1:1 adducts of half-life extending moiety and peptide, or to use bivalent half-life extending moiety to form peptide:half-life extending moiety:peptide adducts, it will be beneficial to use predetermined ratios

of activated half-life extending moiety and peptide material, predetermined concentrations thereof and to conduct the reaction under predetermined conditions (such as duration, temperature, pH, etc.) so as to form a proportion of the described product and then to separate the described product from the other reaction products. The reaction conditions, proportions and concentrations of the reagents can be obtained by relatively simple trial-and-error experiments which are within the ability of an ordinarily skilled artisan with appropriate scaling-up as necessary. Purification and separation of the products is similarly achieved by conventional techniques well known to those skilled in the art.

[00310] Additionally, physiologically acceptable salts of the half-life extending moiety-fused or conjugated to the toxin peptide analogs of this invention are also encompassed within the composition of matter of the present invention.

[00311] The above-described half-life extending moieties and other half-life extending moieties described herein are useful, either individually or in combination, and as further described in the art, for example, in Sullivan et al., Toxin Peptide Therapeutic Agents, US2007/0071764 and Sullivan et al., Toxin Peptide Therapeutic Agents, PCT/US2007/022831, published as WO 2008/088422, which are both incorporated herein by reference in their entireties. The invention encompasses the use of any single species of pharmaceutically acceptable half-life extending moiety, such as, but not limited to, those described herein, in conjugation with the toxin peptide analog, or the use of a combination of two or more like or different half-life extending moieties.

[00312] Linkers. A "linker moiety" as used herein refers to a biologically acceptable peptidyl or non-peptidyl organic group that is covalently bound to an amino acid residue of a toxin peptide analog or other polypeptide chain (e.g., an immunoglobulin HC or LC or immunoglobulin Fc domain) contained in the inventive composition, which linker moiety covalently joins or conjugates the toxin peptide analog or other polypeptide chain to another peptide or polypeptide chain in the composition, or to a half-life extending moiety. In some embodiments of the composition, a half-life extending moiety, as described

herein, is conjugated, i.e., covalently bound directly to an amino acid residue of the toxin peptide analog itself, or optionally, to a peptidyl or non-peptidyl linker moiety (including but not limited to aromatic or aryl linkers) that is covalently bound to an amino acid residue of the toxin peptide analog. The presence of any linker moiety is optional. When present, its chemical structure is not critical, since it serves primarily as a spacer to position, join, connect, or optimize presentation or position of one functional moiety in relation to one or more other functional moieties of a molecule of the inventive composition. The presence of a linker moiety can be useful in optimizing pharamcologial activity of some embodiments of the inventive composition. The linker is preferably made up of amino acids linked together by peptide bonds. The linker moiety, if present, can be independently the same or different from any other linker, or linkers, that may be present in the inventive composition.

[00313] As stated above, the linker moiety, if present (whether within the primary amino acid sequence of the toxin peptide analog, or as a linker for attaching a half-life extending moiety to the toxin peptide analog), can be "peptidyl" in nature (i.e., made up of amino acids linked together by peptide bonds) and made up in length, preferably, of from 1 up to about 40 amino acid residues, more preferably, of from 1 up to about 20 amino acid residues, and most preferably of from 1 to about 10 amino acid residues. Preferably, but not necessarily, the amino acid residues in the linker are from among the twenty canonical amino acids, more preferably, cysteine, glycine, alanine, proline, asparagine, glutamine, and /or serine. Even more preferably, a peptidyl linker is made up of a majority of amino acids that are sterically unhindered, such as glycine, serine, and alanine linked by a peptide bond. It is also desirable that, if present, a peptidyl linker be selected that avoids rapid proteolytic turnover in circulation *in vivo*. Some of these amino acids may be glycosylated, as is well understood by those in the art. For example, a useful linker sequence constituting a sialylation site is $X_1X_2NX_4X_5G$ (SEQ ID NO:279), wherein X_1, X_2,X_4 and X_5 are each independently any amino acid residue.

[00314] In other embodiments, the 1 to 40 amino acids of the peptidyl linker moiety are selected from glycine, alanine, proline, asparagine, glutamine, and lysine. Preferably, a linker is made up of a majority of amino acids that are sterically unhindered, such as glycine and alanine. Thus, preferred linkers include polyglycines, polyserines, and polyalanines, or combinations of any of these. Some exemplary peptidyl linkers are poly$(Gly)_{1-8}$, particularly $(Gly)_3$, $(Gly)_4$ (SEQ ID NO:280), $(Gly)_5$ (SEQ ID NO:281) and $(Gly)_7$ (SEQ ID NO:282), as well as, poly$(Gly)_4$Ser (SEQ ID NO:283), poly$(Gly\text{-}Ala)_{2-4}$ and poly$(Ala)_{1-8}$. Other specific examples of peptidyl linkers include $(Gly)_5$Lys (SEQ ID NO:284), and $(Gly)_5$LysArg (SEQ ID NO:285). Other examples of useful peptidyl linkers are: Other examples of useful peptidyl linkers are:

[00315] $(Gly)_3Lys(Gly)_4$ (SEQ ID NO:286);

[00316] $(Gly)_3AsnGlySer(Gly)_2$ (SEQ ID NO:287);

[00317] $(Gly)_3Cys(Gly)_4$ (SEQ ID NO:288); and

[00318] GlyProAsnGlyGly (SEQ ID NO:289).

[00319] To explain the above nomenclature, for example, $(Gly)_3Lys(Gly)_4$ means Gly-Gly-Gly-Lys-Gly-Gly-Gly-Gly (SEQ ID NO:290). Other combinations of Gly and Ala are also useful.

[00320] Other preferred linkers are those identified herein as "L5" (GGGGS; or "G_4S"; SEQ ID NO:291), "L10" (GGGGSGGGGS; SEQ ID NO:292), "L25" (GGGGSGGGGSGGGGSGGGGSGGGGS; SEQ ID NO:293) and any linkers used in the working examples hereinafter.

[00321] In some embodiments of the compositions of this invention, which comprise a peptide linker moiety, acidic residues, for example, glutamate or aspartate residues, are placed in the amino acid sequence of the linker moiety. Examples include the following peptide linker sequences:

[00322] GGEGGG (SEQ ID NO:294);

[00323] GGEEEGGG (SEQ ID NO:295);

[00324] GEEEG (SEQ ID NO:296);

[00325] GEEE (SEQ ID NO:297);

[00326] GGDGGG (SEQ ID NO:298);

[00327] GGDDDGG (SEQ ID NO:299);

[00328] GDDDG (SEQ ID NO:300);

[00329] GDDD (SEQ ID NO:301);

[00330] GGGGSDDSDEGSDGEDGGGGS (SEQ ID NO:302);

[00331] WEWEW (SEQ ID NO:303);

[00332] FEFEF (SEQ ID NO:304);

[00333] EEEWWW (SEQ ID NO:305);

[00334] EEEFFF (SEQ ID NO:306);

[00335] WWEEEWW (SEQ ID NO:307); or

[00336] FFEEEFF (SEQ ID NO:308).

[00337] In other embodiments, the linker constitutes a phosphorylation site, e.g., $X_1X_2YX_4X_5G$ (SEQ ID NO:309), wherein X_1, X_2, X_4, and X_5 are each independently any amino acid residue; $X_1X_2SX_4X_5G$ (SEQ ID NO:310), wherein X_1, X_2,X_4 and X_5 are each independently any amino acid residue; or $X_1X_2TX_4X_5G$ (SEQ ID NO:311), wherein X_1, X_2, X_4 and X_5 are each independently any amino acid residue.

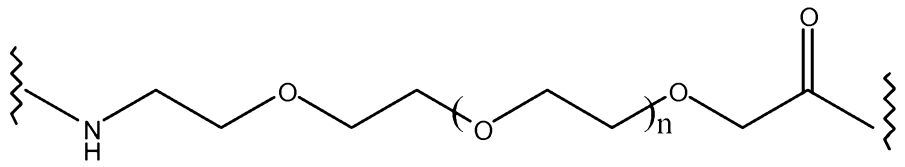
[00338] The linkers shown here are exemplary; peptidyl linkers within the scope of this invention may be much longer and may include other residues. A peptidyl linker can contain, e.g., a cysteine, another thiol, or nucleophile for conjugation with a half-life extending moiety. In another embodiment, the linker contains a cysteine or homocysteine residue, or other 2-amino-ethanethiol or 3-amino-propanethiol moiety for conjugation to maleimide, iodoacetaamide or thioester, functionalized half-life extending moiety.

Another useful peptidyl linker is a large, flexible linker comprising a random Gly/Ser/Thr sequence, for example: GSGSATGGSGSTASSGSGSATH (SEQ ID NO:312) or HGSGSATGGSGSTASSGSGSAT (SEQ ID NO:313), that is estimated to be about the size of a 1 kDa PEG molecule. Alternatively, a useful peptidyl linker may be comprised of amino acid sequences known in the art to form rigid helical structures (e.g., Rigid linker: -AEAAAKEAAAKEAAAKAGG- // SEQ ID NO:314). Additionally, a peptidyl linker can also comprise a non-peptidyl segment such as a 6 carbon aliphatic molecule of the formula $-CH_2-CH_2-$

CH_2-CH_2-CH_2-CH_2-. The peptidyl linkers can be altered to form derivatives as described herein.

[00339] Optionally, a non-peptidyl linker moiety is also useful for conjugating the half-life extending moiety to the peptide portion of the half-life extending moiety-conjugated toxin peptide analog. For example, alkyl linkers such as -NH-$(CH_2)_s$-C(O)-, wherein s = 2-20 can be used. These alkyl linkers may further be substituted by any non-sterically hindering group such as lower alkyl (e.g., C_1-C_6) lower acyl, halogen (e.g., Cl, Br), CN, NH_2, phenyl, *etc.* Exemplary non-peptidyl linkers are PEG linkers (e.g., shown below):

[00340] (III)



wherein n is such that the linker has a molecular weight of about 100 to about 5000 Daltons (Da), preferably about 100 to about 500 Da.

[00341] In one embodiment, the non-peptidyl linker is aryl. The linkers may be altered to form derivatives in the same manner as described herein. In addition, PEG moieties may be attached to the N-terminal amine or selected side chain amines by either reductive alkylation using PEG aldehydes or acylation using hydroxysuccinimido or carbonate esters of PEG, or by thiol conjugation.

[00342] "Aryl" is phenyl or phenyl vicinally-fused with a saturated, partially-saturated, or unsaturated 3-, 4-, or 5 membered carbon bridge, the phenyl or bridge being substituted by 0, 1, 2 or 3 substituents selected from C_{1-8} alkyl, C_{1-4} haloalkyl or halo.

[00343] "Heteroaryl" is an unsaturated 5 , 6 or 7 membered monocyclic or partially-saturated or unsaturated 6-, 7-, 8-, 9-, 10- or 11 membered bicyclic ring,

wherein at least one ring is unsaturated, the monocyclic and the bicyclic rings containing 1, 2, 3 or 4 atoms selected from N, O and S, wherein the ring is substituted by 0, 1, 2 or 3 substituents selected from C_{1-8} alkyl, C_{1-4} haloalkyl and halo.

[00344] Non-peptide portions of the inventive composition of matter, such as non-peptidyl linkers or non-peptide half-life extending moieties can be synthesized by conventional organic chemistry reactions.

[00345] The above is merely illustrative and not an exhaustive treatment of the kinds of linkers that can optionally be employed in accordance with the present invention.

[00346] Compositions of this invention incorporating peptide antagonists of the voltage-gated potassium channel Kv1.3, in particular toxin peptide analogs of the present invention, whether or not conjugated to a half-life extending moiety, are useful as immunosuppressive agents with therapeutic value for autoimmune diseases. For example, such molecules are useful in treating multiple sclerosis, type 1 diabetes, psoriasis, inflammatory bowel disease, and rheumatoid arthritis. (See, e.g., H. Wulff et al. (2003) J. Clin. Invest. 111, 1703-1713 and H. Rus et al. (2005) PNAS 102, 11094-11099; Beeton et al., Targeting effector memory T cells with a selective inhibitor peptide of Kv1.3 channnels for therapy of autoimmune diseases, Molec. Pharmacol. 67(4):1369-81 (2005);1 Beeton et al. (2006), Kv1.3: therapeutic target for cell-mediated autoimmune disease, electronic preprint at //webfiles.uci.edu/xythoswfs/webui/ 2670029.1). Inhibitors of the voltage-gated potassium channel Kv1.3 have been examined in a variety of preclinical animal models of inflammation. Small molecule and peptide inhibitors of Kv1.3 have been shown to block delayed type hypersensitivity responses to ovalbumin [C. Beeton et al. (2005) Mol. Pharmacol. 67, 1369] and tetanus toxoid [G.C. Koo et al. (1999) Clin. Immunol. 197, 99]. In addition to suppressing inflammation in the skin, inhibitors also reduced antibody production [G.C. Koo et al. (1997) J. Immunol. 158, 5120]. Kv1.3 antagonists have shown efficacy in a rat adoptive-transfer experimental autoimmune encephalomyelitis (AT-EAE) model of multiple sclerosis (MS), which model can be employed in assessing the

therapeutic efficacy of the inventive compositions of matter in practicing the inventive method of preventing or mitigating a relapse of a symptom of multiple sclerosis or method of treating an autoimmune disorder. (See, C. Beeton et al., J. Immunol. 166:936 (2001); C. Beeton et al., PNAS 98: 13942 (2001); Sullivan et al., Toxin Peptide Therapeutic Agents, US2007/0071764 , Figure 61, all of which are incorporated herin by reference in their entireties). The Kv1.3 channel is overexpressed on myelin-specific T cells from MS patients, lending further support to the utility Kv1.3 inhibitors may provide in treating MS. Inflammatory bone resorption was also suppressed by Kv1.3 inhibitors in a preclinical adoptive-transfer model of periodontal disease [P. Valverde et al. (2004) J. Bone Mineral Res. 19, 155]. In this study, inhibitors additionally blocked antibody production to a bacterial outer membrane protein, - one component of the bacteria used to induce gingival inflammation. Recently in preclinical rat models, efficacy of Kv1.3 inhibitors was shown in treating pristane-induced arthritis and diabetes [C. Beeton et al. (2006) preprint available at //webfiles.uci.edu/xythoswfs/webui/_xy-2670029_1.]. The Kv1.3 channel is expressed on all subsets of T cells and B cells, but effector memory T cells and class-switched memory B cells are particularly dependent on Kv1.3 [H. Wulff et al. (2004) J. Immunol. 173, 776]. Gad5 / insulin-specific T cells from patients with new onset type 1 diabetes, myelin-specific T cells from MS patients and T cells from the synovium of rheumatoid arthritis patients all overexpress Kv1.3 [C. Beeton et al. (2006) preprint at //webfiles.uci.edu/xythoswfs/webui/_xy-2670029_1.]. Because mice deficient in Kv1.3 gained less weight when placed on a high fat diet [J. Xu et al. (2003) Human Mol. Genet. 12, 551] and showed altered glucose utilization [J. Xu et al. (2004) Proc. Natl. Acad. Sci. 101, 3112], Kv1.3 is also being investigated for the treatment of obesity and diabetes. Breast cancer specimens [M. Abdul et al. (2003)Anticancer Res. 23, 3347] and prostate cancer cell lines [S.P. Fraser et al. (2003) Pflugers Arch. 446, 559] have also been shown to express Kv1.3, and Kv1.3 blockade may be of utility for treatment of cancer. Disorders that can be treated with the inventive compositions of matter, involving Kv1.3 inhibitor toxin peptide analog(s), include multiple sclerosis, type 1 diabetes, psoriasis,

inflammatory bowel disease, contact-mediated dermatitis, rheumatoid arthritis, psoriatic arthritis, asthma, allergy, restinosis, systemic sclerosis, fibrosis, scleroderma, glomerulonephritis, Sjogren syndrome, inflammatory bone resorption, transplant rejection, graft-versus-host disease, and systemic lupus erythematosus (SLE) and other forms of lupus.

[00347] The practice of the inventive method of treating an autoimmune disorder involves administering to a patient, e.g., one who has been diagnosed with an autoimmune disorder, such as, but not limited to, multiple sclerosis, type 1 diabetes, psoriasis, inflammatory bowel disease (IBD, including Crohn's Disease and ulcerative colitis), contact-mediated dermatitis, rheumatoid arthritis, psoriatic arthritis, asthma, allergy, restinosis, systemic sclerosis, fibrosis, scleroderma, glomerulonephritis, Sjogren syndrome, inflammatory bone resorption, transplant rejection, graft-versus-host disease, or lupus, a therapeutically effective amount of the inventive composition of matter, such that at least one symptom of the disorder is alleviated in the patient.

[00348] The practice of the inventive method of preventing or mitigating a relapse of a symptom of multiple sclerosis involves administering to a patient, e.g., one who has previously experienced at least one symptom of multiple sclerosis, a prophylactically effective amount of the inventive composition of matter, such that the at least one symptom of multiple sclerosis is prevented from recurring or is mitigated.

[00349] The diseases and pharmacologically active compositions described herein are merely exemplary and in no way limit the range of inventive pharmacologically active compounds, compositions, and medicaments that can be prepared using the inventive compositions of matter or the diseases and disorders that can be treated with the benefit of the present invention.

[00350] Accordingly, the present invention also relates to the use of one or more of the inventive compositions of matter in the manufacture of a medicament for the treatment or prevention of a disease, disorder, or other medical condition described herein.

[00351] Such pharmaceutical compositions can be configured for administration to a patient by a wide variety of delivery routes, e.g., an intravascular delivery route such as by injection or infusion, subcutaneous, intramuscular, intraperitoneal, epidural, or intrathecal delivery routes, or for oral, enteral, pulmonary (e.g., inhalant), intranasal, transmucosal (e.g., sublingual administration), transdermal or other delivery routes and/or forms of administration known in the art. The inventive pharmaceutical compositions may be prepared in liquid form, or may be in dried powder form, such as lyophilized form. For oral or enteral use, the pharmaceutical compositions can be configured, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, syrups, elixirs or enteral formulas.

[00352] Pharmaceutical Compositions

[00353] In General. The present invention also provides pharmaceutical compositions comprising the inventive composition of matter and a pharmaceutically acceptable carrier. Such pharmaceutical compositions can be configured for administration to a patient by a wide variety of delivery routes, e.g., an intravascular delivery route such as by injection or infusion, subcutaneous, intramuscular, intraperitoneal, epidural, or intrathecal delivery routes, or for oral, enteral, pulmonary (e.g., inhalant), intranasal, transmucosal (e.g., sublingual administration), transdermal or other delivery routes and/or forms of administration known in the art. The inventive pharmaceutical compositions may be prepared in liquid form, or may be in dried powder form, such as lyophilized form. For oral or enteral use, the pharmaceutical compositions can be configured, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, syrups, elixirs or enteral formulas.

[00354] In the practice of this invention the "pharmaceutically acceptable carrier" is any physiologically tolerated substance known to those of ordinary skill in the art useful in formulating pharmaceutical compositions, including, any pharmaceutically acceptable diluents, excipients, dispersants, binders, fillers,

glidants, anti-frictional agents, compression aids, tablet-disintegrating agents (disintegrants), suspending agents, lubricants, flavorants, odorants, sweeteners, permeation or penetration enhancers, preservatives, surfactants, solubilizers, emulsifiers, thickeners, adjuvants, dyes, coatings, encapsulating material(s), and/or other additives singly or in combination. Such pharmaceutical compositions can include diluents of various buffer content (*e.g.*, Tris-HCl, acetate, phosphate), pH and ionic strength; additives such as detergents and solubilizing agents (*e.g.*, Tween® 80, Polysorbate 80), anti-oxidants (*e.g.*, ascorbic acid, sodium metabisulfite), preservatives (*e.g.*, Thimersol®, benzyl alcohol) and bulking substances (*e.g.*, lactose, mannitol); incorporation of the material into particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, etc. or into liposomes. Hyaluronic acid can also be used, and this can have the effect of promoting sustained duration in the circulation. Such compositions can influence the physical state, stability, rate of in vivo release, and rate of in vivo clearance of the present proteins and derivatives. See, e.g., Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing Co., Easton, PA 18042) pages 1435-1712, which are herein incorporated by reference in their entirety. The compositions can be prepared in liquid form, or can be in dried powder, such as lyophilized form. Implantable sustained release formulations are also useful, as are transdermal or transmucosal formulations. Additionally (or alternatively), the present invention provides compositions for use in any of the various slow or sustained release formulations or microparticle formulations known to the skilled artisan, for example, sustained release microparticle formulations, which can be administered via pulmonary, intranasal, or subcutaneous delivery routes. (See, e.g., Murthy et al., Injectable compositions for the controlled delivery of pharmacologically active compound, U.S. Patent No.6,887,487; Manning et al., Solubilization of pharmaceutical substances in an organic solvent and preparation of pharmaceutical powders using the same, U.S. Patent Nos. 5,770,559 and 5,981,474; Lieberman et al., Lipophilic complexes of pharmacologically active inorganic mineral acid esters of organic compounds, U.S. Patent No. 5,002,936; Gen, Formative agent of protein complex, US

2002/0119946 A1; Goldenberg et al., Sustained release formulations, WO 2005/105057 A1).

[00355] One can dilute the inventive compositions or increase the volume of the pharmaceutical compositions of the invention with an inert material. Such diluents can include carbohydrates, especially, mannitol, α-lactose, anhydrous lactose, cellulose, sucrose, modified dextrans and starch. Certain inorganic salts may also be used as fillers, including calcium triphosphate, magnesium carbonate and sodium chloride. Some commercially available diluents are Fast-Flo, Emdex, STA-Rx 1500, Emcompress and Avicell.

[00356] A variety of conventional thickeners are useful in creams, ointments, suppository and gel configurations of the pharmaceutical composition, such as, but not limited to, alginate, xanthan gum, or petrolatum, may also be employed in such configurations of the pharmaceutical composition of the present invention. A permeation or penetration enhancer, such as polyethylene glycol monolaurate, dimethyl sulfoxide, N-vinyl-2-pyrrolidone, N-(2-hydroxyethyl)-pyrrolidone, or 3-hydroxy-N-methyl-2-pyrrolidone can also be employed. Useful techniques for producing hydrogel matrices are known. (E.g., Feijen, Biodegradable hydrogel matrices for the controlled release of pharmacologically active agents, U.S. Patent No. 4,925,677; Shah et al., Biodegradable pH/thermosensitive hydrogels for sustained delivery of biologically active agents, WO 00/38651 A1). Such biodegradable gel matrices can be formed, for example, by crosslinking a proteinaceous component and a polysaccharide or mucopolysaccharide component, then loading with the inventive composition of matter to be delivered.

[00357] Liquid pharmaceutical compositions of the present invention that are sterile solutions or suspensions can be administered to a patient by injection, for example, intramuscularly, intrathecally, epidurally, intravascularly (e.g., intravenously or intraarterially), intraperitoneally or subcutaneously. (See, e.g., Goldenberg et al., Suspensions for the sustained release of proteins, U.S. Patent No. 6,245,740 and WO 00/38652 A1). Sterile solutions can also be administered by intravenous infusion. The inventive composition can be included in a sterile

solid pharmaceutical composition, such as a lyophilized powder, which can be dissolved or suspended at a convenient time before administration to a patient using sterile water, saline, buffered saline or other appropriate sterile injectable medium.

[00358] Implantable sustained release formulations are also useful embodiments of the inventive pharmaceutical compositions. For example, the pharmaceutically acceptable carrier, being a biodegradable matrix implanted within the body or under the skin of a human or non-human vertebrate, can be a hydrogel similar to those described above. Alternatively, it may be formed from a poly-alpha-amino acid component. (Sidman, Biodegradable, implantable drug delivery device, and process for preparing and using same, U.S. Patent No. 4,351,337). Other techniques for making implants for delivery of drugs are also known and useful in accordance with the present invention.

[00359] In powder forms, the pharmaceutically acceptable carrier is a finely divided solid, which is in admixture with finely divided active ingredient(s), including the inventive composition. For example, in some embodiments, a powder form is useful when the pharmaceutical composition is configured as an inhalant. (See, e.g., Zeng et al., Method of preparing dry powder inhalation compositions, WO 2004/017918; Trunk et al., Salts of the CGRP antagonist BIBN4096 and inhalable powdered medicaments containing them, U.S. Patent No. 6,900,317).

[00360] One can dilute or increase the volume of the compound of the invention with an inert material. These diluents could include carbohydrates, especially mannitol, α-lactose, anhydrous lactose, cellulose, sucrose, modified dextrans and starch. Certain inorganic salts can also be used as fillers including calcium triphosphate, magnesium carbonate and sodium chloride. Some commercially available diluents are Fast-Flo™, Emdex™, STA-Rx™ 1500, Emcompress™ and Avicell™.

[00361] Disintegrants can be included in the formulation of the pharmaceutical composition into a solid dosage form. Materials used as disintegrants include but are not limited to starch including the commercial

disintegrant based on starch, Explotab™. Sodium starch glycolate, Amberlite™, sodium carboxymethylcellulose, ultramylopectin, sodium alginate, gelatin, orange peel, acid carboxymethyl cellulose, natural sponge and bentonite can all be used. Insoluble cationic exchange resin is another form of disintegrant. Powdered gums can be used as disintegrants and as binders and these can include powdered gums such as agar, Karaya or tragacanth. Alginic acid and its sodium salt are also useful as disintegrants.

[00362] Binders can be used to hold the therapeutic agent together to form a hard tablet and include materials from natural products such as acacia, tragacanth, starch and gelatin. Others include methyl cellulose (MC), ethyl cellulose (EC) and carboxymethyl cellulose (CMC). Polyvinyl pyrrolidone (PVP) and hydroxypropylmethyl cellulose (HPMC) could both be used in alcoholic solutions to granulate the therapeutic.

[00363] An antifrictional agent can be included in the formulation of the therapeutic to prevent sticking during the formulation process. Lubricants can be used as a layer between the therapeutic and the die wall, and these can include but are not limited to; stearic acid including its magnesium and calcium salts, polytetrafluoroethylene (PTFE), liquid paraffin, vegetable oils and waxes. Soluble lubricants can also be used such as sodium lauryl sulfate, magnesium lauryl sulfate, polyethylene glycol of various molecular weights, Carbowax 4000 and 6000.

[00364] Glidants that might improve the flow properties of the drug during formulation and to aid rearrangement during compression might be added. The glidants can include starch, talc, pyrogenic silica and hydrated silicoaluminate.

[00365] To aid dissolution of the compound of this invention into the aqueous environment a surfactant might be added as a wetting agent. Surfactants can include anionic detergents such as sodium lauryl sulfate, dioctyl sodium sulfosuccinate and dioctyl sodium sulfonate. Cationic detergents might be used and could include benzalkonium chloride or benzethonium chloride. The list of potential nonionic detergents that could be included in the formulation as surfactants are lauromacrogol 400, polyoxyl 40 stearate, polyoxyethylene

hydrogenated castor oil 10, 50 and 60, glycerol monostearate, polysorbate 40, 60, 65 and 80, sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose. These surfactants could be present in the formulation of the protein or derivative either alone or as a mixture in different ratios.

[00366] Oral dosage forms. Also useful are oral dosage forms of the inventive compositionss. If necessary, the composition can be chemically modified so that oral delivery is efficacious. Generally, the chemical modification contemplated is the attachment of at least one moiety to the molecule itself, where said moiety permits (a) inhibition of proteolysis; and (b) uptake into the blood stream from the stomach or intestine. Also desired is the increase in overall stability of the compound and increase in circulation time in the body. Moieties useful as covalently attached half-life extending moieties in this invention can also be used for this purpose. Examples of such moieties include: PEG, copolymers of ethylene glycol and propylene glycol, carboxymethyl cellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone and polyproline. See, for example, Abuchowski and Davis (1981), Soluble Polymer-Enzyme Adducts, Enzymes as Drugs (Hocenberg and Roberts, eds.), Wiley-Interscience, New York, NY, pp 367-83; Newmark, et al. (1982), J. Appl. Biochem. 4:185-9. Other polymers that could be used are poly-1,3-dioxolane and poly-1,3,6-tioxocane. Preferred for pharmaceutical usage, as indicated above, are PEG moieties.

[00367] For oral delivery dosage forms, it is also possible to use a salt of a modified aliphatic amino acid, such as sodium N-(8-[2-hydroxybenzoyl] amino) caprylate (SNAC), as a carrier to enhance absorption of the therapeutic compounds of this invention. The clinical efficacy of a heparin formulation using SNAC has been demonstrated in a Phase II trial conducted by Emisphere Technologies. See US Patent No. 5,792,451, "Oral drug delivery composition and methods."

[00368] In one embodiment, the pharmaceutically acceptable carrier can be a liquid and the pharmaceutical composition is prepared in the form of a solution, suspension, emulsion, syrup, elixir or pressurized composition. The active ingredient(s) (e.g., the inventive composition of matter) can be dissolved, diluted

or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both, or pharmaceutically acceptable oils or fats. The liquid carrier can contain other suitable pharmaceutical additives such as detergents and/or solubilizers (e.g., Tween 80, Polysorbate 80), emulsifiers, buffers at appropriate pH (e.g., Tris-HCl, acetate, phosphate), adjuvants, anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimersol, benzyl alcohol), sweeteners, flavoring agents, suspending agents, thickening agents, bulking substances (e.g., lactose, mannitol), colors, viscosity regulators, stabilizers, electrolytes, osmolutes or osmo-regulators. Additives can also be included in the formulation to enhance uptake of the inventive composition. Additives potentially having this property are for instance the fatty acids oleic acid, linoleic acid and linolenic acid.

[00369] Useful are oral solid dosage forms, which are described generally in Remington's Pharmaceutical Sciences (1990), supra, in Chapter 89, which is hereby incorporated by reference in its entirety. Solid dosage forms include tablets, capsules, pills, troches or lozenges, cachets or pellets. Also, liposomal or proteinoid encapsulation can be used to formulate the present compositions (as, for example, proteinoid microspheres reported in U.S. Patent No. 4,925,673). Liposomal encapsulation can be used and the liposomes can be derivatized with various polymers (e.g., U.S. Patent No. 5,013,556). A description of possible solid dosage forms for the therapeutic is given in Marshall, K., Modern Pharmaceutics (1979), edited by G. S. Banker and C. T. Rhodes, in Chapter 10, which is hereby incorporated by reference in its entirety. In general, the formulation will include the inventive compound, and inert ingredients that allow for protection against the stomach environment, and release of the biologically active material in the intestine.

[00370] The composition of this invention can be included in the formulation as fine multiparticulates in the form of granules or pellets of particle size about 1 mm. The formulation of the material for capsule administration could also be as a powder, lightly compressed plugs or even as tablets. The therapeutic could be prepared by compression.

[00371] Colorants and flavoring agents can all be included. For example, the protein (or derivative) can be formulated (such as by liposome or microsphere encapsulation) and then further contained within an edible product, such as a refrigerated beverage containing colorants and flavoring agents.

[00372] In tablet form, the active ingredient(s) are mixed with a pharmaceutically acceptable carrier having the necessary compression properties in suitable proportions and compacted in the shape and size desired.

[00373] The powders and tablets preferably contain up to 99% of the active ingredient(s). Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, polyvinylpyrrolidine, low melting waxes and ion exchange resins.

[00374] Controlled release formulation can be desirable. The composition of this invention can be incorporated into an inert matrix that permits release by either diffusion or leaching mechanisms e.g., gums. Slowly degenerating matrices can also be incorporated into the formulation, e.g., alginates, polysaccharides. Another form of a controlled release of the compositions of this invention is by a method based on the Oros™ therapeutic system (Alza Corp.), i.e., the drug is enclosed in a semipermeable membrane which allows water to enter and push drug out through a single small opening due to osmotic effects. Some enteric coatings also have a delayed release effect.

[00375] Other coatings can be used for the formulation. These include a variety of sugars that could be applied in a coating pan. The therapeutic agent could also be given in a film-coated tablet and the materials used in this instance are divided into 2 groups. The first are the nonenteric materials and include methylcellulose, ethyl cellulose, hydroxyethyl cellulose, methylhydroxy-ethyl cellulose, hydroxypropyl cellulose, hydroxypropyl-methyl cellulose, sodium carboxymethyl cellulose, providone and the polyethylene glycols. The second group consists of the enteric materials that are commonly esters of phthalic acid.

[00376] A mix of materials might be used to provide the optimum film coating. Film coating can be carried out in a pan coater or in a fluidized bed or by compression coating.

[00377] Pulmonary delivery forms. Pulmonary delivery of the inventive compositions is also useful. The protein (or derivative) is delivered to the lungs of a mammal while inhaling and traverses across the lung epithelial lining to the blood stream. (Other reports of this include Adjei et al., Pharma. Res. (1990) 7: 565-9; Adjei et al. (1990), Internatl. J. Pharmaceutics 63: 135-44 (leuprolide acetate); Braquet et al. (1989), J. Cardiovasc. Pharmacol. 13 (suppl.5): s.143-146 (endothelin-1); Hubbard et al. (1989), Annals Int. Med. 3: 206-12 (α1-antitrypsin); Smith et al. (1989), J. Clin. Invest. 84: 1145-6 (α1-proteinase); Oswein et al. (March 1990), "Aerosolization of Proteins," Proc. Symp. Resp. Drug Delivery II, Keystone, Colorado (recombinant human growth hormone); Debs et al. (1988), J. Immunol. 140: 3482-8 (interferon-γ and tumor necrosis factor α) and Platz et al., U.S. Patent No. 5,284,656 (granulocyte colony stimulating factor). Useful in the practice of this invention are a wide range of mechanical devices designed for pulmonary delivery of therapeutic products, including but not limited to nebulizers, metered dose inhalers, and powder inhalers, all of which are familiar to those skilled in the art. Some specific examples of commercially available devices suitable for the practice of this invention are the Ultravent nebulizer, manufactured by Mallinckrodt, Inc., St. Louis, Missouri; the Acorn II nebulizer, manufactured by Marquest Medical Products, Englewood, Colorado; the Ventolin metered dose inhaler, manufactured by Glaxo Inc., Research Triangle Park, North Carolina; and the Spinhaler powder inhaler, manufactured by Fisons Corp., Bedford, Massachusetts. (See, e.g., Helgesson et al., Inhalation device, U.S. Patent No. 6,892,728; McDerment et al., Dry powder inhaler, WO 02/11801 A1; Ohki et al., Inhalant medicator, U.S. Patent No. 6,273,086).

[00378] All such devices require the use of formulations suitable for the dispensing of the inventive compound. Typically, each formulation is specific to the type of device employed and can involve the use of an appropriate propellant material, in addition to diluents, adjuvants and/or carriers useful in therapy.

[00379] The inventive compound should most advantageously be prepared in particulate form with an average particle size of less than 10 μm (or microns), most preferably 0.5 to 5 μm, for most effective delivery to the distal lung.

[00380] Pharmaceutically acceptable excipients include carbohydrates such as trehalose, mannitol, xylitol, sucrose, lactose, and sorbitol. Other ingredients for use in formulations can include DPPC, DOPE, DSPC and DOPC. Natural or synthetic surfactants can be used. PEG can be used (even apart from its use in derivatizing the protein or analog). Dextrans, such as cyclodextran, can be used. Bile salts and other related enhancers can be used. Cellulose and cellulose derivatives can be used. Amino acids can be used, such as use in a buffer formulation.

[00381] Also, the use of liposomes, microcapsules or microspheres, inclusion complexes, or other types of carriers is contemplated.

[00382] Formulations suitable for use with a nebulizer, either jet or ultrasonic, will typically comprise the inventive compound dissolved in water at a concentration of about 0.1 to 25 mg of biologically active protein per mL of solution. The formulation can also include a buffer and a simple sugar (e.g., for protein stabilization and regulation of osmotic pressure). The nebulizer formulation can also contain a surfactant, to reduce or prevent surface induced aggregation of the protein caused by atomization of the solution in forming the aerosol.

[00383] Formulations for use with a metered-dose inhaler device will generally comprise a finely divided powder containing the inventive compound suspended in a propellant with the aid of a surfactant. The propellant can be any conventional material employed for this purpose, such as a chlorofluorocarbon, a hydrochlorofluorocarbon, a hydrofluorocarbon, or a hydrocarbon, including trichlorofluoromethane, dichlorodifluoromethane, dichlorotetrafluoroethanol, and 1,1,1,2-tetrafluoroethane, or combinations thereof. Suitable surfactants include sorbitan trioleate and soya lecithin. Oleic acid can also be useful as a surfactant. (See, e.g., Bäckström et al., Aerosol drug formulations containing hydrofluoroalkanes and alkyl saccharides, U.S. Patent No. 6,932,962).

[00384] Formulations for dispensing from a powder inhaler device will comprise a finely divided dry powder containing the inventive compound and can also include a bulking agent, such as lactose, sorbitol, sucrose, mannitol,

trehalose, or xylitol in amounts which facilitate dispersal of the powder from the device, e.g., 50 to 90% by weight of the formulation.

[00385] Nasal delivery forms. In accordance with the present invention, intranasal delivery of the inventive composition of matter and/or pharmaceutical compositions is also useful, which allows passage thereof to the blood stream directly after administration to the inside of the nose, without the necessity for deposition of the product in the lung. Formulations suitable for intransal administration include those with dextran or cyclodextran, and intranasal delivery devices are known. (See, e.g, Freezer, Inhaler, U.S. Patent No. 4,083,368).

[00386] Transdermal and transmucosal (e.g., buccal) delivery forms). In some embodiments, the inventive composition is configured as a part of a pharmaceutically acceptable transdermal or transmucosal patch or a troche. Transdermal patch drug delivery systems, for example, matrix type transdermal patches, are known and useful for practicing some embodiments of the present pharmaceutical compositions. (E.g., Chien et al., Transdermal estrogen/progestin dosage unit, system and process, U.S. Patent Nos. 4,906,169 and 5,023,084; Cleary et al., Diffusion matrix for transdermal drug administration and transdermal drug delivery devices including same, U.S. Patent No. 4,911,916; Teillaud et al., EVA-based transdermal matrix system for the administration of an estrogen and/or a progestogen, U.S. Patent No. 5.605,702; Venkateshwaran et al., Transdermal drug delivery matrix for coadministering estradiol and another steroid, U.S. Patent No. 5,783,208; Ebert et al., Methods for providing testosterone and optionally estrogen replacement therapy to women, U.S. Patent No. 5,460,820). A variety of pharmaceutically acceptable systems for transmucosal delivery of therapeutic agents are also known in the art and are compatible with the practice of the present invention. (E.g., Heiber et al., Transmucosal delivery of macromolecular drugs, U.S. Patent Nos. 5,346,701 and 5,516,523; Longenecker et al., Transmembrane formulations for drug administration, U.S. Patent No. 4,994,439).

[00387] Buccal delivery of the inventive compositions is also useful. Buccal delivery formulations are known in the art for use with peptides. For

example, known tablet or patch systems configured for drug delivery through the oral mucosa (e.g., sublingual mucosa), include some embodiments that comprise an inner layer containing the drug, a permeation enhancer, such as a bile salt or fusidate, and a hydrophilic polymer, such as hydroxypropyl cellulose, hydroxypropyl methylcellulose, hydroxyethyl cellulose, dextran, pectin, polyvinyl pyrrolidone, starch, gelatin, or any number of other polymers known to be useful for this purpose. This inner layer can have one surface adapted to contact and adhere to the moist mucosal tissue of the oral cavity and can have an opposing surface adhering to an overlying non-adhesive inert layer. Optionally, such a transmucosal delivery system can be in the form of a bilayer tablet, in which the inner layer also contains additional binding agents, flavoring agents, or fillers. Some useful systems employ a non-ionic detergent along with a permeation enhancer. Transmucosal delivery devices may be in free form, such as a cream, gel, or ointment, or may comprise a determinate form such as a tablet, patch or troche. For example, delivery of the inventive composition can be via a transmucosal delivery system comprising a laminated composite of, for example, an adhesive layer, a backing layer, a permeable membrane defining a reservoir containing the inventive composition, a peel seal disc underlying the membrane, one or more heat seals, and a removable release liner. (E.g., Ebert et al., Transdermal delivery system with adhesive overlay and peel seal disc, U.S. Patent No. 5,662,925; Chang et al., Device for administering an active agent to the skin or mucosa, U.S. Patent Nos. 4,849,224 and 4,983,395). These examples are merely illustrative of available transmucosal drug delivery technology and are not limiting of the present invention.

[00388] Dosages. The dosage regimen involved in a method for treating the above-described conditions will be determined by the attending physician, considering various factors which modify the action of drugs, e.g. the age, condition, body weight, sex and diet of the patient, the severity of any infection, time of administration and other clinical factors. Generally, the daily regimen should be in the range of 0.1-1000 micrograms of the inventive compound per kilogram of body weight, preferably 0.1-150 micrograms per kilogram.

[00389] The following working examples are illustrative and not to be construed in any way as limiting the scope of the present invention.

EXAMPLES

Example 1

Kv1.3 and Kv1.1 Electrophysiology

[00390] Cell lines expressing Kv1.1 through Kv1.7. CHO-K1 cells were stably transfected with human Kv1.3, or for counterscreens (see, Example 6), with hKv1.4, hKv1.6, or hKv1.7; HEK293 cells were stably expressing human $K_V1.3$ or with human Kv1.1. Cell lines were from Amgen or BioFocus DPI (A Galapagos Company). CHO K1 cells stably expressing hKv1.2, for counterscreens, were purchased from Millipore (Cat#.CYL3015).

[00391] Whole cell patch clamp electrophysiology. Whole-cell currents were recorded at room temperature using MultiClamp 700B amplifier from Molecular Devices Corp. (Sunnyvale, CA), with 3-5MΩ pipettes pulled from borosilicate glass (World Precision Instruments, Inc). During data acquisition, capacitive currents were canceled by analogue subtraction, no series resistance compensation was used, and all currents were filtered at 2 kHz. The cells were bathed in an extracellular solution containing 1.8 mM $CaCl_2$, 5 mM KCl, 135 mM NaCl, 5 mM Glucose, 10 mM HEPES, pH 7.4, 290-300 mOsm. The internal solution containing 90 mM KCl, 40 mM KF, 10 mM NaCl, 1 mM $MgCl_2$, 10 mM EGTA, 10 mM HEPES, pH 7.2, 290-300 mOsm. The currents were evoked by applying depolarizing voltage steps from -80 mV to +30 mV every 30 s (Kv1.3) or 10 s (Kv1.1) for 200 ms intervals at holding potential of -80 mV. To determine IC50, 5-6 peptide or peptide conjugate concentration at 1:3 dilutions were made in extracellular solution with 0.1 % BSA and delivered locally to cells with Rapid Solution Changer RSc-160 (BioLogic Science Instruments). Currents were achieved to steady state for each concentration. Data analysis was performed using pCLAMP (version 9.2) and OriginPro (version 7), and peak

currents before and after each test article application were used to calculate the percentage of current inhibition at each concentration.

[00392] PatchXpress®, planar patch-clamp electrophysiology. Cells were bathed in an extracellular solution containing 1.8 mM $CaCl_2$, 5 mM KCl, 135 mM NaCl, 5 mM Glucose, 10 mM HEPES, pH 7.4, 290-300 mOsm. The internal solution contained 90 mM KCl, 40 mM KF, 10 mM NaCl, 1 mM $MgCl_2$, 10 mM EGTA, 10 mM HEPES, pH 7.2, 290-300 mOsm. Usually 5 peptide or peptide conjugate concentrations at 1:3 dilutions are made to determine the IC50s. The peptide or peptide conjugates are prepared in extracellular solution containing 0.1% BSA. Dendrotoxin-k and Margatoxin were purchased from Alomone Labs Ltd. (Jerusalem, Israel); ShK toxin was purchased from Bachem Bioscience, Inc. (King of Prussia, PA); 4-AP was purchased from Sigma-Aldrich Corp. (St. Louis, MO). Currents were recorded at room temperature using a PatchXpress® 7000A electrophysiology system from Molecular Devices Corp. (Sunnyvale, CA). The voltage protocols for hKv1.3 and hKv1.1 are shown in Table 4E in Example 6 below. An extracellular solution with 0.1% BSA was applied first to obtain 100% percent of control (POC), then followed by 5 different concentrations of 1:3 peptide or peptide conjugate dilutions for every 400 ms incubation time. At the end, excess of a specific benchmark ion channel inhibitor (Table 4E in Example 6) was added to define full or 100% blockage. The residual current present after addition of benchmark inhibitor, was used in some cases for calculation of zero percent of control. The benchmark inhibitors for Kv1.3 and Kv1.1 are described in Table 4E in Example 6. Each individual set of traces or trial were visually inspected and either accepted or rejected. The general criteria for acceptance were:

[00393] 1. Baseline current must be stable

[00394] 2. Initial peak current must be >300 pA

[00395] 3. Intitial Rm and final Rm must >300 Ohm

[00396] 4. Peak current must achieve a steady-state prior to first compound addition.

[00397] The POC was calculated from the average peak current of the last 5 sweeps before the next concentration compound addition and exported to Excel for IC50 calculation.

[00398] IonWorks, high-throughput, planar patch-clamp electrophysiology. Electrophysiology was performed on CHO cells stably expressing hKv1.3 and HEK293 cells stably expressing hKv1.1. The procedure for preparation of the "Assay Plate" containing ShK analogues and conjugates for IWQ electrophysiology was as follows: all analogues were dissolved in extracellular buffer (PBS, with 0.9 mM Ca^{2+} and 0.5 mM Mg^{2+}) with 0.3% BSA and dispensed in the row H of 96-well polypropylene plates at the concentration of 100 nM from column 1 to column 10. Column 11 and 12 were reserved for negative and positive controls, then serial diluted at 1:3 ratio to row A. IonWorks Quattro (IWQ) electrophysiology and data analysis were accomplished as follows: re-suspended cells (in extracellular buffer), the Assay Plate, a Population Patch Clamp (PPC) PatchPlate as well as appropriate intracellular (90 mM potassium gluconate, 20 mM KF, 2 mM NaCl, 1 mM $MgCl_2$, 10 mM EGTA, 10 mM HEPES, pH 7.35) and extracellular buffers were positioned on IonWorks Quattro. When the analogues were added to patch plates, they were further diluted 3-fold from the assay plate to achieve a final test concentration range from 33.3 nM to 15 pM with 0.1% BSA. Electrophysiology recordings were made from the CHO-Kv1.3 and HEK-Kv1.1 cells using an amphotericin-based perforated patch-clamp method. Using the voltage-clamp circuitry of the IonWorks Quattro, cells were held at a membrane potential of –80 mV and voltage-activated K+ currents were evoked by stepping the membrane potential to +30 mV for 400 ms. K+ currents were evoked under control conditions i.e., in the absence of inhibitor at the beginning of the experiment and after 10-minute incubation in the presence of the analogues and controls. The mean K+ current amplitude was measured between 430 and 440ms and the data were exported to a Microsoft Excel spreadsheet. The amplitude of the K+ current in the presence of each concentration of the analogues and controls was expressed as a percentage of the K+ current of the pre-compound current amplitude in the same well. When these % of control values were plotted

as a function of concentration, the IC50 value for each compound could be calculated using the dose-response fit model 201 in Excel fit program which utilizes the following equation:

$$\% \text{ of control} = y_{min} + \left(\frac{y_{max} - y_{min}}{1 + \left(\frac{conc.}{IC_{50}} \right)^{n}} \right)$$

where ymin is the minimum y-value of the curve, ymax is the maximum y-value of the curve, conc. is the test concentration and n is the Hill slope of the curve.

Example 2

Measuring Bioactivity in Human Whole Blood

[00399] Ex vivo assay to examine impact of toxin peptide analog Kv1.3 inhibitors on secretion of IL-2 and IFN-g. The potency of ShK analogs and conjugates in blocking T cell inflammation in human whole blood was examined using an ex vivo assay that has been described earlier (see Example 46 of WO 2008/088422 A2, incorporated herein by reference in its entirety). In brief, 50% human whole blood is stimulated with thapsigargin to induce store depletion, calcium mobilization and cytokine secretion. To assess the potency of molecules in blocking T cell cytokine secretion, various concentrations of Kv1.3 blocking peptides and peptide-conjugates were pre-incubated with the human whole blood sample for 30-60 min prior to addition of the thapsigargin stimulus. After 48 hours at 37°C, 5% CO_2, conditioned medium was collected and the level of cytokine secretion was determined using a 4-spot electrochemilluminescent immunoassay from MesoScale Discovery. Using the thapsigargin stimulus, the cytokines IL-2 and IFN-g were secreted robustly from blood isolated from multiple donors. The IL-2 and IFN-g produced in human whole blood following thapsigargin stimulation were produced from T cells, as revealed by intracellular cytokine staining and fluorescence-activated cell sorting (FACS) analysis.

[00400] Kv1.3 is the major voltage-gated potassium channel present on T cells. Allowing for K^+ efflux, Kv1.3 provides the driving force for continued Ca^{2+}

influx which is necessary for the sustained elevation in intracellular calcium needed for efficient T cell activation and cytokine secretion. Kv1.3 inhibitors have been shown earlier to suppress this calcium flux induced by TCR ligation (G.C. Koo et al., 1999, Cell. Immunol. 197, 99-107). Thapsigargin-induced store-depletion and TCR ligation elicits similar patterns of Ca^{2+} mobilization in isolated T cells (E. Donnadieu et al., 1991, J. Biol. Chem. 267, 25864-25872), but we have found thapsigargin gives a more robust response in whole blood. Therefore, we developed a bioassay whereby the bioactivity of Kv1.3 inhibitors is assessed by examining their ability to block thapsigargin-induced cytokine secretion from T cells in human whole blood. Since whole blood is a complex fluid containing high protein levels, the activity of peptides and peptide conjugates in this whole blood assay has an additional advantage in assessing the molecules stability over 48 hours in a biologically relevant fluid. The whole blood assay provides important confirmation of the Kv1.3 potency of molecules determined by electrophysiology (ePhys), since ePhys assays are generally of short duration (<1-2 hours) and use physiological saline containing no protein. The longer duration of the whole blood assay may allow for more effective determination of equilibrium binding kinetics relative to ePhys studies which are of short duration.

[00401] For example, ShK-Dap22 (SEQ ID NO:317) is a Lys22 analog of ShK, reported earlier to have improved Kv1.3 versus Kv1.1 selectivity (Kalman et al., ShK-Dap22, a potent Kv1.3-specific immunosuppressive polypeptide, J. Biol. Chem. 273(49):32697-707 (1998)). The ShK-Dap22 molecule was reported to have similar potency to native ShK and to provide potent blockade of Kv1.3 with an IC50 of about 23 pM as measured by whole cell patch clamp electrophysiology (K. Kalman et al., 1998, ibid). We also found by whole cell patch clamp electrophysiology that ShK-Dap22 (purchased from Bachem) potently blocks Kv1.3 with an IC50 of about 12 pM (see, Example 3, Example 5, and Table 4E, below), but find it is about 300 times less potent in blocking IL-2 production from human whole blood (IC50 of about 3763 pM). In contrast, native ShK is about equipotent in blocking Kv1.3 and IL-2 whole blood responses. Consistent with our whole blood findings that ShK-Dap22 has reduced potency, R.E. Middleton et

al. (Biochemistry 42, 13698-13707, 2004) report by radioligand binding competition that ShK-Dap22 has a about 700-fold weaker equilibrium binding affinity for Kv1.3 compared to native ShK. Therefore, it appears that patch-clamp electrophysiology alone sometimes fails to reveal a toxin peptide analog's true potency or affinity, and alternative methods should be employed to decipher whether any significant changes have occurred in the toxin peptide analog's equilibrium binding affinity.

[00402] For this reason, we developed a parallel screening approach that tested all toxin peptide analogs and conjugates for potency in two distinct assays: (a) an electrophysiology assay to measure impact on Kv1.3 or Kv1.1, and (b) the 48 hour human whole blood assay described herein, which we believe measures equilibrium binding. Using the later assay, a toxin peptide analog PEGylated at its N-terminus with linear PEG, 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16; see, Example 4, below) was found to provide potent blockade of whole blood IL-2 secretion with an IC50 of 0.092 nM (n= 14) and an IC95 of 1.013 nM (n=14). The bioactivity of toxin peptide analogs and conjugates in the whole blood assay of inflammation is provided in other examples and tables disclosed herein.

Example 3

Toxin Peptide Analogs

[00403] All ShK amino acid residue positions except Cys residues were each substituted at a single position with either an Ala, an Arg, a Lys, a Glu, or a 1-Nal residue. Conventional solid phase synthetic methods were employed to make and purify the toxin peptides and toxin peptide analogs. (See, e.g., Example 4 hereunder). Kv1.3 and Kv1.1 activity were measured using electrophysiology (IWQ) and and an ex vivo whole blood assay (WB) to examine impact of Kv1.3 inhibitor toxin peptide analogs on secretion of IL-2 and IFN-γ. Electrophysiology (IWQ) data and human whole blood (WB) functional assay data were collected as described in Example 1 and Example 2 and were analyzed. Electrophysiology and whole blood assay results for the Ala, Arg, Lys, Glu, or 1-Nal scans are described in Tables 6-10 below. We found in the IWQ and WB assays that single residue changes of specific ShK residues, in addition to the previously identified position 22, can uniquely modify the Kv1.3 inhibition potency and/or Kv1.3 selectivity (i.e., inhibition of Kv1.3 versus Kv1.1) of the toxin peptide analogs. General conclusions are represented in Figure 3, which are supported by (a)-(c) below.

[00404] (a) Certain single substitution toxin peptide analogs at various positions in relation to SEQ ID NO:4 were found to significantly reduce Kv1.3 inhibition activity (i.e., IC50 increased) compared to the native ShK sequence (SEQ ID NO:1). These included the following positions, with the amino acid residues that reduced Kv1.3 activity in parentheses: Asp5 (K,R,E, Nal, [D5A not done]), Ile7(K,R,E, Nal), Arg11 (A,E, Nal), Ser20(R,E, Nal), Met21(K,R,E, Nal), Lys22(R,E, Nal), Tyr23(R,E, Nal, [Y23K not done]), Phe27(K,R,A, Nal, [F27E not done]). These ShK residues (D5, I7, R11, S20, M21, K22, Y23, F27) can be considered key binding sites for Kv1.3 and were found to cluster to a single face of the 3-dimensional solution structure of ShK (Figure 1C).

[00405] (b) Certain single substitution toxin peptide analogs at various positions in relation to SEQ ID NO:4 were found to improve Kv1.3 inhibition activity (i.e., IC50 decreased) compared to the native ShK sequence (SEQ ID

NO:1). These included the following positions, with the amino acid residues that improved Kv1.3 activity in parentheses: Ser2(E), Ile4(K,E,A), Ser10(R,E), Phe15(A), Lys18(R,A), Lys30(R,E), Thr31(Nal), Thr34(Nal).

[00406] (c) Certain single substitution toxin peptide analogs at various positions in relation to SEQ ID NO:4 were found to improve selectivity for Kv1.3 (versus Kv1.1; i.e. IC50 Kv1.1 / IC50 Kv1.3 increased) compared to the native ShK sequence (SEQ ID NO:1), while having no substantial effect on Kv1.3 inhibition activity. These included the following positions, with the amino acid residues that improved selectivity for Kv1.3 in parentheses: Ile7(K), Ser10(A), Gln16(K, Nal), Ser20(K,R), Lys22(A), Tyr23(A, [Y23K not done]), Ser26(Nal), Phe27(Nal [F27E not done]), Arg29(K, Nal). Analysis of the atomic structure of ShK revealed that several key residues improving Kv1.3 selectivity upon analoging, were located at the periphery and surrounding those ShK residues critical for Kv1.3 binding, described above in part (a). These residues (I7, S10, Q16, S20, S26, R29) that, when converted to an analog, result in improved Kv1.3 selectivity, are light shaded in Figure 1D which shows a space filling stereo model of the NMR structure of ShK. Analogs of some residues critical for Kv1.3 binding, also improve selectivity, such as Ile7(K), Lys22(A), Tyr23(A) and Phe27(1-Nal). However for several of these analogs, this gain in selectivity is sometimes accompanied by an unacceptably large loss in potency that makes them undesirable.

[00407] The data indicated that amino acid residues at positions 8, 13, and 33, relative to SEQ ID NO:4 can be substituted with the scanning residues (Arg, Lys, Ala, 1-Nal, and Glu) without a major loss in Kv1.3 inhibition potency.

[00408] The same cannot be said for residue Asp5, where Arg, Glu, 1-Nal, and lysine substitutions were not well tolerated. We observed a major loss of Kv1.3 inhibition activity. (Substitution of Ala at position 5 has yet to be assayed since this molecule is very difficult to prepare.) Position 5 is quite sensitive, even the change from Asp (short side chain acidic) to Glu (long side chain acidic residue) was not well tolerated. From the entire multipass ShK scan, we found the most intolerant residues of substitutions were Asp5 (most intolerant), Ser20,

Lys22 and Tyr23. ShK residues Lys22 and Tyr23 have been reported earlier as important binding sites for Kv1.3. Whereas Ala analogs of Lys22 and Tyr23 showed reduced activity, all other analogs of these residues were inactive. The Lys22(A) and Tyr23(A) analogs were 7-10 times less active against Kv1.3 and 35-56 times less active in the human whole blood assay of T cell inflammation compared to native ShK. The ShK-Dap22 analog reported earlier to have improved Kv1.3 selectivity (K. Kalman et al., 1998, ibid), was 56 times less active in this whole blood assay. Therefore, despite the slight improvement in Kv1.3 selectivity conferred by Ala and diaminoproprionic acid (Dap) analogs of Lys22 of ShK, the large reduction in potency negated much of the benefit of the slight selectivity improvement.

[00409] We confirmed that, in accordance with literature observations, alanine substitution at position 16 was not found to confer selectivity. (See, e.g., Kem et al., ShK toxin compositions and methods of use, US Patent No. 6,077,680). However, when position 16 was changed from the Gln residue in the native ShK sequence (SEQ ID NO:1) to a basic lysine residue, high potency and very good selectivity were obtained (Table 5 and Table 10). Interestingly, the same result was not obtained with arginine (Table 6), another basic residue, but compared to lysine the Arg side chain is nearly 1 Å longer. Furthermore, through its guanido functionality, arginine imparts a bulky planar configuration to the positive charge compared with the pyramidal configuration in the primary amine. Good Kv1.3 selectivity was also observed with the introduction of a large bulky neutral group, such as 1-Naphthylalanine (1-Nal), at position 16 (Table 8). However, the 1-Nal modification at position 16 did reduce the potency of the molecule for Kv1.3 inhibition (>5-fold reduction) and in the whole blood assay (about 325-500-fold reduction in activity). Kv1.3 selectivity was also observed from the introduction of 1-Nal at positions 26 or 27 of ShK, although again like with 1-Nal at position 16, these substitutions also led to a reduction in potency in the whole blood assay.

[00410] Another observation we made based on the data in Table 11 (Position 16 toxin peptide analogs), was that improved Kv1.3 selectivity was

obtained, without a substantial change in Kv1.3 activity measured by IWQ or WB assays, by changing the polar by neutral Gln side chain in the native ShK sequence to a long basic residue (e.g., Lys). Selectivity for Kv1.3 (compared to Kv1.1) was gradually lost as the side chain was shortened by 1 methylene group at a time (Lys>Orn>Dab>Dap). Interestingly, a His16 substitution (basic, aryl residue) failed to yield a selective molecule. Improved Kv1.3 selectivity over Kv1.1 was obtained by by changing the polar by neutral Gln side chain to a large aromatic (aryl) residue, Ahp. Other toxin peptide analogs such as [Ala23]ShK and [Arg30]ShK also conferred Kv1.3 selectivity, albeit with significantly less potency of inhibition at Kv1.3.

[00411] In the preparation of the toxin peptide analog molecules a +16 Da side product was observed during the synthesis, folding, and lyophilization processes. Given that the ShK peptide contains a single methionine residue at position 21 (see SEQ ID NO:1) and this sulfur containing residue is well-documented to oxidize to its Met[O] form (+16 Da) it was desirable to replace the residue at position 21 relative to SEQ ID NO:4 with another non-oxidizable residue. A well known isosteric substitute for Met is norleucine (Nle), where the isoteric side chain group consists of the same number of atoms but differs by the substitution of the sulfur atom (present in the Met residue) with a methylene (-CH_2-) unit in the Nle residue. The incorporation of Nle was reasonably well tolerated at position 21, although Kv1.3 inhibition potency was slightly reduced (Table 17). Position scans at residue 21 showed that this position affects Kv1.3 potency and selectivity. Aside from Nle subsitution, Met[O], Asn, Gln, Tyr, Val, Leu, Abu, Chg, Phe and Nva were reasonably well tolerated as determined by IWQ assay. Of these changes Asn, Tyr, Val, Leu, Abu, Chg, Phe and Nva still resulted in good selectivity for Kv1.3 over Kv1.1. In the whole blood assay, [Lys16, Nle21]ShK-Ala (SEQ ID NO:236), [Lys16, Met(O)21]ShK-amide (SEQ ID NO:257), and [Lys16, Nva21]ShK-amide (SEQ ID NO:260) showed more potency with IL-2 response. (Table 15 and Table 17).

[00412] To explore more ShK structure-activity and also avoid a C-terminal cysteinyl carboxylate, we sought to identify more appropriate C-terminal

functionality. C-terminal cysteinyl carboxylate may increase chemical synthetic difficulties related to their preparation. We examined C-terminal amidation which involves the changing of the C-terminus from a free carboxylate to a carboxamide (Table 5 and Table 14). Changing [Lys16]ShK (SEQ ID NO:13) to [Lys16]ShK-amide (SEQ ID NO:14) resulted in a molecule with roughly equivalent activity in blocking Kv1.3 (IWQ IC50 = 0.2 nM) and WB IL-2 secretion (IC50 = 0.1 nM). However, combination of the Lys16, Nle 21 double subtitutions with C-terminal amidation resulted in a molecule (SEQ ID NO:15) with improved Kv1.3 selectivity (over Kv1.1), that despite being equipotent in blocking Kv1.3 (IWQ IC50 = 0.15 nM) had about 7-fold less activity in the WB IL-2 assay of inflammation (IC50 = 0.82 nM) relative to [Lys16]ShK (SEQ ID NO:13; IC50 = 0.11) (See Table 5 and Table 14). Interestingly, [His16; Nle21]ShK-amide (SEQ ID NO:157; Table 11), which substitutes Lys16 with the basic and larger histidine residue at residue 16, is potent at Kv1.3 but does not show selectivity for Kv1.3 (IWQ IC50 = 139 pM) over Kv1.1 (IWQ IC50 = 15 pM). Of the toxin peptide analogs containing a C-terminal amide (Table 14), the following analogs showed improved Kv1.3 selectivity and retained high potency (IC50 < 270 pM) in blocking Kv1.3 and WB IL-2 secretion:

[Ala15, Lys16]ShK-amide (SEQ ID NO:186);

[Glu4, Lys16]ShK-amide (SEQ ID NO:188);

[Lys16, Glu30]ShK-amide (SEQ ID NO:190);

[Lys16, Glu18]ShK-amide (SEQ ID NO:195);

[Glu9, Lys16, Nle21]ShK-amide (SEQ ID NO:222);

[Lys16,29;Ala30]ShK-amide (SEQ ID NO:210);

[Lys16,29;Trp27]ShK-amide (SEQ ID NO:202);

[Glu4; Lys16, 29;Arg30]ShK-amide (SEQ ID NO:201);

[Glu4;Lys16,29;Arg18,30]ShK-amide (SEQ ID NO:212);

[Glu4,9;Lys16,29;Arg18,30]ShK-amide (SEQ ID NO:198);

[Glu4,10; Lys16,29; Arg18,30]ShK-amide (SEQ ID NO:199); and

[Glu4, 9,10; Lys16,29; Arg18,30]ShK-amide (SEQ ID NO:200).

[00413] In order to solve potential issues with C-terminal cysteinyl carboxylate peptides, we explored the extension of the C-terminus with additional residues. Unexpectedly, addition of a single alanine residue following the C-terminal Cys35 residue of [Lys16]ShK (SEQ ID NO:13), caused a significant further improvement in Kv1.3 specificity over Kv1.1. As shown in Table 15 and Table 16, extension of the C-terminus of ShK by addition of a non-Cys residue allows for the reintroduction of the native C-terminal negative charge and potentially increase potency. Of particular interest, the Lys16 analog of ShK with an Ala residue added after the C-terminal Cys35 residue (SEQ ID NO:235, Table 4H and Table 15), retained Kv1.3 activity and showed a dramatic 262 fold improved selectivity for Kv1.3 over neuronal Kv1.1 by PatchXpress (PX) electrophysiology (Table 4H) and 158 fold improved selectivity by IonWorks (IWQ) electrophysiology (Table 15). This [Lys16]ShK-Ala (SEQ ID NO:235) toxin peptide analog provided potent blockade of IL-2 secretion from human whole blood (IC50 = 138 pM, Table 4H) and is one of the most Kv1.3 selective (over Kv1.1) two-amino-acid-change analogs of ShK that we have identified. Other amino acid analogs of ShK with a C-terminal extension that were potent in the whole blood assay (IC50 < 500 pM in blocking IL-2 secretion) and had over 200 fold Kv1.3 versus Kv1.1 selectivity, included (see Table 15):
[Lys16,Nle21]ShK-Ala (SEQ ID NO:236);
[Glu4,Lys16, Nva21]ShK-Ala (SEQ ID NO:242),
[Glu4,Lys16, Nle21]ShK-Ala (SEQ ID NO:243);
[Lys16, Nle21]ShK-Glu (SEQ ID NO:244); and
[Lys16, Nle21]ShK-Tyr (SEQ ID NO:245).

Example 4

Preparation of Peptides and PEGylated Peptides, including Nα-(20 kDa PEG)-ShK (SEQ ID NO:8), Nα-(20 kDa PEG)-[Lys16]ShK (SEQ ID NO: 16), Nα-(20 kDa PEG)-[Lys16]ShK-Ala (SEQ ID NO:316), and Nα-brPEG-[Lys16]ShK (SEQ ID NO:315)

[00414] Peptide Synthesis. N^{α}-Fmoc, side-chain protected amino acids and H-Cys(Trt)-2Cl-Trt resin were purchased from Novabiochem, Bachem, or Sigma Aldrich. The following side-chain protection strategy was employed: Asp(OtBu), Arg(Pbf), Cys(Trt), Glu(OtBu), His(Trt), Lys(N^{ε}-Boc), Ser(OtBu), Thr(OtBu) and Tyr(OtBu). ShK (SEQ ID NO:1), RSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCGTC (SEQ ID NO:13), or other toxin peptide analog amino acid sequences, were synthesized in a stepwise manner on an CS Bio peptide synthesizer by SPPS using DIC/HOBt coupling chemistry at 0.2 mmol equivalent scale using H-Cys(Trt)-2Cl-Trt resin (0.2 mmol, 0.32 mmol/g loading). For each coupling cycle, 1 mmol N^{α}-Fmoc-amino acid was dissolved in 2.5 mL of 0.4 M 1-hydroxybenzotriazole (HOBt) in N,N-dimethylformamide (DMF). To the solution was added 1.0 mL of 1.0 M N,N'-diisopropylcarbodiimide (DIC) in DMF. The solution was agitated with nitrogen bubbling for 15 min to accomplish pre-activation and then added to the resin. The mixture was shaken for 2 h. The resin was filtered and washed three times with DMF, twice with dichloromethane (DCM), and three times with DMF. Fmoc deprotections were carried out by treatment with 20% piperdine in DMF (5 mL, 2 x 15 min). The first 23 residues were single coupled through repetition of the Fmoc-amino acid coupling and Fmoc removal steps described above. The remaining residues were double coupled by performing the coupling step twice before proceeding with Fmoc-removal.

[00415] Following synthesis, the resin was then drained, and washed sequentially with DCM, DMF, DCM, and then dried in vacuo. The peptide-resin was transferred to a 250-mL plastic round bottom flask. The peptide was deprotected and released from the resin by treatment with triisopropylsilane (1.5 mL), 3,6-dioxa-1,8-octane-dithiol (DODT, 1.5 mL), water (1.5 mL),

trifluoroacetic acid (TFA, 20 mL), and a stir bar, and the mixture was stirred for 3 h. The mixture was filtered through a 150-mL sintered glass funnel into a 250-mL plastic round bottom flask. The mixture was filtered through a 150-mL sintered glass funnel into a 250-mL plastic round bottom flask, and the filtrate was concentrated in vacuo. The crude peptide was precipitated with the addition of cold diethyl ether, collected by centrifugation, and dried under vacuum.

[00416] Peptide Folding. The dry crude linear peptide (about 600 mg), for example [Lys16]ShK peptide (SEQ ID NO:13) or [Lys16]ShK-Ala (also known as [Lys16, Ala36]-ShK; SEQ ID NO:235) peptide, was dissolved in 16 mL acetic acid, 64 mL water, and 40 mL acetonitrile. The mixture was stirred rapidly for 15 min to complete dissolution. The peptide solution was added to a 2-L plastic bottle that contained 1700 mL of water and a large stir bar. To the thus diluted solution was added 20 mL of concentrated ammonium hydroxide to raise the pH of the solution to 9.5. The pH was adjusted with small amounts of acetic acid or NH_4OH as necessary. The solution was stirred at 80 rpm overnight and monitored by LC-MS. Folding was usually judged to be complete in 24 to 48 h, and the solution was quenched by the addition of acetic acid and TFA (pH = 2.5). The aqueous solution was filtered (0.45 μm cellulose membrane).

[00417] Reversed-Phase HPLC Purification. Reversed-phase high-performance liquid chromatography was performed on an analytical (C18, 5 μm, 0.46 cm × 25 cm) or a preparative (C18, 10 μm, 2.2 cm × 25 cm) column. Chromatographic separations were achieved using linear gradients of buffer B in A (A = 0.1% aqueous TFA; B = 90% aq. ACN containing 0.09% TFA) typically 5-95% over 35 min at a flow rate of 1 mL/min for analytical analysis and 5-65% over 90 min at 20 mL/min for preparative separations. Analytical and preparative HPLC fractions were characterized by ESMS and photodiode array (PDA) HPLC, combined and lyophilized.

[00418] Mass Spectrometry. Mass spectra were acquired on a single quadrupole mass spectrometer equipped with an Ionspray atmospheric pressure ionization source. Samples (25 μL) were injected into a moving solvent (10 μL/min; 30:50:20 ACN/MeOH containing 0.05% TFA) coupled directly to the

ionization source via a fused silica capillary interface (50 μm i.d.). Sample droplets were ionized at a positive potential of 5 kV and entered the analyzer through an interface plate and subsequently through an orifice (100-120 μm diameter) at a potential of 60 V. Full scan mass spectra were acquired over the mass range 400-2200 Da with a scan step size of 0.1 Da. Molecular masses were derived from the observed m/z values.

[00419] PEGylation, Purification and Analysis. Peptide, e.g., [Lys16]ShK (SEQ ID NO:13) or [Lys16]ShK-Ala (SEQ ID NO:235), was selectively PEGylated by reductive alkylation at its N-terminus, using activated linear or branched PEG. Conjugation was performed at 2 mg/ml in 50 mM NaH_2PO_4, pH 4.5 reaction buffer containing 20mM sodium cyanoborohydride and a 2 molar excess of 20 kDa monomethoxy-PEG-aldehyde (NOF, Japan). Conjugation reactions were stirred for approximately 5 hrs at room temperature, and their progress was monitored by RP-HPLC. Completed reactions were quenched by 4-fold dilution with 20 mM NaOAc, pH 4 and chilled to 4°C. The PEG-peptides were then purified chromatographically at 40C; using SP Sepharose HP columns (GE Healthcare, Piscataway, NJ) eluted with linear 0-1M NaCl gradients in 20mM NaOAc, pH 4.0. Eluted peak fractions were analyzed by SDS-PAGE and RP-HPLC and pooling determined by purity >97%. Principle contaminants observed were di-PEGylated toxin peptide analog. Selected pools were concentrated to 2-5 mg/ml by centrifugal filtration against 3 kDa MWCO membranes and dialyzed into 10 mM NaOAc, pH 4 with 5% sorbitol. Dialyzed pools were then sterile filtered through 0.2 micron filters and purity determined to be >97% by SDS-PAGE (Figure 5A, Figure 5C, Figure 5E) and RP-HPLC (Figure 5B, Figure 5D, and Figure 5F). Reverse-phase HPLC was performed on an Agilent 1100 model HPLC running a Zorbax® 5μm 300SB-C8 4.6 x 50 mm column (Agilent) in 0.1% TFA/H_2O at 1 ml/min and column temperature maintained at 40°C. Samples of PEG-peptide (20 μg) were injected and eluted in a linear 6-60% gradient while monitoring wavelength 215 nm.

Example 5

Nα-20 kDa-PEG-[Lys16]ShK (SEQ ID NO:16) and other PEGylated Toxin Peptide Analogs; Pharmacokinetics in Mammalian Species; Safety Pharmacology Assay; and Isolated Rabbit Heart Assay

[00420] Conjugation of 20 kDa PEG greatly increased the exposure of a toxin peptide in vivo, as demonstrated by a comparison of the pharmacokinetics (PK) of ShK-L5 (SEQ ID NO:17) versus Nα-(20 kDa PEG)-ShK (SEQ ID NO:8). (see Figure 4). ShK-L5 is a potent peptide inhibitor of Kv1.3 described in earlier publications. (Beeton et al., Targeting effector memory T cells with a selective peptide inhibitor of Kv1.3 channnels for therapy of autoimmune diseases, Molec. Pharmacol. 67(4):1369-81 (2005); Chandy et al., Analogs of ShK toxin and their uses in selective inhibition of Kv1.3 potassium channels, WO 2006/042151 A2; Pennington et al., Engineering a stable and selective peptide blocker of the Kv1.3 channel in T lymphocytes, Molecular Pharmacology Fast Forward, published January 2, 2009 as doi:10.1124/mol.108.052704 (2009)). ShK-L5 (SEQ ID NO:17) contains an N-terminal phophotyrosine moiety attached to ShK via an 2-(2-(2-aminoethoxy)ethoxy)acetic acid (AEEA) linker. Despite reports that this molecule has improved selectivity for mouse Kv1.3 versus Kv1.1, in our hands we found the ShK-L5 molecule blocked human Kv1.3 and Kv1.1 with about equipotency (Table 4H, Table 5 and Figure 6C-D). Whether the differences in our findings versus those published, results from differences in the channel species (mouse versus human) used or differences in the cell lines, is unclear. More recently, M.W. Pennington et al. described ShK-192 (SEQ ID NO:438) as a stable and selective peptide blocker of the Kv1.3 channel; ShK-192 (SEQ ID NO:438) was identified by Pennington et al. as a more stable analog than ShK-L5 which showed pH-related hydrolysis and oxidation byproducts that were exacerbated by increasing temperatures. (Pennington et al., Engineering a stable and selective peptide blocker of the Kv1.3 channel in T lymphocytes, Molec. Pharmacol. 75(4):762-73 (2009)). Based on this report, we synthesized and tested ShK-192 for activity on human Kv1.3 and human Kv1.1. Whereas our studies on the ShK-L5 molecule did not show increased selectivity at Kv1.3 over Kv1.1 (Figures 6C

and 6D), the newer ShK-192 analog showed improved Kv1.3 selectivity (Figure 29A) relative to Kv1.1 (Figure 29B). ShK-192 inhibited human Kv1.3 with an IC50 of 0.039±0.005 nM, which was about 87 times more potent than its IC50 on human Kv1.1 (3.39±1.61 nM). Whether the improved Kv1.3 selectivity of ShK-192 relative to ShK-L5 is a result of its improved stability, or is due to other reasons, is unknown.

[00421] Regardless, we also found that ShK conjugated with a N-terminal 20 kDa PEG moiety (SEQ ID NO:8) was Kv1.3 non-selective and had roughly equivalent potency on neuronal Kv1.1 (Table 5), despite being highly potent and having an extended half-life in vivo. In contrast, [Lys16]ShK toxin peptide analog containing a N-terminal 20kDa PEG moiety (SEQ ID NO:16) had greatly improved Kv1.3 selectivity (Table 5 and Figure 6A-B), showing 1060 fold greater activity against Kv1.3 versus Kv1.1(Table 4H). In addition, the 20kDa-PEG-[Lys16]ShK conjugate (SEQ ID NO:16) retained high potency (sub-nM IC50) in the human whole blood assay of T-cell inflammation. Figure 30A-B shows representative dose-response curves from three lots of 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16) for inhibition of IL-2 (Figure 30A) and IFNγ (Figure 30B) secretion. The average IC50 of 20kDa-PEG-[Lys16]ShK (all lots tested) for inhibition of IL-2 and IFNγ secretion from thapsigargin stimulated T cells in human whole blood obtained from multiple donors, was 0.109±0.081 nM (n=34) and 0.240±0.163 nM (n=34), respectively. Cyclosporin A was about 2000-3000 less active, inhibiting IL-2 and IFNγ production with IC50 values of 334.8±172.2 nM (n=64) and 495.2±307.8 nM (n=64), respectively. Other than its potent activity in blocking T cell responses in human whole blood, 20kDa-PEG-[Lys16]ShK also potently inhibited T cell IL-17 secretion from cynomolgus monkey whole blood stimulated with thapsigargin (Figure 31, Example 8) exhibiting an average IC50 of 0.09 nM in experiments using blood collected from seven different monkeys. Myelin-specific proliferation of the rat T effector memory cell line, PAS, was also potently (IC50 = 0.17 nM) blocked by the 20kDa-PEG-[Lys16]ShK molecule (Figure 32, Example 9). Therefore, 20kDa-PEG-[Lys16]ShK was equally effective in inhibiting T cell responses of three

distinct species: human, monkey and rat. Whereas, the [Lys16]ShK toxin peptide analog alone (SEQ ID NO:13) showed modest Kv1.3 selectivity (Kv1.1/Kv1.3 IC_{50} ratio = 15-fold), N-terminal conjugation of this peptide with a 20 kDa PEG moiety further enhanced its selectivity, with the conjugate having about 1000-fold selectivity for Kv1.3 over neuronal Kv1.1. Further ion channel counterscreens revealed that the novel 20kDa-PEG-[Lys16]ShK conjugate was 680 fold selective over Kv1.2, about 500-fold selective over Kv1.6 and >10000 fold selective over Kv1.4, Kv1.5 and Kv1.7 (Table 4A). Importantly, the conjugate did not impact ion channels that are known to serve a role in human cardiac action potential, exhibiting >10000 fold selectivity over Nav1.5, Cav1.2, Kv4.3, KvLQT1/minK and hERG (Table 4A, see Example 6). The conjugated toxin peptide analog 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16) also showed no impact on the calcium-activated K^+ channels, KCa3.1 (IKCa1) and BKCa.

[00422] To further characterize the pharmacology of the conjugate, it was tested for ex vivo stability in plasma and pharmacokinetics in vivo. The 20kDa-PEG-[Lys16]ShK conjugate was stable in rat, cyno and human plasma for 48 hours at 37°C (Figure 10A-D; see, Example 7). Pharmacokinetic studies in mouse, rat, dog and cyno (Figure 11A-B, see, Example 8) indicated the conjugate was stable in vivo and had a prolonged half-life. As shown in Figure 11C, 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16) given as a single 0.5 mg/kg subcutaneous dose to cynomolgus monkeys showed a prolonged half-life, achieving a Cmax at 8 hrs of 254 nM (1031 ng/ml) and day 7 serum levels of 28.4 nM (115 ng/ml). The day 7 serum concentration after this single dose was about 28 times greater than the conjugate's IC_{95} (1.0 nM) in blocking inflammation in human whole blood and about 315 times its IC_{50} (0.09 nM) in this assay (performed as described in Example 2). Therefore, the pharmacokinetics of PEG-[Lys16]ShK in cynos suggests weekly dosing in humans is possible and that very small doses would be needed to block inflammation. Further details on the pharmacokinetics of 20kDa-PEG-[Lys16]ShK(SEQ IDD NO:16) administered subcutaneously are provided below and in Table 4B, Table 4C, Table 4D, and Table 4D(a), below.

[00423] The efficacy of 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16) molecule in an animal model of multiple sclerosis was determined using the adoptive-transfer (AT)-EAE model, as described in Example 9. PEG-[Lys16]ShK delayed disease onset and caused dose-dependent reduction in disease severity (Figure 7 and Figure 8A-D). The molecule was highly potent in this model and 4.4 μg/kg/day of PEG-[Lys16]ShK (SEQ ID NO:16) was estimated as the effective dose causing 50% reduction in disease severity (ED50), based on EAE score at day 7.

[00424] To further assess the pharmacology and safety of the 20kDa-PEG-[Lys16]ShK molecule (SEQ ID NO:16) in vivo, a 12-week pharmacology study was performed in cynomolgus monkeys (Example 10). The 12-week study involved three pre-dose baseline measure over two weeks, weekly 0.5 mg/kg SC dosing for one month and six weeks of follow-up analysis. Further details of the study are provided in Example 10 and Table 4F, below. Based on earlier pharmacokinetic studies on the 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16) molecule weekly dosing at 0.5 mg/kg would provide excess target coverage ranging from 28 times IC_{95} at Cmin to 249 times IC_{95} at Cmax. The PEG-[Lys16]ShK conjugate (SEQ ID NO:16) was well tolerated. The animals gained weight throughout the study (Figure 9D), and there were no changes in CBCs and blood chemistry relative to pre-dose baseline measures (Table 4G, Example 10). Using the cyno whole blood pharmacodynamic assay described in Example 8, T cell inflammation was suppressed for the entire one month dosing period and no changes were detected over time (Figure 9A-B). Upon repeat dosing, there was good correlation between predicted and observed serum drug trough levels (Figure 9C) over the one month dosing period. Foreign peptides or proteins can sometimes be immunogenic and produce clearing or neutralizing antibodies that can alter serum drug levels and/or pharmacodynamic coverage. Since no such alterations were observed in cynomolgus monkeys after repeat dosing with 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16), these data imply that this PEG-conjugate did not produce neutralizing or clearing antibodies in monkeys.

[00425] There are typically three reasons cited for peptides having a short half-life in vivo: target-mediated clearance, rapid proteolytic degradation or rapid renal clearance. For a given class of peptides the reason for short half-life is often not obvious. Although there are established methods for reducing renal clearance (e.g., PEGylation, Fc fusion), these methods would be futile if the peptide in question is either proteolytically susceptible (rapidly hydrolysed) in vivo or cleared rapidly as a result of target-mediated clearance or non-specific (charge) binding. In addition, since only large PEG or Fc fusions reduce renal clearance, it needs to be determined empirically whether a given class of peptides retains potency following conjugation. Conjugates that exhibit a significant loss in potency, erase any benefit gained by improved in vivo half-life since the level of target coverage in animals is directly dependent on both the molecules potency and stability in vivo. For a new class of peptides, it is not obvious that one can successfully form conjugates to a large PEG or Fc moiety and retain activity. The reasons for this are that distinct classes of peptides have distinct primary and tertiary structures, whose biophysical stability can be significantly altered upon conjugation. Any significant reduction in stability (in vitro or in vivo) would reduce the molecule's activity and eliminate its utility as a therapeutic.

[00426] The native ShK peptide (SEQ ID NO:1) is a potent Kv1.3/Kv1.1 inhibitor that has a short half-life in vivo (C. Beeton et al., 2001, Proc. Natl. Acad. Sci. 98, 13942-13947). The major cause of ShK's poor stability in vivo is unknown, with Norton et al. (Current Medicinal Chemistry 11, 3041-3052, 2004) indicating more detailed analyses of its absorption, distribution, metabolism and excretion were necessary to identify the cause. To assess whether proteolysis was the major cause, Beeton et al. (J. Biol. Chem. 283: 988-997, 2008) described an extensive effort to generate a D-diastereomer analog of ShK containing D-amino acids in order to limit proteolysis and improve circulating half-life. The D-allo-ShK molecule was about 2800 times less active than native ShK in blocking the Kv1.3 target. The authors reported that the D-allo analog of ShK did not improve its half-life in vivo, suggesting proteolysis did not explain the short half-life of ShK. To improve the therapeutic potential of ShK, we generated novel PEG and

Fc conjugates of ShK and demonstrated they have prolonged half-life in vivo (Example 8), and importantly remain highly Kv1.3 potent. Since our molecules are highly potent against Kv1.3 and have prolonged half-life, our data imply that Kv1.3 target-mediated clearance does not explain the short half-life of the ShK peptide. In addition, since it is unlikely our conjugates would protect against proteolysis, our findings imply that proteolysis may not play a role in ShK's short half-life in vivo. A reduction in renal clearance may be the key reason our ShK conjugates have prolonged half-life in vivo, but to determine this definitively, further studies would be necessary to assess absorption, distribution, metabolism and excretion of the conjugate.

[00427] In our efforts to identify Kv1.3 inhibitors with improved therapeutic potential, we performed systematic multi-pass analoging of ShK to identify analogs with improved Kv1.3 versus Kv1.1 selectivity, formed PEG or Fc-conjugates of the analogs and tested each for potency, selectivity and stability in vivo. The afore-mentioned [Lys16]ShK analog (single amino acid change of ShK Gln16 to Lys16; SEQ ID NO:13) was highly potent and showed improved selectivity, that was unexpectedly further enhanced upon conjugation to PEG. Of particular importance, this conjugate retained high potency and blocked Kv1.3 and T cell inflammation with an IC50 in the picomolar range. Despite the success here, several ShK analogs identified with improved Kv1.3 selectivity, after conjugation with a 20kDa PEG moiety showed too large a reduction in potency to be therapeutically useful. Some examples include 1-Nal16 (SEQ ID NO:11) and 1-Nal27 (SEQ ID NO:95) analogs of ShK, that as free peptides showed sub nM potency in blocking Kv1.3 and had improved Kv1.3/Kv1.1 selectivity (Table 8), but as PEG conjugates showed about 57 times less activity (SEQ ID NO:159 and SEQ ID NO:160, respectively; Table 12). On the other hand, the [Lys16]ShK-Ala toxin peptide analog (SEQ ID NO:235; see, Example 3) was N-terminally conjugated with a 20 kDa PEG moiety. The Nα-20kDa-PEG-[Lys16]ShK-Ala conjugate (SEQ ID NO:316) retained high potency, blocked Kv1.3 and T cell inflammation with sub-nM IC50 and exhibited >3500 fold selectivity for Kv1.3 over neuronal Kv1.1 (Table 4H). This conjugate, with an N-terminal 20 kDa-PEG

moiety and a C-terminal alanine addition, represents one of the most potent and selective conjugates yet identified.

[00428] Conjugation of a peptide or protein with PEG molecules of differing size (molecular weight) and form can significantly affect the bioactivity, pharmacokinetics, distribution and metabolism of the molecule. To explore the impact of alternative PEG forms other than Nα-20 kDa-PEG, the Kv1.3-selective [Lys16]ShK (SEQ ID NO:13) peptide inhibitor of Kv1.3 was N-terminally conjugated with either a 30 kDa PEG moiety (SEQ ID NO:158) or a branched PEG moiety comprised of two 10 kDa PEG units (Nα-brPEG-[Lys16]ShK; SEQ ID NO:315). The purified brPEG-[Lys16]ShK molecule (SEQ ID NO:315) was a potent blocker of T cell cytokine secretion from whole blood (IC50 = 198 pM for blocking IL-2) and exhibited about 750-fold selectivity for lymphocyte Kv1.3 over neuronal Kv1.1 (Table 4H). The Nα-30kDa-PEG-[Lys16]ShK molecule (SEQ ID NO:158) was also highly active with an IC50 of 282 pM in blocking thapsigargin-stimulated IL-2 secretion from human whole blood (Table 12). Given the Nα-20kDa PEG-[Lys16]ShK (SEQ ID NO:16) peptide conjugate had an IC50 of about 100 pM in this same assay, these data imply that the Kv1.3 selective [Lys16]ShK toxin peptide analog (SEQ ID NO:13) tolerates and retains activity upon N-terminal conjugation with PEG moieties of a variety of different sizes and shapes.

[00429] Comparison of PK characteristics across mammalian species. PEG-[Lys16]ShK (SEQ ID NO:16) preclinical pharmacokinetics performed in mouse, rat, cynomolgus monkey and dog were generally supportive of a weekly dosing regimen in humans. Details on the pharmacokinetics of 20kDa-PEG-[Lys16]ShK(SEQ IDD NO:16) administered subcutaneously are provided in Table 4B, Table 4C, and Table 4D, below. The preclinical pharmacokinetics were generally less favorable in rodents than non-rodent species when considering parameters such as exposure, half life and bioavailability. Rat pharmacokinetics were the least favorable of the four species that were tested and should present an on-going challenge to using this species effectively for experiments requiring consistent or long-term exposure of PEG-[Lys16]ShK (SEQ ID NO:16) (e.g.,

pharmacology or toxicology). There is also a growing body of evidence that rats exhibit a species specific sensitivity to mast cell degranulation caused by dosing of PEG-[Lys16]ShK (SEQ ID NO:16) (See, Example 10). The mast cell degranulation is accompanied by the series of physical changes which may have an effect on rat pharmacokinetics including drug adsorption, distribution and clearance. (Example 10).

[00430] Preclinical pharmacokinetic parameters for PEG-[Lys16]ShK (SEQ ID NO:16) administered intravenously are shown in Table 4D(a), below. The doses for the intravenous (IV) experiments were 0.2 mg/kg for all species, while subcutaneous administration (SC) was performed at either 0.5 or 2 mg/kg. In general, the pharmacokinetic properties of PEG-[Lys16]ShK (SEQ ID NO:16) were more favorable in dog and cynomolgus monkey when compared to the rodent species. Volume of distribution at steady state (Vss) was small in the case of mouse, cynomolgus monkey and dog. Rat was an outlier when considering its Vss, which was approximately 3-4 times larger per unit weight than the other three species. Both IV and SC PK experiments indicated that exposure in rat was mediated by a large initial distribution phase which was less pronounced in other species. Terminal elimination half-lives measured after IV dosing were 12.0 hr, 16.1 hr, 21.0 hr and 28.3 hr for rat, mouse, cynomolgus monkey and dog, respectively, indicating delineation between rodent and non-rodent species, concerning terminal half-life. PEG-[Lys16]ShK (SEQ ID NO:16) was observed to exhibit variable absorption across species upon subcutaneous dosing. Mouse had a more rapid subcutaneous absorption than other species; T_{max} was obtained after 4 hrs, compared to mean T_{max} values from 8 hr to 40 hr for other species. Relative ranking of the subcutaneous absorption rate constants (K_a) following subcutaneous injection were Mouse (0.244 h^{-1}) > Cynomolgus monkey (0.197 h^{-1}) > Dog (0.191 h^{-1}) > Rat (0.065 h^{-1}).

[00431] In more detail, clearance values for PEG-[Lys16]ShK (SEQ ID NO:16) measured after IV dosing (0.2 mg/kg) were 43.9, 8.2, 5.94 and 2.68 mL/hr/kg, for rat, mouse, cynomolgus cynomolgus monkey and dog, respectively. These clearance values resulted in terminal elimination half-lives of 12.0, 16.1,

21.0 and 28.3 hours, respectively for PEG-[Lys16]ShK (SEQ ID NO:16). Clearance was roughly proportional to glomerular filtration in these species and provided further support to the expectation that renal filtration is a main route of elimination for PEG-[Lys16]ShK (SEQ ID NO:16). Volumes of distribution at steady state (Vss) measured after IV dosing (0.2 mg/kg) were 87.3, 289, 68.4, and 77.0 mL/kg for mouse, rat, cynomolgus cynomolgus monkey and dog, respectively (Table 4D(a)). Rat had a 3 times larger volume of distribution than the next species making it an outlier in this regard.

[00432] Bioavailabilities ranged from 15% to 100% in the various subcutaneous PK experiments that were performed. Bioavailability was observed to increase with increasing dose in the rat. Exposure upon subcutaneous dosing was species dependent, with some delineation observed between rodent and non-rodents. As an example of this, exposure in rat was less than 4% of that observed in cynomolgus monkey and dog after giving a 0.5 mg/kg subcutaneous dose of PEG-[Lys16]ShK (SEQ ID NO:16) ($AUC_{0\text{-}inf}$ of 3,220, 137,000, and 103,000 ng•hr/mL for rat, cynomolgus monkey and dog, respectively). The difference in exposure is attributed to a combination of lower bioavailability, higher volume of distribution, and higher elimination in rat as compared to non-rodent species. In general, subcutaneous exposure in Sprague-Dawley rats specifically could be characterized as either low or inconsistent across the various experiments that were performed where PK was measured, although it was usually consistent within individual rat experiments.

[00433] PEG-[Lys16]ShK (SEQ ID NO:16) was tested for dose proportionality in male Sprague-Dawley rats using subcutaneous doses of 0.1, 0.5, 2.0 and 5.0 mg/kg. Exposures were twice that predicted from previous subcutaneous experiments in rats and increased at a greater than dose proportional rate, probably due to an increase in bioavailability with increasing dose. PEG-[Lys16]ShK (SEQ ID NO:16) was also tested for dose proportionality in female C57BL/6 mice using subcutaneous doses of 0.1, 0.3, 0.6 and 2.5 mg/kg per animal. There was a similar tendency in this species for greater than dose proportional increases with dose, which added an additional 20-35 % greater AUC

than would have been expected from the next lower dose. This study confirmed results of high exposure observed previously in toxicology studies using female C57BL/6 mice and also confirmed that there was a 6 to 8-fold increase in exposure in female C57BL/6 versus male CD-1 mice when given a similar dose.

[00434] Safety Pharmacology Assay. Safety pharmacology studies investigate the potential undesirable pharmacodynamic effects of chemicals or pharmaceutical compounds on vital organs or systems which are essential for sustaining life. The International Conference on Harmonisation (ICH) Safety Expert Working Group has developed a hierarchy of organ systems with respect to their life supporting functions. The most important functions are those of the cardiovascular, respiratory or central nervous systems. Drug induced effects on these systems should be investigated prior to the first administration of substances to humans. Other organ systems (e.g. the renal or gastrointestinal system), the functions of which can transiently be disrupted by adverse pharmacodynamic effects without causing irreversible harm, are of less immediate investigative concern (S. Whitebread et al., DDT, 10:1421-1433 (2005); R. Porsolt et al., Drug. Dev. Res., 64:83-89 (2005)). PEG-[Lys16]ShK (SEQ ID NO:16) was tested in the Cerep safety pharmacology assay at 10 μM to evaluate binding capacity to various human targets such as receptors, enzymes and ion channels. Of 151 targets, there were 9 hits (>50% inhibition; Table 4D(b)), 5 of which were ion channels. IC50 values were determined for each hit and a functional assay was performed for neuropeptide Y1. Compared to the IC50 value for the K Channel (expected target), the IC50's for the other hits are >25 fold higher. In addition, there was no impact on Neuropeptide Y1 in a functional assay. These data imply that these off-target binding events are unlikely to lead to unexpected toxicities.

[00435] Isolated rabbit heart assay. Potential cardiovascular effect of 1 μM PEG-[Lys16]ShK (SEQ ID NO:16) was evaluated in the isolated rabbit heart assay. In this preparation, the test article was perfused through the coronary circulation and cardiac electrical (ECG), mechanical (left ventricular contractility), and hemodynamics (coronary blood flow) were assessed after 20 min of perfusion. There was no effect of PEG-[Lys16]ShK (SEQ ID NO:16) on

ECG, contractility or hemodynamic parameters in the isolated heart model, which indicates that it was devoid of any effect cardiac ion channel function.

Table 4A. Potency and selectivity of Nα-(20 kDa PEG)-[Lys16]ShK (SEQ ID NO: 16).

Assay	IC50 nM (n)
Human whole blood (TG induced IL-2)	0.092 (14)
Kv1.1	997 (3)
Kv1.2	639 (3)
Kv1.3	0.94 (5)
Kv1.4	>10000 (3)
Kv1.5	>30000 (3)
Kv1.6	466 (7)
Kv1.7	>10000 (3)
IKCa1	>10000 (4)
BKCa	>10000 (4)
hERG (IKr)	>10000 (3)
Nav1.5 (INa)	>30000 (3)
Cav1.2 (ICa)	>30000 (3)
Kv4.3 (Ito)	>30000 (3)
KvLQT1/minK (IKs)	>30000 (4)

Table 4B. Pharmacokinetic data showing PEG-ShK (SEQ ID NO:8) has a prolonged half-life in Sprague-Dawley rats compared to the small peptide ShK-L5 (SEQ ID NO:17). Administration was by subcutaneous injection.

CMPD (n)	Dose (mg/kg)	Tmax (h)	Cmax (ng/ml)	AUC0-t (ng•hr•mL^{-1})	AUC0-inf (ng•hr•mL^{-1})	DNAUC (0-inf)	CL/F (mL•hr^{-1}•kg^{-1})	MRT (h)
PEG-ShK (3)	2	24±0	1138 ±404	42347 ±10,228	42374 ±10,237	21187	49±14	37±3
PEG-ShK (2)	0.3	24	165	6011	6,020	20065	52	27
ShK-L5 (6)[a]	0.2	0.5	50	93	97	487	2052	2

[a] Beeton et al., Molec. Pharmacol. 67(4):1369-81 (2005).

Table 4C. Pharmacokinetic data for PEG-[Lys16]ShK (SEQ ID NO:16) in mice (dose = 2.0 mg/kg), rats (dose = 2.0 mg/kg) and cynomolgus monkeys (dose = 0.5 mg/kg) administered by subcutaneous injection.

Species	HL (h)	Tmax (h)	Cmax (ng/ml)	AUC0-t (ng•hr•mL^{-1})	AUC0-inf (ng•hr•mL^{-1})	Vz/F (ml/kg)	CL/F (mL•hr^{-1}•kg^{-1})	MRT (h)
Cyno	64.5 ±14.9	8.0	1010 ±105	71500 ±607	74900 ±3260	621 ±143	6.68 ±0.29	87 ±16
Mouse*	14.9	4.0	1860	37000	37000	1170	54.1	16.6
Rat	N/A	40 ±14	531 ±90	21900 ±2770	21900 ±2760	N/A	92 ±13	36 ±2

* Sparse sampling PK experiment. No standard deviations were calculated for PK parameters.

Table 4D. Pharmacokinetic data for PEG-[Lys16]ShK (SEQ ID NO:16) administered by subcutaneous injection (dose = 0.5 mg/kg) to dog (beagle) or monkey (cyno).

Species	HL (h)	Tmax (h)	Cmax (ng/ml)	AUC0-t ($ng \cdot hr \cdot mL^{-1}$)	AUC0-inf ($ng \cdot hr \cdot mL^{-1}$)	Vz/F (ml/kg)	CL/F ($mL \cdot hr^{-1} \cdot kg^{-1}$)	MRT (h)
Cyno(a)	64.5 ±14.9	8.0	1010 ±105	71500 ±607	74900 ±3260	621 ±143	6.68 ±0.29	87 ±16
Cyno(b)	37.4 ±13.2	18.7 ±9.2	1480 ±258	125000 ±31600	137000 ±45500	197 ±9.3	3.90 ±1.10	70.5 ±20
Beagle	42.6 ±4.21	18.7 ±9.24	1270 ±347	95200 ±31300	103000 ±37300	322 ±98	5.37 ±2.14	66.1 ±13.5

Table 4D(a). Preclinical pharmacokinetics of of PEG-[Lys16]ShK (SEQ ID NO:16) in mouse, rat, cynomolgus monkey and dog. *= Bioavailability calculated using 0.2 mg/kg IV dose

Parameter	Mouse (Male CD-1)	Rat (Male SD)	Monkey (Male Cynomolgus)	Dog (Male Beagle)
IV $t_{1/2}$ (h)				
0.2 mg/kg (1)	16.1 (1)	12.0±3.7 (1)	21.0±13.7 (1)	28.3±4.2 (1)
2.0 mg/kg (2)	ND (2)	16.8 (2)	ND (2)	ND (2)
IV MRT (h)				
0.2 mg/kg (1)	11.0 (1)	6.6± 0.7 (1)	13.3±7.5 (1)	27.2±8.6 (1)
2.0 mg/kg (2)	ND (2)	8.5±0.5 (2)	ND(2)	ND (2)
IV Cl (mL/h/kg)				
0.2 mg/kg (1)	8.2 (1)	43.9±1.6 (1)	5.94±2.30 (1)	2.68±0.54 (1)
2.0 mg/kg (2)	ND (2)	60±17 (2)	ND(2)	ND (2)
IV Vss (mL/kg)				
0.2 mg/kg (1)	87.3 (1)	289.3±19.3 (1)	68.4±24.7 (1)	77.0±8.4 (1)
2.0 mg/kg (2)	ND (2)	511±170 (2)	ND (2)	ND (2)
SC $AUC_{0\rightarrow\infty}$(ng·h/mL)				
0.5 mg/kg (1)	24300 (1)	4570±160 (1)	36700±11700 (1)	76500±14000 (1)
2.0 mg/kg (2)	ND (2)	35500±9900 (2)	ND (2)	ND (2)
SC T_{max} (h)			8±0.0 (1a)	
0.5 mg/kg (1)	ND (1)	24.0±0.0 (1)	18.7±9.2 (1b)	18.7±9.2 (1)
2.0 mg/kg (2)	4.0 (2)	40±14 (2)	ND (2)	ND (2)
SC C_{max} (ng/mL)			1031±105 (1a)	
0.5 mg/kg (1)	ND (1)	94.5±30 (1)	1480±258 (1b)	1270±347 (1)
2.0 mg/kg (2)	1860 (2)	531±90 (2)	ND (2)	ND (2)
SC $AUC_{0\rightarrow\infty}$(ng·h/mL)			75000±3,260 (1a)	
0.5 mg/kg (1)	ND (1)	3220±781 (1)	137000±45500 (1b)	103000±37300 (1)
2.0 mg/kg (2)	37000 (2)	21900±2,760 (2)	ND (2)	ND (2)
SC Bioavailability(%)*	15.2 (2)	28 (1), 62 (2)	82 (1a), >100 (1b)	54 (1)

Table 4D(b). PEG-[Lys16]ShK (SEQ ID NO:16) was tested in the Cerep safety pharmacology assay. Hits are shown. ND = Not done

Target	**% Inhibition**	**IC50 (μM)**	**Functional Assay**
NMDA	71	10	ND
Glycine (Strychnine insensitive)	101	1.7	ND
Lysophosphotodic A1	62	5.5	ND
Neuropeptide Y1	51	22	No agonistic or antagonistic effects
Ca Channel (N)	58	3.0	ND
K Channel (KATP)	54	2.7	ND
K Channel (Kv)	90	0.023	ND
Nitric Oxide (endothelial)	90	0.59	ND
Phosphodiestrase 1B	68	27	ND

Example 6

Ion channel counterscreens

[00436] Kv1.1, Kv1.2, Kv1.3, Kv1.4, Kv1.6 and Kv1.7, PatchXpress®, planar patch-clamp electrophysiology. Ion channel currents were recorded at room temperature using PatchXpress® 7000A electrophysiology system from MDC using methods and cells described in Example 1 above. The voltage protocols for each channel are shown in Table 4E, below.

Table 4E. Voltage protocols and recording conditions.

Ion Channel	Voltage Step	Pulse Duration	Time between pulses	Holding Potential	Benchmark Inhibitor
hKv1.1	From −80mV to +30mV	200ms	10s	−80mV	10nM DTX-k
hKv1.2	From −80mV to +60mV	400ms	10s	−80mV	1nM MgTx
hKv1.3	From −80mV to +30mV	200ms	30s	−80mV	1nM ShK
hKv1.4	From −80mV to +30mV	200ms	30s	−80mV	1mM 4-AP
hKv1.6	From −80mV to +60mV	500ms	15s	−80mV	1mM 4-AP
hKv1.7	From −80mV to +30mV	1000ms	15s	−80mV	1mM 4-AP

[00437] Cardiac ion channel counterscreens (hERG, hKvLQt1/hminK, hNav1.5, hKv1.5, hCav1.2, hKv4.3).

[00438] Cell lines. HEK293 cells stably transfected with hKvLQT1/hminK and hERG were from Amgen or Cytomyx, Inc. HEK293 cells stable transfected with human hNav1.5 were purchased from Cytomyx, Inc. HEK293 cells stably expressing hKv4.3 and CHO cells stably expressing hKv1.5 were from ChanTest. CHO cells stably expressing the human L-type calcium channel Cav1.2 were from ChanTest and contained the human CACNA1C gene encoding hCav1.2 and coexpressed the beta 2 subunit encoded by human CACNB2 and alpha2delta1 encoded by the CACNA2D1 gene.

[00439] FASTPatch® studies were performed at ChanTest to examine the impact of peptides and conjugates on the cloned human L-type calcium channel hCav1.2, cloned hKv4.3 and cloned hKv1.5 involved PatchXpress (Model 7000A, Molecular Devices, Union City, CA) electrophysiology at room temperature. The extracellular recording solution (HB-PS) contained 137 mM NaCl, 4 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES and 10 mM Glucose adjusted to pH 7.40 with NaOH. The intracellular recording solution for hKv4.3 and hKv1.5 contained 130 mM potassium aspartate, 5 mM MgCl2, 5 mM EGTA, 4 mM ATP

and 10 mM HEPES adjusted to pH 7.2 with KOH. The intracellular solution for hCav1.2 contained 130 mM cesium aspartate, 5 mM $MgCl_2$, 5 mM EGTA, 4 mM ATP, 2 mM EDTA, 1 mM $CaCl_2$, 0.1 mM GTP and 10 mM HEPES adjusted to pH 7.2 with N-methyl-D-glucamine. In preparation for recording, intracellular solution is loaded into the intracellular compartments of the *Seal*chip$_{16}$ planar electrode. Cell suspensions are pipetted into the extracellular compartments of the *Seal*chip$_{16}$ planar electrode. After establishing a whole-cell configuration, membrane currents are recorded using dual-channel patch clamp amplifiers in the PatchXpress® system. Before digitization, the currents were low-pass filtered at one-fifth of the sampling frequency. Three concentrations of peptide conjugates (test article) diluted into HB-PS with 1% BSA are applied at five minute intervals to naïve cells. Solution exchange were performed in quadruplicate and the duration of exposure to each test article concentration was five minutes. Vehicle controls were also applied to naïve cells and after a solution exchange positive controls are applied to verify sensitivity to ion channel blockade. All positive controls were diluted into HB-PS with 0.3% DMSO. Positive controls for blockade of channels included: nifedipine (0.01 μM) which produced about 75% hCav1.2 current block, flecainide (0.1 mM) which produced about 75% inhibition of the hKv4.3 current and 4-aminopyridine (2 mM) which blocked about 80% of the hKv1.5 current. Valid whole-cell recordings must meet the following criteria: (1) membrane resistance (Rm) $\geq$ 200 MΩ , (2) leak current $\leq$ 25% channel current. The test procedures for hCav1.2, hKv4.3 and hKv1.5 were as follows:

[00440] a.) hCav1.2 test procedure. Onset and steady state block of hCav1.2/β2/α2δ channels were measured using a stimulus voltage pattern consisting of a depolarizing test pulse (duration, 200 ms; amplitude, 10 mV) at 10-s intervals from a -40 mV holding potential. Test article concentrations may be applied cumulatively in ascending order without washout between applications. Peak current was measured during the step to 10 mV. Saturating concentration of nifedipine (10 μM) is added at the end of each experiment to block hCav1.2 current. Leak current was digitally subtracted from the total membrane current record.

[00441] b.) hKv4.3 test procedure. Onset and steady state block of hKv4.3 current were measured using a pulse pattern with fixed amplitudes (depolarization: 0 mV for 300 ms) repeated at 10-s intervals from a holding potential of –80 mV. Peak and sustained test pulse current amplitudes were measured during the step to zero mV.

[00442] c.) hKv1.5 test procedure. Onset and steady state block of hKv1.5 current were measured using a pulse pattern with fixed amplitudes (depolarization: +20 mV amplitude, 300 ms duration) repeated at 10-s intervals from a holding potential of –80 mV. Current amplitude was measured at the end of the step to +20 mV.

[00443] Counterscreens against the cloned human Nav1.5 sodium channel using the PatchXpress® system. The extracellular (HB-PS2) recording solution contained 70 mM NaCl, 67 mM N-methyl-D-glucamine, 4 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 10 mM Glucose adjusted to pH 7.4 with HCl. The internal recording solution contained 130 mM CsF, 10 mM NaCl, 10 mM EGTA, 2 mM $MgCl_2$, 10 mM HEPES adjusted to pH 7.20 with CsOH. Stock solutions of reference standard or test articles were diluted into HB-PS2 prior to application. Test articles included either peptides or peptide conjugates described herein. Lidocaine (1 – 30 μM) was the reference standard. A standardized step protocol is used to elicit ionic current through the hNav1.5 sodium channel. Cells are held at −80 mV. Onset and steady state block of hNav1.5 sodium current due to Test Article was measured using a pulse pattern with fixed amplitudes (conditioning prepulse: −120 mV for 50 ms; depolarizing test step to –30 mV for 20 ms) repeated at 10-s intervals. Currents are filtered at 3 kHz and acquired at 10 kHz, in episodic mode. When a good recording was established, cells were washed for 2 minutes, following by applying control vehicle for 5 minutes. Then control and each concentration of test article was applied for 5 minutes. There were 3 additions for each concentration with 1-minute interval. Dispense speed was 40 μL/s with suction on. To determine IC_{50}, Test Article at 1 μM, 3 μM, 10 μM and 30 μM was applied to cells (n = 3 cells) cumulatively (without washout between test article concentrations) in ascending order, to each cell (n = 3 where

n = number of cells). Each concentration of test article was applied for 5 minutes. There were 3 additions for each concentration with a 1-minute interval. Electrophysiological data acquisition was performed using PatchXpress Commander v1.4 (Axon Instruments, Union City, CA) and analyses was performed using DataXpress v1.4 (Axon Instruments, Union City, CA). The 5 peak currents before and after test article application were used to calculate the percentage of current inhibition at each concentration. Acceptance criteria for a good recording include: (1) seal resistance > 200 MΩ, (2) access resistance < 10 MΩ, (3) peak tail current > 200 pA, (4) leakage current < 25% of the peak tail current, (5) rundown < 2.5%/minute in control vehicle.

[00444] Counterscreens against the human IKs (hKvLQT1 + hminK) potassium channel using the PatchXpress® system. The extracellular recording solution was HB-PS. The internal recording solution contained 20 mM KF, 90 mM KCl, 10 mM NaCl, 10 mM EGTA, 5 mM K_2ATP, 1 mM $MgCl_2$, 10 mM HEPES adjusted to pH 7.20 with KOH. Stock solutions of reference standard or test articles were diluted into HB-PS prior to application. Test articles included either peptides or peptide conjugates described herein. Chromanol 293B (0.3 - 10 μM) was the reference standard. A standardized step protocol was used to elicit ionic current through the IKs potassium channel. Cells were held at −80 mV. Onset and steady state block of IKs potassium current due to Test Article was measured using a pulse pattern with fixed amplitudes (depolarizing test step to +50 mV for 5s) repeated at 10-s intervals. Currents is filtered at 3 kHz and acquired at 10 kHz, in episodic mode. When a good recording was established, cells were washed for 2 minutes, following by applying control vehicle for 5 minutes. Then control and each concentration of test article were applied for 5 minutes. There were 3 additions for each concentration with 1 minute interval. Dispense speed was 40 μL/s with suction on. Test article at 1 μM, 3 μM, 10 μM and 30 μM were applied to cells (n = 3 cells) cumulatively (without washout between test article concentrations) in ascending order, to each cell (n = 3 where n = number of cells). Each concentration of test article was applied for 5 minutes. There were 3 additions for each concentration with a 1 minute interval.

Electrophysiological data acquisition was performed using PatchXpress Commander v1.4 (Axon Instruments, Union City, CA) and analyses is performed using DataXpress v1.4 (Axon Instruments, Union City, CA). The 5 peak currents before and after test article application were used to calculate the percentage of current inhibition at each concentration. Acceptance criteria for a good recording includes: (1) seal resistance > 200 MΩ, (2) access resistance < 10 MΩ, (3) peak tail current > 200 pA, (4) leakage current < 25% of the peak current, (5) rundown < 2.5%/minute in control vehicle.

[00445] Counterscreens against the human IKr (hERG or hKv11.1) potassium channel by conventional whole cell patch clamp electrophysiology. One to 2 drops of the cell suspension is added to a 35 mm poly-d-lysine coated cover slip for overnight incubation before electrophysiology experiments. Whole-cell currents were recorded from single cells by using tight GΩ seal configuration of the patch-clamp technique. A 35 mm cover slip was transferred to the recording stage after rinsing and replacing the culture medium with extracellular recording buffer containing 135 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 10 mM HEPES, and 5 mM Glucose (pH was adjusted to 7.40 with NaOH and osmolarity was set at 300 mOsm). Cells were continuously perfused with the extracellular recording buffer via one of the glass capillaries arranged in parallel and attached to a motorized rod, which places the glass capillary directly on top of the cell being recorded. For hERG profiling, the recording pipette solution contained 130 mM KF, 2 mM $MgCl_2$, 10 mM EGTA, and 10 mM HEPES adjusted to pH 7.40 with KOH and osmolarity set at 280 mOsm. Experiments were performed at room temperature and recorded using Multiclamp 700A amplifier (Molecular Devices Inc.). Pipette resistances were typically 2-3 MΩ. Cells were held at a potential of -80 mV. To achieve a baseline or reference point for the peak outward tail current, a step to -50 mV for 500 ms was used. This was followed by a depolarizing step to +20 mV for 2 s to drive the channels to the inactivated state. A step back to -50 mV for 2s allowed the inactivation to be relieved and peak hERG current to be measured. Pulses were repeated once every 10 s. Total hERG current was measured as the difference between the peak current at the

repolarizing -50 mV step and the baseline current at -50 mV. Test articles (up to 10 μM), which included the peptides and peptide conjugates described herein, were mixed into the extracellular recording buffer containing 0.1% bovine serum albumin (BSA) and subsequently transferred to glass perfusion reservoirs. Electronic pinch valves controlled the flow of the test articles from the reservoirs onto the cell being recorded. IC50 values and curve fits were estimated using the four parameter logistic fit of XLfit software. The hERG channel inhibitor, cisapride, was used to validate the assay.

[00446] Counterscreens against calcium-activated potassium channels human IKCa1 and BKCa by conventional whole cell patch clamp electrophysiology. CHO IKCa and BKCa cell lines were obtained from BioFocus DPI (A Galapagos Company). One to 2 drops of the hIKCa1 or BKCa cell suspension is added to a 35 mm poly-d-lysine coated cover slip for overnight incubation before electrophysiology experiments. Whole-cell currents were recorded from single cells by using tight GΩ seal configuration of the patch-clamp technique. A 35 mm cover slip was transferred to the recording stage after rinsing and replacing the culture medium with the extracellular recording buffer containing 135 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 10 mM HEPES, and 5 mM Glucose (pH was adjusted to 7.40 with NaOH and osmolarity was set at 300 mOsm). Cells were continuously perfused with the extracellular recording buffer via one of the glass capillaries arranged in parallel and attached to a motorized rod, which places the glass capillary directly on top of the cell being recorded. The recording pipette solution contained 130 mM potassium aspartate, 1 mM $MgCl_2$, 1.26 mM $CaCl_2$, 2 mM EGTA, 2 mM Mg-ATP and 10 mM HEPES adjusted to pH 7.40 with KOH and osmolarity set at 280 mOsm. Experiments were performed at room temperature and recorded using Multiclamp 700A amplifier (Molecular Devices Inc.). Cells were held at potential of −80 mV. Both BK and IK currents were activated as calcium ion diffused into the cell from recording pipette solution. Activation of the calcium dependent outward potassium current by calcium diffusion generally takes 3 to 5 min for full activation. Outward currents were continuously monitored at holding potential of

+50 mV before and during drug exposure. Alternatively, 400 ms voltage ramps from −120 to +60 mV were repeated once every 10 s to characterize the current voltage relation for both channels before and during drug exposure. Test articles (up to 10 μM), which included the peptides and peptide conjugates described herein, were mixed into the extracellular recording buffer containing 0.1% bovine serum albumin (BSA) and subsequently transferred to glass perfusion reservoirs. Electronic pinch valves controlled the flow of the test articles from the reservoirs onto the cell being recorded. Pipette resistances were typically 2-3 MΩ. IC50 values and curve fits were estimated using the four parameter logistic fit of XLfit software. A IKCa and BK peptide inhibitor, charybdotoxin (100 nM), was applied at the conclusion of the assay procedures for pharmacological validation of the assay.

Example 7

Plasma Stability Studies

[00447] The stability of 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16) in rat, cynomolgus monkey and human plasma was tested by spiking the peptide conjugate into 100% plasma to a final concentration of 200 ng/mL and incubating for various periods of time at 37°C. At the end of each incubation period, samples were frozen until analysis. As a control, an aliquot of conjugate stock solution was diluted into plasma and immediately frozen to generate a time zero, untreated control. Analysis of stability included an ELISA analysis of drug levels (see Example 8, "Protocol 1") to confirm retention of the ShK immunoreactivity and whole blood assessment of potency to confirm retention of its bioactivity in blocking T cell responses. To assess bioactivity, serial dilutions of each stability sample were tested in the human whole blood bioassay described in Example 2 which measures T cell IL-2 and IFN-g production after thapsigargin stimulation. In brief, stability samples or standard were pre-incubated with whole blood for 30-60 minutes, prior to addition of thapsigargin to induce cytokine secretion. Supernatants were removed 48 hours later to measure the level of cytokine secretion. Since Kv1.3 inhibitors suppress cytokine secretion in this assay, the

level of cytokine suppression is a direct measure of the peptide conjugates bioactivity. As a control to define full T cell activation and cytokine secretion in the absence of inhibitor, each plate contained whole blood samples that were treated with thapsigargin alone or unstimulated. Dilutions from plasma stability study samples that provide 30% - 70% inhibition of the thapsigargin-induced cytokine response were used in analysis of stability. Figure 10A-D, indicates 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16) is stable when incubated in rat, cyno and human plasma for up to 48 hrs at 37degC and retains bioactivity and immunoreactivity. Figure 10A shows [Lys16]ShK (SEQ ID NO:13) structural moiety of SEQ ID NO:16 is stable in plasma over time as indicated by retention of the immunological epitope quantified by ELISA analysis as described in Example 8, below. Recovery % refers to the ELISA measured level of 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16) in the plasma stability sample, compared to the expected or initial 200 ng/ml concentration. As an example, a plasma stability sample measured to have 160 ng/ml, would be reported with a recovery % of 80. Figures 10B-D show the bioactivity of various dilutions of the plasma stability sample added to the thapsigargin whole blood assay to measure the extent of cytokine inhibition as a measure of the bioactivity of the 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16) molecule. The final dilution factor of these samples is listed to the right of Figure 10B and Figure 10D. Roughly half of the IL-2 cytokine response (measured in relative light units, RLU) was suppressed by 0.93 – 2.78% of the plasma stability samples and there was no significant change in the level of response (RLU units) over time at 37°C which indicates the 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16) molecule retains bioactivity and was stable in plasma.

Example 8

Pharmacokinetic & Pharmacodynamic Studies

[00448] Antibodies to ShK. Rabbit polyclonal and mouse monoclonal antibodies to ShK were generated by immunization of animals with the Fc-ShK

peptibody conjugate. Anti-ShK specific polyclonal antibodies were affinity purified from antisera to isolate only those antibodies specific for the ShK portion of the conjugate. Following fusion and screening, hybridomas specific for ShK were selected and isolated. Mouse anti-ShK specific monoclonal antibodies were purified from the conditioned media of the clones. By ELISA analysis, purified anti-ShK polyclonal and monoclonal antibodies reacted only to the ShK peptide alone and did not cross-react with Fc.

[00449] Pharmacokinetic (PK) studies on 20kDa-PEG-ShK (SEQ ID NO:8) and 20 kDa-PEG-[Lys16]ShK (SEQ ID NO:16) peptide conjugates in mice, rats, beagles and monkeys. Single subcutaneous doses were delivered to animals and serum was collected at various time points after injection. Studies in rats, dogs (beagles), and cynomolgus monkeys involved two to three animals per dose group, with blood and serum collection occuring at various time points over the course of the study. Male Sprague-Dawley (SD) rats (about 0.3 kg), male beagles (about 10 kg) and male cynomolgus monkeys (about 4 kg) were used in the studies described herein (n = 3 animals per dose group). Approximately 5 male CD-1 mice were used per dose and time point in our mouse pharmacokinetic studies. Serum samples were stored frozen at -80°C, until analysis in an enzyme-linked immunosorbent assay (ELISA).

[00450] A brief description of the ELISA protocol for detecting serum levels of PEG-ShK and PEG-[Lys16]ShK is provided below:

[00451] (1) Protocol 1, (a)-(g) below, detects PEG-ShK and PEG-[Lys16]ShK, as well as the ShK and [Lys16]ShK peptides alone:

[00452] (a) Streptavidin microtiter plates were coated with 250 ng/ml biotinylated-anti-ShK mouse monoclonal antibody (mAb2.10, Amgen) in I block buffer [per liter: 1000 mL 1XPBS without $CaCl_2$, $MgCl_2$, 5 ml Tween 20 (Thermo Scientific), 2 g I block reagent (Tropix)] at 4°C, incubated overnight without shaking.

[00453] (b) Plates were washed three times with KPL wash buffer (Kirkegaard & Perry Laboratories).

[00454] (c) Standards (STD), quality controls (QC) and sample dilutions were prepared with 100% pooled sera, then diluted 1/5 (pretreatment) in I block buffer. Pretreated STDs, QCs and samples were added to the washed plate and incubated at room temperature for 2 hours. (Serial dilutions of STDs, QCs were prepared in 100% pooled sera. Samples needing dilution were also prepared with 100% pooled sera. The pretreatment was done to both stds, QCs and samples to minimize the matrix effect.)

[00455] (d) Plates were washed three times with KPL wash buffer.

[00456] (e) A HRP-labeled rabbit anti-ShK polyclonal Ab at 250 ng/ml in I block buffer was added and plates were incubated at room temperature for 1 hour with shaking.

[00457] (f) Plates were again washed three times with KPL wash buffer and the Femto [Thermo Scientific] substrate was added.

[00458] (g) The plate was read with a Lmax II 384 (Molecular Devices) luminometer.

[00459] Pharmacokinetic (PK) studies on Fc-, Ig-, or Ab conjugates of ShK and [Lys16]ShK were performed in male SD rats. Single subcutaneous doses were delivered to animals and serum was collected at various time points after injection. Three animals were used per dose group, with blood and serum collection occuring at various time points over the course of the study. Serum samples were stored frozen at -80°C, until analysis in an enzyme-linked immunosorbent assay (ELISA). A brief description of the ELISA protocol for detecting serum levels of Fc-, Ig-, or Ab-conjugates of ShK and [Lys16]ShK is provided below. Protocol 2, below detects both the human Ig, Fc or Ab portion of the molecule, as well as the ShK peptide portion. Protocol 3, below is an early assay that detects the human Fc region alone and was used for early assessment of serum levels of Fc-ShK peptibodies in rodent pharmacokinetic studies. A brief description of these ELISA protocols is provided:

[00460] (2) Protocol 2, (a)-(g) below, detects both the human Ig, Fc or Ab portion of the molecule, as well as the ShK peptide portion:

[00461] (a) Streptavidin microtiter plates were coated with 250 ng/ml biotinylated-anti-ShK mouse monoclonal antibody (mab 2.10, Amgen) in I block buffer [per liter: 1000 ml 1XPBS without $CaCl_2$, $MgCl_2$, 5 ml Tween 20 (Thermo Scientific), 2 g I block reagent (Tropix)] at 4°C, overnight without shaking;

[00462] (b) Plates were washed three times with KPL wash buffer (Kirkegaard & Perry Laboratories)

[00463] (c) Standards (STD), quality controls (QC) and sample dilutions with 100% pooled sera were prepared, then were diluted 1/5 (pretreatment) in I block buffer. Pretreated STDs, QCs and samples were added to the washed plate. Incubation was at room temperature for 2hours. (Serial dilutions of STDs, QCs were prepared in 100% pooled sera. Samples needing dilution were also prepared with 100% pooled sera. The pretreatment was done to both stds, QCs and samples to minimize the matrix effect.);

[00464] (d) Plates were washed three times with KPL wash buffer;

[00465] (e) A HRP-labeled Ab35 (against human IgG Fc) at 150 ng/ml in I block buffer was added and plates were incubated at room temperature for 1hour with shaking.

[00466] (f) Plates were washed three times with KPL wash buffer and the Femto [Thermo Scientific] substrate was added;

[00467] (g) The plate was read with a Lmax II 384 [Molecular Devices] luminometer.

[00468] (3) Protocol 3, (a)-(h) below, is an early assay that detects the human Fc region alone and was used for early assessment of serum levels of Fc-ShK peptibodies in rodent pharmacokinetic studies:

[00469] (a) Costar 3590 96-well EIA/RIA plates were coated with 0.1 mL/well of 2 μg/mL Goat anti-HuFc, $F(ab')_2$, (Sigma I-3391) diluted in 1x Coating Buffer (10x Coating Buffer: 1.59 g Na_2CO_3, 2.93 g $NaHCO_3$ in 100 ml H_2O). Plates were sealed and incubated at 4°C overnight;

[00470] (b) Plates were washed three times with PBST (PBS + 0.1% Tween-20) and blocked by addition of 0.3 ml of blotto (PBS, 0.1% Tween-20, 5%

non-fat dry milk) to each well and incubated for 1 h at room temperature (RT) with shaking;

[00471] (c) Plates were washed with a KP Wash Solution (Cat #50-63-00, KPL, Gaithersburg, MD);

[00472] (d) Diluted serum samples and controls/standards in Dilution Buffer (PBS, 0.1% BSA, 0.1% Tween-20) plus rat serum, if needed, were brought to 10% rat serum final and 0.1ml sample was added per well. Plates were incubated at room temperature with shaking for 1 hour;

[00473] (e) Plates were washed with a KP Wash Solution (Cat #50-63-00, KPL, Gaithersburg, MD);

[00474] (f) A HRP labeled secondary antibody (Pierce #31416-HRP Goat α-Hu IgG Fc) was diluted 1:5000 in PBST and then 100 µl/well is added and incubated at RT with shaking for 1 hour;

[00475] (g) Plates were washed with a KP Wash Solution (Cat #50-63-00, KPL, Gaithersburg, MD) and 100 µl/well of ABTS substrate (ABTS Microwell Substrate 1-Component, Cat#50-66-018, KPL) was added;

[00476] (h) At appropriate times after substrate addition and shaking, the plate was read with a SpectraMax340 [Molecular Devices] plate reader.

[00477] Comparison of the rat pharmacokinetics of our novel toxin conjugates to the published pharmacokinetic profile of the small ShK-L5 (SEQ ID NO:17) peptide. The pharmacokinetics of a single 0.2 mg/kg subcutaneous dose of ShK-L5 (SEQ ID NO:17) in rats was reported by Beeton et al. (Mol. Pharmacol. 67, 1369-1381, 2005). ShK-L5 was reported to have a circulating half-life estimated to be about 50 min. Beeton et al. (ibid) provide two figures showing serum ShK-L5 concentrations (nM) verus time after a single 0.2 mg/kg subcutaneous injection. Based on the data from those figures, a single 0.2 mg/kg dose of ShK-L5 was estimated to provide the following ng/ml serum concentration per unit time in minutes after subcutaneous injection: 10(5), 23(10), 28(20), 50(30), 34(60), 19(120), 10(180), 2(420), where values in parenthesis are time in minutes and the ShK-L5 molecular weight (MW) was set to about 4050 Da. Figure 4 illustrates that 20kDa-PEG-ShK (SEQ ID NO:8) molecule has an

extended half-life and provides much greater exposure in rats than the ShK-L5 (SEQ ID NO:17) molecule. Because only the peptide portion of 20kDa-PEG-ShK (SEQ ID NO:8) was used in calculating mg/mL stock concentrations, and this ShK peptide portion (4055 Da) is of similar MW to ShK-L5 (SEQ ID NO:17), equivalent mg/kg doses of these two molecules generate approximately equivalent nmol/kg doses. The calculated pharmacokinetic values from this analysis of ShK-L5 (SEQ ID NO:17) and the 20kDa-PEG-ShK (SEQ ID NO:8) molecule, is provided in Table 4B. The clearance (CL/F) of PEG-ShK (SEQ ID NO:8) in rats is about 40 times slower than ShK-L5 (SEQ ID NO:17) and the mean residence time (MRT) of PEG-ShK is 13-18 times longer. Example 12 and Tables 4I-K, provide results from rat pharmacokinetic studies on exemplary Fc, Ig and Ab-ShK toxin peptide analog conjugate embodiments that demonstrate they have extended half-life in vivo and improved therapeutic potential. The anti-KLH-Ab-[Lys16]ShK Ab molecules, in both bivalent and monovalent forms (represented schematically in Figure 12G and Figure 12F, respectively), exhibit an extended half-life in vivo, which is approximately 32-60 times as long as that reported by Beeton et al. (2005, ibid) for ShK-L5. (See, Example 12, Figure 21) Since the anti-KLH-Ab-[Lys16] ShK Ab molecules have a MW of about 150 kDa, a 6 mg/kg dose of these molecules on a nmol/kg basis would be equivalent to a 0.16 mg/kg of the smaller (4 kDa) ShK (SEQ ID NO:1) or ShK-L5 (SEQ ID NO:17) peptide. Therefore, the 0.2 mg/kg PK study on ShK-L5 provides a nmol/kg dose that is very similar (within 25%) to that achieved with a 6 mg/kg dose of the larger anti-KLH-Ab-[Lys16]ShK Ab molecules. Our monovalent anti-KLH-Ab-[Lys16]ShK molecule exhibited very slow clearance in rats (CL/F = 10.9 $mL \bullet hr^{-1} \bullet kg^{-1}$), a rate that was about 188 times slower than ShK-L5 (CL/F = 2052 $mL \bullet hr^{-1} \bullet kg^{-1}$). The monovalent KLH-Ab-[Lys16]ShK molecule provided about 6100 times greater drug exposure in rats (AUC0-inf = 594000 $ng \bullet hr \bullet mL^{-1}$) compared to the small ShK-L5 peptide (AUC0-inf = 97 $ng \bullet hr \bullet mL^{-1}$) (see Table 4B and Table 4J). If one normalizes for MW differences between these two molecules (150 kDa / 4 kDa), the monovalent KLH-Ab-[Lys16]ShK molecule provided about 163 times greater exposure (based on AUC0-inf) than the small ShK-L5 (SEQ ID

NO:17) molecule. The monovalent aKLH-AbLoop-[Lys16]ShK Ab molecule, described in detail in Example 11 and Example 12, exhibited the slowest clearance in rats of all the novel toxin-conjugates that we have examined (Figure 22 and Table 4L). This molecule's clearance (CL/F = 3.11 $mL \cdot hr^{-1} \cdot kg^{-1}$) was about 660 times slower than ShK-L5.

[00478] Ex vivo cynomolgus monkey whole blood assay to measure the potency of 20kDa-PEG-[Lys16]ShK and its level of pharmacodynamic coverage in vivo. The potency and level of coverage of cynomolgus monkey T cell responses was determined with an ex vivo whole blood assay measuring thapsigargin-induced IL-4, IL-5 and IL-17. To determine potency of peptides and peptide conjugates, cynomolgus whole blood was obtained from healthy, naïve, male monkeys in a heparin vacutainer. DMEM complete media was Iscoves DMEM (with L-glutamine and 25 mM Hepes buffer) containing 0.1% human albumin (Gemini Bioproducts, #800-120), 55 μM 2-mercaptoethanol (Gibco), and 1X Pen-Strep-Gln (PSG, Gibco, Cat#10378-016). Thapsigargin was obtained from Alomone Labs (Jerusalem, Israel). A 10 mM stock solution of thapsigargin in 100% DMSO was diluted with DMEM complete media to a 40 μM, 4X solution to provide the 4X thapsigargin stimulus for calcium mobilization. The Kv1.3 inhibitor peptide ShK (Stichodacytla helianthus toxin, Cat# H2358) and the BKCa1 inhibitor peptide IbTx (Iberiotoxin, Cat# H9940) were purchased from Bachem Biosciences, whereas the Kv1.1 inhibitor peptide DTX-k (Dendrotoxin-K) was from Alomone Labs (Israel). The calcineurin inhibitor cyclosporin A (CsA) is available commercially from a variety of vendors. Whereas the BKCa inhibitor IbTx and the Kv1.1 inhibitor DTX-k do not inhibit the cytokine response, the Kv1.3 inhibitor ShK and the calcineurin inhibitor CsA inhibit the cytokine response and are used routinely as standards or positive controls. Ten 3-fold serial dilutions of standards, ShK analogs or ShK-conjugates were prepared in DMEM complete media at 4X final concentration and 50 μl of each were added to wells of a 96-well Falcon 3075 flat-bottom microtiter plate. Whereas columns 1-5 and 7-11 of the microtiter plate contained inhibitors (each row with a separate inhibitor dilution series), 50 μl of DMEM complete media alone was added to the

8 wells in column 6 and 100 µl of DMEM complete media alone was added to the 8 wells in column 12. To initiate the experiment, 100 µl of whole blood was added to each well of the microtiter plate. The plate was then incubated at 37°C, 5% CO_2 for one hour. After one hour, the plate was removed and 50 µL/well of the 4X thapsigargin stimulus (40 µM) was added to all wells of the plate, except the 8 wells in column 12. The plates were placed back at 37°C, 5% CO_2 for 48 hours. To determine the amount of IL-4, IL-5 and IL-17 secreted in whole blood, 100 µL of the supernatant (conditioned media) from each well of the 96-well plate was transferred to a storage plate. For Meso Scale Discovery (MSD) electrochemiluminesence analysis of cytokine production, the supernatants (conditioned media) were tested using MSD Multi-Spot Custom Coated plates (Meso Scale Discovery, Gaithersburg, MD). The working electrodes on these plates were coated with seven Capture Antibodies (hIL-2. hIL-4, hIL-5, hIL-10, hTNFa, hIFNg and hIL-17) in advance. After blocking plates with MSD Human Serum Cytokine Assay Diluent, and then washing with PBS containing 0.05% of BSA, 25 µL/well of conditioned medium was added to wells of the MSD plate. The plates were covered and placed on a shaking platform for 1hr. Next, 25 µL of a cocktail of Detection Antibodies in MSD Antibody Diluent were added to each well. The cocktail contained seven Detection Antibodies (hIL-2, hIL-4, hIL-5, hIL-10, hTNFa, hIFNg and hIL-17) at 1 µg/mL each. The plates were covered and placed on a shaking platform overnight (in the dark). The next morning the plates were washed three times with PBS buffer. 150 µl of 2X MSD Read Buffer T was added to wells of the plate before reading on the MSD Sector Imager. Since the 8 wells in column 6 of each plate received only the thapsigargin stimulus and no inhibitor, the average MSD response here was used to calculate the "High" value for a plate. The calculate "Low" value for the plate was derived from the average MSD response from the 8 wells in column 12 which contained no thapsigargin stimulus and no inhibitor. Percent of control (POC) is a measure of the response relative to the unstimulated versus stimulated controls, where 100 POC is equivalent to the average response of thapsigargin stimulus alone or the "High" value. Therefore, 100 POC represents 0% inhibition of the response. In

contrast, 0 POC represents 100% inhibition of the response and would be equivalent to the response where no stimulus is given or the "Low" value. To calculate percent of control (POC), the following formula is used: [(MSD response of well) – ("Low")] / [("High") – ("Low")] x 100. The potency of the molecules in whole blood was calculated after curve fitting from the inhibition curve (IC) and IC50 was derived using standard curve fitting software. Although we describe here measurement of cytokine production using a high throughput MSD electrochemillumenescence assay, one of skill in the art can readily envision lower throughput ELISA assays are equally applicable for measuring cytokine production.

[00479] Ex vivo cynomolgus monkey pharmacodynamic (PD) assay to measure the level of T cell Kv1.3 coverage in vivo following dosing of animals. To determine the level of target coverage in monkeys prior to or following dosing with the Kv1.3 inhibitor 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16), whole blood was removed and tested for level of T cell cytokine secretion following thapsigargin stimulation. Example 10 provides the results using this assay of levels of coverage in a 12-week study in cynomolgus monkeys, involving one month of weekly dosing with 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16). To determine level of suppression in animals, prior to thapsigargin addition, each whole blood sample was split into two aliquots, one aliquot being untreated to measure level of drug in the animal and the second aliquot spiked with an excess of 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16; 100 nM) as a control of full suppression. After preincubation at 37°C, 5% CO_2 for one hour, thapsigargin is added to blood to stimulate cytokine secretion and conditioned media is collected 48 hours later. A detailed description of the procedure follows. Cynomolgus whole blood was collected from untreated or 20kDa-PEG-[Lys16]ShK-treated healthy, naïve, male cynomolgus monkeys by arm-pull into a heparin vacutainer. Monkeys voluntarily presented arms for a grape incentive, allowing for injections and blood draws in the absence of any sedatives or stress. DMEM complete media was Iscoves DMEM (with L-glutamine and 25 mM Hepes buffer) containing 0.1% human albumin (Gemini Bioproducts, #800-120), 55 μM 2-

mercaptoethanol (Gibco), and 1X Pen-Strep-Gln (PSG, Gibco, Cat#10378-016). Thapsigargin was obtained from Alomone Labs (Jerusalem, Israel). A 10 mM stock solution of thapsigargin in 100% DMSO was diluted with DMEM complete media to a 40 μM, 4X solution to provide the 4X thapsigargin stimulus for calcium mobilization. PEG peptide inhibitor of Kv1.3, 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16) was prepared in DMEM complete media to a 400 nM, 4X final concentration. The assay was set up in a 96-well Falcon 3075 flat-bottom microtiter plate. 100 μl/well of DMEM complete media was added to columns 1 and 2 as a negative control. Columns 3-7 received 50 μl/well of DMEM complete media, while columns 8-12 received 50 μl/well of the 4X 20kDa-PEG-[Lys16]ShK inhibitor (400 nM) in DMEM complete media. To initiate the experiment, 100 μL of whole blood from one monkey was added to each well of one row of the microtiter plate. The plate was then incubated at 37°C, 5% CO_2 for one hour. After one hour, the plate was removed and 50 μl of the 4X thapsigargin stimulus (40 μM) was added to wells in columns 3-12. The plates were placed back at 37°C, 5% CO_2 for 48 hours. To determine the amount of IL-4, IL-5 and IL-17 secreted in whole blood, 100 μL of the supernatant (conditioned media) from each well of the 96-well plate was transferred to a storage plate. For MSD electrochemiluminesence analysis of cytokine production, supernatants (conditioned media) were tested on MSD Multi-Spot Custom Coated plates (Meso Scale Discovery, Gaithersburg, MD). The working electrodes on these plates were coated with seven Capture Antibodies (hIL-2. hIL-4, hIL-5, hIL-10, hTNFa, hIFNg and hIL-17) in advance. After blocking plates with MSD Human Serum Cytokine Assay Diluent, and then washing with PBS containing 0.05% of BSA, 25 μl of conditioned media was added to the MSD plate. The plates were covered and placed on a shaking platform for 1hr. Next, 25 μL/well of a cocktail of Detection Antibodies in MSD Antibody Diluent were added to each well. The cocktail contained seven Detection Antibodies (hIL-2, hIL-4, hIL-5, hIL-10, hTNFa, hIFNg and hIL-17) at 1 μg/mL each. The plates were covered and placed on a shaking platform overnight (in the dark).The next morning the plates were washed three times with PBS buffer. 150 μl of 2X MSD Read Buffer T was

added to the plate before reading on the MSD Sector Imager. Since the wells in columns 3-7 of each plate received only the thapsigargin stimulus and had no spike with excess inhibitor, the average MSD response here was used to calculate the "Test" value in a row for each animal to detect the in vivo level of drug present in each animal. The calculated "Negative Control" value for each animal, was calculated from the average MSD response in columns 1-2 of each row, which received neither thapsigargin nor inhibitor spike. The "Positive Control" value for the animal was derived from the average MSD response from the 5 wells in columns 8-12 which contained thapsigargin stimulus and a spike of exogenous inhibitor. Percent of Inhibition (POI) is a measure of the relative thapsigargan-induced cytokine response of the same blood samples either spiked with exogenous inhibitor or untreated, where 100 POI is equivalent to the average response of thapsigargin stimulus plus exogenous inhibitor spike. Therefore, 100 POI represents 100% inhibition of the response. In contrast, 0 POI represents 0% inhibition of the response. To calculate percent of inhibition (POI), the following formula is used: [("Positive Control" ECL counts) – ("Negative Control" ECL counts)] / [("Test" ECL counts) – ("Negative Control" ECL counts)] x 100. Percent of control (POC) is calculated using the formula, POC = (100 – POI). Although we describe here measurement of cytokine production using a high throughput MSD electrochemilumenescence assay, one of skill in the art can readily envision lower throughput ELISA assays are equally applicable for measuring cytokine production.

Example 9

Adoptive-Transfer EAE Model of Efficacy

[00480] Using an adoptive transfer experimental autoimmune encephalomyelitis (AT-EAE) model of multiple sclerosis in rats described earlier [C. Beeton et al. (2001) J. Immunol. 166, 936], we examined the activity in vivo of our Kv1.3 selective 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16) and compared its efficacy to the less selective 20kDa-PEG-ShK molecule (SEQ ID NO:8). The 20kDa-PEG-[Lys16]ShK molecule delivered subcutaneously (SC) daily at 10, 100

and 1000 μg/kg (day −1 to day 7) significantly reduced disease severity and increased survival, whereas rats treated with vehicle developed severe disease and died (Figure 7 and Figure 8A-D). Rats treated daily with 0.04 μg/kg 20kDa-PEG-ShK also showed significant disease amelioration.

[00481] The encephalomyelogenic CD4+ rat T cell line, PAS, specific for myelin-basic protein (MBP) was kindly provided by Dr. Evelyne Beraud. The maintenance of these cells in vitro and their use in the AT-EAE model has been described earlier [C. Beeton et al. (2001) PNAS 98, 13942]. PAS T cells were maintained in vitro by alternating rounds of antigen stimulation or activation with MBP and irradiated thymocytes (2 days), and propagation with T cell growth factors (5 days). Activation of PAS T cells (3×10^5/ml) involved incubating the cells for 2 days with 10 μg/ml MBP and 15×10^6/ml syngeneic irradiated (3500 rad) thymocytes. On day 2 after in vitro activation, $10\text{-}15 \times 10^6$ viable PAS T cells were injected into 6-12 week old female Lewis rats (Charles River Laboratories) by tail IV. Daily subcutaneous injections of vehicle (2% Lewis rat serum in PBS), 20kDa-PEG-[Lys16]ShK or 20kDa-PEG-ShK were given from day −1 to day 7 (Figure 7 and Figure 8A-D), where day −1 represents 1 day prior to injection of PAS T cells (day 0 in Figure 7). Serum was collected by retro-orbital bleeding at day 4 and by cardiac puncture at day 8 (end of the study) for analysis of levels of inhibitor. Rats were weighed on days −1 and days 4 - 8. Animals were scored blinded once a day from the day of cell transfer (day 0) to day 3, and twice a day from day 4 to day 8. Clinical signs were evaluated as the total score of the degree of paresis of each limb and tail. Clinical scoring ("EAE Score" in Figure 7 and Figure 8A-D): 0 = No signs, 0.5 = distal limp tail, 1.0 = limp tail, 2.0 = mild paraparesis, ataxia, 3.0 = moderate paraparesis, 3.5 = one hind leg paralysis, 4.0 = complete hind leg paralysis, 5.0 = complete hind leg paralysis and incontinence, 5.5 = tetraplegia, 6.0 = moribund state or death. Rats reaching a score of 5.5 were euthanized. The in vivo activity of other conjugates in this model, are described within the Examples disclosed herein. Under some circumstances prior to testing peptide conjugates for efficacy in vivo, conjugates were tested in vitro for their activity in inhibiting antigen (myelin)-mediated

proliferation (3H-thymidine incorporation) of the rat T effector memory cell line, PAS. The methods employed here were similar to those described in C. Beeton et al., PNAS 98, 13942 (2001), and are well known to those skilled in the art.

[00482] Treatment of rats with the Kv1.3 blocker 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16) prior to the onset of EAE caused a lag in the onset of disease, inhibited the progression of disease, and prevented death in a dose-dependent manner (Figure 7). Onset of disease in rats treated with vehicle alone occured on day 4.0, whereas onset was delayed until day 4.5 to day 5.0 in animals treated with 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16). In addition to delaying onset, treatment with 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16) significantly reduced disease severity in a dose-dependent manner. Whereas vehicle treated rats on day 6 had severe disease (EAE score of 6) and were sacrificed, animals treated with the two highest doses of 20kDa-PEG-[Lys16] ShK (SEQ ID NO:16) had only mild disease and an EAE score of about 1. The 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16) molecule provided potent blockade of experimental autoimmune encephalomyelitis and had a calculated ED50 of about 4.4 μg/kg, based on a dose response assessment of the data of day 7, pm (Figure 8C).

Example 10

Pharmacology Studies in Cynomolgus Monkeys; Determination of PEG-[Lys16]ShK (SEQ ID NO:16) in Cerebral Spinal Fluid (CSF); Toxicologic Exploration & Mast Cell Degranulation Studies

[00483] A repeat-dose pharmacology study was designed and implemented in order to investigate the long-term effects of the 20kDa-PEG-[Lys16]ShK molecule (SEQ ID NO:16) in nonhuman primates. Prior to initiating the study, 6 to10 male cynomolgus monkeys were profiled for a period of 3-10 weeks to allow for assessment of the end-points stability over time and selection of 6 cynos for the study. End-points measured, included complete blood counts (CBCs), blood chemistry, FACS analysis of lymphocyte subsets and the ex vivo whole blood PD assay measuring cytokine response and target coverage, as described in Example 8. Subsets analyzed by FACS included: lymphocytes, $CD4^+$, $CD4^+$ naïve, $CD4^+$

T_{CM}, $CD4^+$ T_{EM}, $CD4^+CD28^-CD95^-$, $CD8^+$, $CD8^+$ naïve, $CD8^+$ T_{CM}, $CD8^+$ T_{EM}, $CD8^+CD28^-CD95^-$, B cells, NK cells, and NKT cells. Monkeys with the highest level of CD4+ effector memory T cells were chosen. Using the cyno whole blood PD assay, 20kDa-PEG-[Lys16]ShK had an IC_{50} of 0.09±0.08 nM for blockade of the IL-17 response and an IC_{50} of 0.17±0.13 nM for blockade of the IL-4 response (n = 7 monkeys). A 0.5 mg/kg weekly 20kDa-PEG-[Lys16]ShK dose was selected which would provide excess target coverage for a period of one month. This dose, based on early pharmacokinetic studies (see, Example 4) was estimated to provide about 28 fold coverage (28 nM) of the 20kDa-PEG-[Lys16]ShK IC_{95} (1.0 nM) in human whole blood. Table 4F illustrates the design of the 12-week cynomolgus pharmacology study.

[00484] Male Chinese cynomolgus monkeys were used in this study that were naïve (no earlier exposure to drugs). Care was taken to avoid undue stress. All injections and blood draws were done by arm-pull, with the monkeys voluntarily presenting their arm for a grape incentive. The study involved baseline measures for two weeks (3 predose samples), one month of Kv1.3 block (qw dosing of 20kDa-PEG-[Lys16]ShK [SEQ ID NO:16]) and 6 weeks follow-up analysis.

[00485] Excess coverage of the Kv1.3 target for one month by 20kDa-PEG-[Lys16]ShK was well tolerated. Clinical pathology measures of hematology and blood chemistry showed no changes relative to baseline measures (Table 4G) over the entire one month period. Animal weight increased progressively over the study and PEG-[Lys16]ShK had no impact on weight gain (Figure 9D). The PD assay of coverage, as measured by IL-4 and IL-17 serum concentration (Figure 9A-B), indicated the target was fully suppressed over the entire one month dosing period (from week 3 though week 6) and there was no evidence of loss of coverage over time. Based on this finding, there was no evidence of a clearing antibody response to the [Lys16]ShK conjugate or compensatory ion channels, since both of these responses would be expected to result in release of the PD suppression over time. In addition, since predicted and observed serum drug levels of 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16) matched closely (Figure 9C),

these data also indicate that the PEG-[Lys16]ShK (SEQ ID NO:16) conjugate did not generate an immunogenic response resulting in clearing antibodies over the one month dosing period. Others have suggested that PEGylation of peptides and proteins can reduce immunogenicity and have a beneficial effect in shielding or protecting peptides and proteins from an immunological response (F.M. Veronese and A. Mero, Biodrugs 22: 315 (2008)). Since ShK (SEQ ID NO:1) and [Lys16]ShK (SEQ ID NO:13) are peptides foreign to cynos, PEGylated toxin peptide (or toxin peptide analog)-conjugates may have enhanced protection over the unconjugated, free peptides alone. Alternatively, it was thought that ShK (SEQ ID NO:1) and [Lys16]ShK (SEQ ID NO:13) may be intrinsically of low immunogenicity since these peptides have a very small compact structure. After dosing cessation, there was no evidence of a "rebound" effect based on cytokine response, blood chemistry and cellular profiling. During dosing and follow-up analysis there were no changes in CD4 or CD8 T cell subsets, NK cells, NKT cells and B cells relative to pre-dose, baseline measures. In conclusion, 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16) provided potent blockade of T cell responses in cynomolgus monkeys and was well tolerated at doses providing excess coverage of the target for an extended period of time.

[00486] Although the 12-week pharmacology study on PEG-[Lys16]ShK (SEQ ID NO:16) indicated weekly dosing for one month provided sustained coverage and expected drug levels, further analysis of serum samples from this study indicated 4 of the 6 monkeys had anti-drug antibodies which emerged during the third week of dosing (week 5, Table 4F) and peaked at week 9. To examine further the immunogenicity of PEG-[Lys16]ShK (SEQ ID NO:16), 5 of the 6 cynomolgus monkeys dosed more than a year earlier with PEG-[Lys16]ShK (SEQ ID NO:16) were available and re-challenged with drug. The study involved baseline measures for two weeks, 0.5 mg/kg PEG-[Lys16]ShK (SEQ ID NO:16) weekly dosing for seven weeks and four weeks of follow up analysis. Pharmacokinetics were measured after the first and sixth week of dosing, and serum and blood were collected weekly throughout the study to measure drug exposure, CBCs and blood chemistry, levels of anti-drug antibody and the

pharmacodynamic level of target coverage. PEG-[Lys16]ShK (SEQ ID NO:16) was well tolerated upon re-challenge. All monkeys gained weight over the course of the study and there were no changes in CBCs or blood chemistry. Upon re-challenge, an antibody response in 3 of the 5 animals with binding specificity against the [Lys16]ShK peptide was detected, and antibody concentrations to the drug exceeding 10 μg/ml were observed in two monkeys. These 2 animals also tested positive for neutralizing antibodies in a bioassay. In the re-challenge study, after the sixth dose of PEG-[Lys16]ShK (SEQ ID NO:16) (0.5 mg/kg, weekly), exposure as measured by area under the curve ($AUC_{0\text{-}96h}$) was observed to increase by 1.8, 2.0, 3.0, 8.8 and 9.2 fold in the five monkeys, using an LC-MS/MS method detecting total drug levels. The changes in AUC for PEG-[Lys16]ShK (SEQ ID NO:16) upon multiple dosing were also accompanied by increases in Cmax and apparent half-life. While higher than expected accumulation occurred in all animals, the highest AUC increases (8.8 and 9.2 fold) were observed in animals having the highest antibody concentration. The same two monkeys exhibited extended whole blood pharmacodynamic (PD) coverage after dosing cessation, which was consistent with the higher exposure to PEG-[Lys16]ShK (SEQ ID NO:16). It can be concluded that the development of anti-drug antibodies in these two animals may have been associated with the excessive accumulation of drug and extended PD impact in these animals. Because more, not less, pharmacodynamic coverage was observed in the two animals showing anti-drug antibodies, it is possible that prolonged suppression of the Kv1.3-dependent whole blood PD response is caused by both elevated drug levels and a PEG-ShK[Lys16] (SEQ ID NO:16) affinity for T-cell Kv1.3 that is higher than the PEG-ShK[Lys16] (SEQ ID NO:16) affinity for the antibody. Further studies are necessary to clarify this.

[00487] Despite the occurrence of anti-drug antibodies in monkeys, it is unclear whether a similar response would occur in humans since preclinical immunogenicity is not predictive of human immunogenicity (Ponce et al. Regulatory Toxicology and Pharmacology **54**, 164–182 (2009)). Exenatide represents an example of an approved drug for treatment of type 2 diabetes

mellitus that shows anti-drug antibodies in humans, but remains safe and effective (Faludi et al. Peptides **30**, 1771–1774 (2009); Malone et al. Expert Opin. Investig. Drugs **18**, 359-367 (2009); Schnabel et al. Peptides **27**, 1902-1910 (2006)).

[00488] Determination of PEG-[Lys16]ShK (SEQ ID NO:16) in Cerebral Spinal Fluid (CSF). To address concerns that ShK peptide-based inhibitors have some propensity for non specific targeting of the Kv1.1 (a potassium channel that has been shown to be important in the regulation of neuronal activity in the brain), we evaluated the ability of PEG-[Lys16]ShK (SEQ ID NO:16) to enter cerebral spinal fluid (CSF). PEG-[Lys16]ShK (SEQ ID NO:16) concentrations were evaluated in the CSF of cynomolgus monkeys and Sprague-Dawley rats. In one study, PEG-[Lys16]ShK (SEQ ID NO:16) was measured in the CSF of monkeys 24 and 48 hours after subcutaneous administration. These data are shown in Table 4G(a) below. CSF concentrations were small in comparison to serum concentrations for both monkeys and rats. As shown in Table 4G(a), the highest concentration of PEG-[Lys16]ShK (SEQ ID NO:16) measured in the CSF of monkeys was 7.62 ng/mL. This sample was observed visually to be contaminated with blood. The highest concentration measured in an uncontaminated sample was 3.83 ng/mL, which represents a serum (ng/mL) to CSF (ng/mL) ratio of 292. Table 4G(b) shows the CSF concentrations of PEG-[Lys16]ShK (SEQ ID NO:16) measured in rats. The highest concentrations were 43 ng/mL and 12.7 ng/mL, representing serum (ng/mL) to CSF (ng/mL) ratios of 46.5 and 470, respectively. Both these samples were taken from rats sacrificed early in the study (6 hrs). Of the rats that survived to the end of the study (48 hr), only one had an PEG-[Lys16]ShK (SEQ ID NO:16) concentration that could be detected above the assay limit of quantitation (1 ng/mL). This CSF sample had a concentration of 5.70 ng/mL, which was nearly identical to the measured serum concentration, making it an outlier compared to all other samples and supporting the conclusion that it was also contaminated with serum during sampling.

[00489] Mast cell degranulation studies. Because the native peptide ShK impacts Kv1.3 and neuronal Kv1.1, concerns have been expressed that a small fraction of drug crossing the blood-brain barrier could cause off-target impacts on

neuronal Kv1.1 and paralysis (Kalman et al., J. Biol. Chem. 273, 32697-32707 (1998)). In pharmacokinetic studies of ShK, PEG-ShK and PEG-[Lys16]ShK (SEQ ID NO:16) in Sprague-Dawley rats, we noted severe adverse events immediately following injection of large IV bolus doses. Shortly after 2.0 and 5.0 mg/kg bolus IV injections of PEG-[Lys16]ShK (SEQ ID NO:16) which generated serum C_0/C_{max} levels greater than 10,000 ng/ml (>2500 nM) which were more than 25,000 times the molecules IC_{50} in blocking T cell cytokine production in whole blood (0.1 nM), rats were moribund and at the bottom of the cage. Two of the three animals died at the 5 mg/kg high dose group, whereas all animals receiving the 2 mg/kg dose survived. Other observations included discolored skin (blue paws, ears) and thick blood. Initially these transient effects were attributed to an impact on neuronal Kv1.1, however, treated rats exhibited no seizures, expressed other symptoms inconsistent with a CNS impact (e.g. thick blood) and the Kv1.3 selective PEG-[Lys16]ShK conjugate (SEQ ID NO:16) caused similar effects despite being 1000 fold less potent on Kv1.1. In single-dose subcutaneous pharmacokinetic studies in rats, whereas animals receiving low doses (0.1 and 0.5 mg/kg) appeared normal and gained weight, 2 of 3 animals treated with 5.0 mg/kg were lethargic, lost weight and exhibited mild swelling of the forelimb and hindlimb 24 hours post-dose. The occurrence of symptoms 24 hours post-dose correlated with Cmax and the animals recovered over time and gained weight. Interestingly, these effects were not observed in mouse, cyno or dog pharmacokinetic studies despite equal or higher exposure. In an attempt to understand the unique findings in rats, literature was reviewed for studies that described molecules that produced related symptoms and exhibited species selectivity. Basic secretagogues, such as mast cell degranulating peptide (MCDP), were reported to show a differential impact on mast cells from different species, with rat showing increased sensitivity to some cationic molecules (Barrett & Pearce Int. Archs. Allergy appl Immun. **72**, 234-238 (1983); M. Mousli et al. Immunopharmacology **27**, 1-11 (1994); Keller Int. Arch. Allergy **34**, 139-144 (1968); Leung & Pearce Br. J. Pharmac. **81**, 693-701 (1984)). Since ShK is a basic peptide like MCDP and mast cell degranulation could cause swelling,

discolored skin and vascular collapse, we embarked on studies to test the hypothesis that the adverse events in rats following large bolus doses of PEG-[Lys16]ShK are due to mast cell degranulation and an anaphylactoid response. Sera from rats receiving 2 and 5 mg/kg bolus IV or subcutaneous injections of PEG-[Lys16]ShK were tested for levels of histamine. As shown in Figure 37, histamine levels were profoundly elevated 5 and 15 minutes following bolus IV injection of PEG-[Lys16]ShK achieving levels that were greater than 2,000 ng/ml which quickly declined over time. This level of histamine would be expected to cause anaphylactoid shock and vascular collapse. Subcutaneous adminstration of PEG-[Lys16]ShK at doses of 0.1, 0.5, 2.0 and 5.0 mg/kg produced 24 hour post-dose C_{max} drug levels of 52 ng/ml (13 nM), 233 ng/ml (58 nM), 988 ng/ml (247 nM) and 2570 ng/ml (642 nM) corresponding to drug levels that were 130-6420 times greater than the molecules IC_{50} (0.1 nM) in the whole blood pharmacodynamic assay of target coverage. As shown in Figure 38, at the 2.0 and 5.0 mg/kg dose groups, a slight elevation in serum histamine was observed 24-48 hours after dosing. In the 5 mg/kg highest dose group, the two animals (rat #10 and #12) with mild swelling of the limbs exhibited increased serum histamine, whereas the animal (rat #11) without these symptoms did not show significant histamine elevation.

[00490] To examine further the impacts of PEG-[Lys16]ShK in rats, further toxicology studies were undertaken. In addition, toxicology was assessed in a second species, cynomolgus monkeys. In vitro studies were also performed to directly assess the effect of PEG-[Lys16]ShK on mast cells. Further details on these studies are provided in the sections below.

[00491] In one toxicology study, PEG-[Lys16]ShK (SEQ ID NO:16) was administered by intravenous infusion (over 30 minutes) to male Sprague Dawley rats at single doses of 0.04, 0.12, 0.2, 0.4, 1.36, and 4.0 mg/kg (n=1-3/dose group). At 4.0 mg/kg, 1 animal was found dead and the other was euthanized due to clinical signs of hypoactivity/lethargy, lateral recumbence, discolored skin (blue paws, tail, and/or ears), and coldness to touch. Hypoactivity and lateral recumbence were also observed at 0.4 mg/kg. The 1 animal given 1.36 mg/kg

was euthanized due to clinical signs of swelling and discolored skin. Swelling was observed in all test article-treated animals, except for the 1 animal dosed at 0.12 mg/kg. The severity of swelling was not dose dependent, and all clinical observations noted in all groups that survived to their scheduled euthanasia resolved by approximately 24 hours postdose. At 4.0 and 1.36 mg/kg, test article-related effects included degeneration/necrosis of renal tubular epithelium, necrosis/depletion of thymic lymphocytes, inflammation (increased neutrophils and monocytes), and altered fluid homeostasis (dehydration, decreased sodium and chloride, and decreased albumin and globulin). In animals given 0.4 and 4.0 mg/kg, there were vacuoles in the renal tubular epithelium, similar to those described following administration of pegylated biomolecules (Bendele et al. Short Communication: Renal tubular vacuolation in animals treated with polyethylene-glycol-conjugated proteins, Tox Sci. 42:152-157 (1998)). Other changes noted at lower doses were limited to decreased lymphocytes and triglycerides at 0.4 mg/kg.

[00492] In another toxicology study, PEG-[Lys16]ShK (SEQ ID NO:16) was administered by subcutaneous injection once weekly to male Sprague Dawley rats (n=5/dose group) at doses of 0.1, 0.5, or 2.0 mg/kg/dose for 14 days (2 doses). At the 0.5- and 2.0-mg/kg dose levels, all animals showed clinical signs of discolored skin (red), which was more severe in the high dose group. In addition, chromorhinorrhea was noted in animals dosed at 2.0 mg/kg. All clinical signs were resolved by day 11. A decrease in body weight was noted in the 2-mg/kg dose group 48 hours after both doses and a decrease in body weight gain (days 1-14) was noted in the 2.0 mg/kg dose group compared with controls. Hematologic effects in animals given $\geq$ 0.5mg/kg/day were consistent with increased red cell turnover, and correlated with increased spleen weights, and increased splenic hemopoiesis. Increased neutrophils and eosinophils were noted in all test article-treated animals. Increased bone marrow eosinophil hematopoeisis and splenic hemopoiesis was noted histologically at all doses of PEG-[Lys16]ShK (SEQ ID NO:16).

[00493] In another toxicology study, PEG-[Lys16]ShK (SEQ ID NO:16) was administered by subcutaneous injection to male Sprague Dawley rats (n=5/dose group) at doses of 0.1, 0.3, 0.7, 2.5, or 5.0 mg/kg/dose once every 72 hours for 12 days (4 doses). Exposures increased with increasing dose up to 2.5 mg/kg, but exposures were similar in rats given 2.5 and 5.0 mg/kg. All animals survived the 12-day treatment period. Redness in the ears and paws was observed in all animals 24 hours after each dose in the 0.3, 0.7, 2.5 and 5.0 mg/kg/dose groups. In the 0.1 mg/kg/dose group, one of five animals showed redness, but only after the second dose. There was a decrease in body weight and body weight gain noted in the 2.5 and 5.0 mg/kg/dose groups. Hematologic effects were noted at doses $\geq$ 2.5 mg/kg/day and were consistent with increased red cell turnover. An increase in platelet production was noted at doses $\geq$ 0.7 mg/kg/dose. In the kidney, vacuoles were noted in the proximal convoluted tubules of rats given 5 mg/kg/day. Urine chemistry values were increased in a few animals/group at 0.5 or 2.0 mg/kg/day, but these changes were inconsistent among and within animals, and considered unlikely to be related to treatment. There were no test article-related effects on biomarkers of renal injury (beta-2 microglobulin, calbindin, clusterin, cystatin C, glutathione S-transferase alpha, kidney injury molecule-1, neutrophil gelatinase-associated lipocalin, osteopontin, tissue inhibitor of metalloproteinase-1, and vascular endothelial growth factor). There was a dose-dependent increase in incidence/severity of hematopoiesis in the bone marrow, spleen, liver, and mesenteric lymph node of treated rats. This change was more prominent in the spleen and bone marrow compared with the liver and mesenteric lymph node. In the spleen, the increased hematopoiesis was composed of erythroid, myeloid, and platelet precursors; whereas in the bone marrow, it was primarily composed of myeloid precursors. The majority of the myeloid precursors in the spleen and bone marrow were associated with eosinophil precursors. In the liver and mesenteric lymph node, hematopoiesis was composed of scattered groups of erythroid and eosinophil precursors. In the tibia and/or femur, there was osteoblastic new bone formation in the medullary canal, which was primarily composed of woven bone. In addition, there was increased porosity

of cortical bone accompanied by a subtle increase in woven bone. In the adrenal gland of most treated rats, there was minimal hypertrophy of the cortex. In 1 rat given 5.0 mg/kg/dose, there was erosion of the glandular epithelium. In the kidney, vacuoles were noted in the proximal convoluted tubules of rats given 5 mg/kg/dose. These vacuoles are consistent with PEG-associated renal vacuoles and were not associated with any other renal tubular change (Bendele et al. Short Communication: Renal tubular vacuolation in animals treated with polyethylene-glycol-conjugated proteins, Tox Sci. 42:152-157 (1998)).

[00494] In another toxicology study, a single subcutaneous dose of PEG-[Lys16]ShK (SEQ ID NO:16) (1 mg/kg in A4S and DPBS vehicle), relatively Kv1.3-inactive PEG-[1-Nal 16]ShK (SEQ ID NO:159) (1 mg/kg), anti-KLH Ab-[Lys16]ShK (heterotetramer of SEQ ID NOS:338, 339, 338, 342) (37 mg/kg), Fc/Fc-[Lys16]ShK (heterodimer of SEQ ID NOS:337, 348) (14 mg/kg), or PEG (5 mg/kg) was administered to male Sprague Dawley rats (n=3/dose group). There was no mortality nor effects on body weights in test article-treated rats. The dose given to each group was intended to provide an equimolar equivalent of the ShK peptide or an equivalent load of PEG. On a molar basis, measured AUC exposure for groups given PEG-[Lys16]ShK (SEQ ID NO:16) (in A4S and DPBS) and anti-KLH Ab-[Lys16]ShK were roughly equal. Compared with PEG-[Lys16]ShK (SEQ ID NO:16) (in A4S and DPBS) and anti-KLH Ab-[Lys16]ShK (heterotetramer of SEQ ID NOS:338, 339, 338, 342), molar AUC exposures for the groups given PEG-[1-Nal 16]ShK (SEQ ID NO:159) and Fc/Fc-[Lys16]ShK (heterodimer of SEQ ID NOS:337, 348) were ~6-8X and ~0.5X, respectively. Rats given PEG-[Lys16]ShK (SEQ ID NO:16) in both A4S and DPBS had red ears/paws, while rats given PEG alone did not. Rats in all treatment groups given a ShK construct had similar pathology findings. In the bone marrow, there were increased numbers of cells of the myeloid series (increased myeloid:erythroid ratio), many of which were eosinophils. In the spleen, there was increased hemopoiesis with equal erythroid and myeloid cell populations. Increased levels in serum histamine were noted in all groups given a ShK construct. This study demonstratesd that different constructs (PEG, Fc/Fc, anti-KLH) using the active or

inactive ShK moiety can cause similar changes in rats. Further, these changes were independent of the vehicle used and are not caused by PEG alone.

[00495] In a separate study, PEG-[Lys16]ShK (SEQ ID NO:16) at 5 mg/kg was administered as a single subcutaneous injection to male Sprague Dawley, Lewis, Wistar, or Fischer rats (n=3/dose group/strain) to determine if the changes in previous studies were rat strain specific. There were no mortalities or test article-related effects on body weight. All 4 rat strains exhibited test article-related clinical observations of chromorhinorrhea, chromodacryorrhea, and/or discolored skin (red), and had statistically significant increases in plasma histamine levels compared with their respective vehicle control group. Although there were some minor differences in mean exposures (AUC_{0-48}) and C_{max} between strains, the PEG-[Lys16]ShK (SEQ ID NO:16) serum concentration profiles were generally similar for all 4 rat strains. Plasma fibrinogen was consistently increased to a similar degree in all strains, and was usually accompanied by other indicators of inflammation, including increased neutrophils and globulins and decreased albumin. One Fischer rat had adrenal necrosis and focal degeneration/necrosis of the papillary muscle in the heart. A similar but less severe change in the adrenal gland of 2 Wistar rats was minimal, multifocal necrosis of individual cortical cells. In the Sprague Dawley, Lewis and Wistar strains, there was a cellular infiltrate in the adrenal cortex and/or medulla. This infiltrate was either an inflammatory response to adrenal cortical necrosis, extramedullary hematopoiesis, or a combination of inflammation and hematopoiesis. Adrenal cortex hypertrophy was noted in the Sprague Dawley and Wistar strains, which may be due to physiologic stress and/or the effects of mast cell degranulation and histamine release on ACTH and corticosterone secretion (Bugajski et al., Influence of cyclooxygenase inhibitors on the central histaminergic stimulation of hypothalamic-pituitary-adrenal axis, *J Phys and Pharm.* 54(4):643-652 (2003)). There was lymphoid depletion in the cortex of the thymus, the periarteriolar lymphoid sheaths of the spleen, and the paracortex of the mesenteric lymph node. Lymphoid depletion was most pronounced in the thymus of the Fischer rat, whereas lymphoid depletion in the spleen and lymph

node occurred in the Sprague Dawley, Lewis, and Wistar strains. Additional changes in the spleen included increased hemopoiesis, which involved both erythroid and myeloid series and generally correlated with increased spleen weight in the Sprague Dawley, Lewis and Wistar strains, and sinus neutrophilia, which correlates with the increased neutrophils in the CBC. There was increased cellularity in the bone marrow, which was primarily due to an increase in early myeloid progenitors. Kupffer cells in the livers of Sprague Dawley and Wistar strains were prominent, which is likely due to increased activity (e.g. increased phagocytosis, release of cytokines). In the ear pinna of all treated rats, small veins in the superficial dermis were congested and the endothelium was lined by marginated neutrophils; this change is related to the clinical observation of red skin. In the deep dermis/subcutis of the injection site of treated rats, there was an inflammatory infiltrate composed primarily of macrophages, with fewer lymphocytes and neutrophils. The nature of the inflammatory infiltrate is consistent with the injection of foreign material into the subcutis. A less severe inflammatory infiltrate was noted in controls and was primarily composed of lymphocytes. In the kidney, vacuoles were noted in the proximal convoluted tubules of all treated rats at this 5 mg/kg dose of PEG-[Lys16]ShK (SEQ ID NO:16). These vacuoles are consistent with PEG-associated renal vacuoles, and were not associated with any other renal tubular change (Bendele, ibid.).

[00496] In an assessment of positive wheal and flare reactions, PEG-[Lys16]ShK (SEQ ID NO:16) was administered by intradermal injection to male Sprague Dawley rats at various dose levels ranging between 0.4 to 40,000 pmol in a 50 µl injection volume. To determine the incidence of positive wheal reactions at 30 minutes post-dose, a total of 8 rats on two separate days were given PBS negative control and a series of doses ranging from 0.4 to 4,000 pmol. To determine whether intradermal injection could induce a systemic response, groups of 3 rats were given a single dose of 40, 400, 4,000 or 40,000pmol. Mean exposures (AUC_{0-24}) were roughly dose proportional from 400 to 40,000 pmol for PEG-[Lys16]ShK (SEQ ID NO:16), but there was no systemic exposure measured at 40 pmol. There were no mortalities during the study. Thirty minutes after

intradermal injection, there was a noticeable wheal reaction (edema), the size of which was dose dependent. There was no flare reaction (redness due to vascular congestion) noted after 30 minutes, but redness at the injection site was noted at 8 and 24 hours postdose and correlated with inflammation noted histologically. Positive wheal reactions were noted in individual animals at doses $\geq$ 0.4 pmol. The incidence of positive wheal reactions was dose responsive, and it plateaued (100% incidence) at 400 pmol. The increase in wheal area and wheal thickness was also dose responsive. Dispersion of mast cell granules and margination/congestion of blood vessels were noted histologically at dose levels of 40, 400, and 4,000 pmol 30 minutes postdose. At 24 hours postdose coagulative necrosis of the dermis and epidermis was noted in 2 of 3 animals given 40,000 pmol. In addition, doses of 400, 4,000, and 40,000 were associated with test article-related inflammation, and mast cells were no longer apparent in toluidine blue stained sections. Compared with the inflammation noted at 24 hours, there was a significant reduction in inflammation at 72 hours and no inflammation at 168 hours. Increased fibrinogen and histamine levels in rats dosed with 40,000 pmol suggest that systemic exposure in this group induced a systemic response. The NOEL for the systemic reaction was 4,000 pmol. This study demonstrates that there is a large margin between a wheal reaction and a systemic response, suggesting that relevant intradermal doses could be administered to humans with low risk of an undesirable systemic reaction.

[00497] In a further rat study, PEG-[Lys16]ShK (SEQ ID NO:16) was administered by subcutaneous injection to telemeterized male Sprague Dawley rats (n=3/dose group) at doses of 0.7 and 2.5 mg/kg and mean arterial pressure and heart rate were monitored continuously for 24 hours. At the 24 hour timepoint after dosing, there was a dose-dependent decrease in mean arterial pressure and an increase in heart rate (reflex-mediated) relative to vehicle-treated rats (Table 4G(c)). In addition, PEG-[Lys16]ShK (SEQ ID NO:16) exhibited clinical observations of redness, swelling and pinna irritation, which were considered consistent with histamine release.

[00498] In another study, a dose of 2.5 mg/kg PEG-[Lys16]ShK (SEQ ID NO:16) was administered by subcutaneous injection to telemeterized female Sprague Dawley rats (n=5/dose group) following pretreatment with Compound 48/80, which is known to cause mast cell degranulation and depletion of granule contents (Blom et al., A method for determining whether hypotension caused by novel compounds in preclinical development results from histamine release. J Pharm and Toxicol Methods, 49:31-37 (2004)). Pretreatment with Compound 48/80 caused complete inhibition of PEG-[Lys16]ShK (SEQ ID NO:16)-induced hypotension. The cause of the decreased mean arterial pressure and was attributed to mast cell degranulation, and the increased heart rate was attributed to a reflex response.

[00499] In summary, the adverse observations at higher doses in the in vivo rat toxicology studies were consistent with the biologic consequences of mast cell activation/degranulation and the release of a variety of preformed and/or synthesized inflammatory mediators. Histamine level is a reliable marker of mast cell degranulation, and was elevated in some animals treated with test-article. Histamine is a potent vasoactive mediator that produces increases in blood flow and vasodilation, which explains many of the clinical observation, as well as the results of the telemetry studies. Histamine can also produce gastric ulcers in rats (Hase et al., Prostaglandin E2 aggravates gastric mucosal injury induced by histamine in rats through EP1 receptors, *Life Sci.* 74:629-641 (2003)), and increase ACTH and corticosterone secretion, which may have caused adrenal hypertrophy (Bugajski et al., Influence of cyclooxygenase inhibitors on the central histaminergic stimulation of hypothalamic-pituitary-adrenal axis, *J Phys and Pharm.* 54(4):643-652 (2003)). Histamine may play a role in bone metabolism and has been shown to regulate osteoclastogenesis, which may have caused the bone changes following repeat dosing (Biosse-Duplan et al., Histamine promotes osteoclastogenesis through the differential expression of histamine receptors on osteoclasts and osteoblasts. *Am J Pathol.* 174:1426-1434 (2009)). Other mast cell-derived cytokines, such as IL-3, IL-5 and GM-CSF, are important in eosinophil maturation and chemotaxis, and can promote eosinophil hematopoiesis

in tissues, which was noted in several studies (Shakoory et al., The role of human mast cell-derived cytokines in eosinophil biology, *J Interferon and Cytokine Res.* 24:271-281 (2004)).

[00500] Interestingly, the putative mast cell related effects, which were observed in Sprague-Dawley,Lewis, Wistar, and Fischer rat strains, were not observed in mouse, cynomolgus monkey, or dog studies despite equal or higher exposure than in the rats.

[00501] For example, in a study in mice, PEG-[Lys16]ShK (SEQ ID NO:16) was administered daily by subcutaneous injection to female C57BL/6 mice (n=3/dose group) at doses of 0.6, 2.5, or 5 mg/kg/day for 14 days. PEG-[Lys16]ShK (SEQ ID NO:16) exposure at the end of day 14 was 833±185 ng/ml (208±46 nM), 10066±2219 ng/ml (2516±555 nM), and 31334±5800 ng/ml (7834±1450 nM) for mice receiving daily doses of 0.6, 2.5 and 5.0 mg/kg, respectively. All mice survived the dosing period. No test article-related clinical observations or body weight changes were noted in any animal. There was dose-dependent vacuole formation in the renal tubular epithelium, and no vacuole formation in hepatic sinusoidal macrophages or resident macrophages in mesenteric lymph node and spleen.

[00502] In another study in cynomolgus monkey, PEG-[Lys16]ShK (SEQ ID NO:16) was administered by subcutaneous injection to male cynomolgus monkeys (n=3/dose group) at doses of 0.7 mg/kg every third day or 0.1, 0.5, or 2.0 mg/kg weekly for 2 weeks. No adverse effects were seen in cynomolgus monkeys administered PEG-[Lys16]ShK (SEQ ID NO: 16). There were no treatment-related effects on body weight. There were no test article-related organ weight changes, or macroscopic or microscopic findings. Further, no qualitative or quantitative electrocardiogram (ECG) abnormalities were attributable to administration of PEG-[Lys16]ShK (SEQ ID NO:16) at doses of 0.1, 0.5, or 2 mg/kg/dose. The only findings considered possibly test-article related were minimal increases in serum urea nitrogen and serum creatinine for animals given 0.7 mg/kg every third day. No histopathologic correlates to the clinical pathology changes were observed. Renal vacuoles were not observed, and there were no

changes in urinary biomarkers of renal injury (alpha-1 microglobulin, beta-2 microglobulin, calbindin, clusterin, connective tissue growth factor, creatinine, cystatin C, glutathione S-transferase alpha, kidney injury molecule-1, microalbumin, neutrophil gelatinase-associated lipocalin, osteopontin, Tamm-Horsfall protein, tissue inhibitor of metalloproteinase-1, trefoil factor 3, and vascular endothelial growth factor). Thus, the No-Observable-Adverse-Effect-Level in cynomolgus monkey is >2 mg/kg. In this study, the 2 mg/kg dose group had a mean AUC value of 584,000 ng x hr / mL. Binding antibodies against PEG-[Lys16]ShK (SEQ ID NO:16) were detected in 17% (2/12) of the PEG-[Lys16]ShK (SEQ ID NO:16) treated animals. Both of the antibody-positive animals were in the 0.5 mg/kg dose group. The detected antibodies were capable of binding to both PEG-[Lys16]ShK (SEQ ID NO:16) and the ShK peptide (SEQ ID NO:1). Antibodies were not detectable in the low or high dose groups.

[00503] Renal tubule vacuolization is a well recognized consequence of dosing with PEGylated compounds. It is noteworthy that PEG-[Lys16]ShK (SEQ ID NO:16) caused renal vacuoles in mice and rats (NOEL = ~20X), but not in cynomolgus monkeys (NOEL >~150X). Moreover, there was no evidence of overt renal toxicity, including the evaluation of sensitive biomarkers of renal toxicity, in any species.

[00504] In an additional study of wheal and flare reactions, cynomolgus monkeys (n=3) received intradermal injections of 0.01, 0.1, 1.0 and 10 μg Compound 48/80 (positive control for mast cell degranulation) and 0.4, 4, 40, 400, 4000, 40000 pmol PEG-[Lys16]ShK (SEQ ID NO:16) per dose site. The two compounds were given at least 7 days apart and each animal received the complete range of doses on a single day. Positive wheal reactions were noted at 30 minutes post-dose in all 3 animals at the dose sites receiving 10 μg of Compound 48/80, and 40,000 pmol of PEG-[Lys16]ShK (SEQ ID NO:16). The 40,000 pmol of PEG-[Lys16]ShK (SEQ ID NO:16) dose sites were also positive at 4 hours post-dose. One of three dose sites receiving 4,000 pmol of PEG-[Lys16]ShK (SEQ ID NO:16) had a positive reaction 30 minutes post-dose only. Wheal reactions were not observed at dose sites receiving 0.4 to 400 pmol PEG-

[Lys16]ShK (SEQ ID NO:16). Compound 48/80-induced wheal reactions were larger, but persisted for a shorter duration than the PEG-[Lys16]ShK (SEQ ID NO:16)-induced wheal reactions. Histologically, there were no PEG-[Lys16]ShK (SEQ ID NO:16)-related findings. In contrast, Compound 48/80 caused slight inflammation in sites given 10 μg, and there were decreased toluidine blue positive mast cells at sites given ≥0.1 μg. Compared to the positive wheal reactions in the rat, the positive wheal reactions in the cynomolgus monkey were smaller and not associated with histologic evidence of a biologic effect. Based on the data from this study, it is uncertain if the PEG-[Lys16]ShK (SEQ ID NO:16)-induced wheal reactions in the cynomolgus monkey are due to mast cell degranulation followed by edema, or to the injection of high concentration of PEGylated peptide.

[00505] Telemeterized male cynomolgus monkeys were used to evaluate the impact of PEG-[Lys16]ShK (SEQ ID NO:16) on mean arterial pressure, heart rate and cardiac intervals. Several weeks prior to the administration of test article, each animal was treated with vehicle (5 ml/kg) or Compound 48/80 (0.3 or 3 mg/kg SC) to validate the animal model. Subcutaneous injection of 3 mg/kg Compound 48/80 caused a reduction in systolic and mean arterial pressure in the cynomolgus monkey, whereas administration of vehicle did not have any impact on these parameters. After validation of the animal model, PEG-[Lys16]ShK (SEQ ID NO:16) was administered at doses of 0.5 or 2.0 mg/kg and monitored continuously for 72 hours. PEG-[Lys16]ShK (SEQ ID NO:16) did not have any effect on hemodynamics or cardiac intervals compared to vehicle control treatment.

[00506] Mast cell degranulation leading to a systemic response occurred at low exposures of PEG-[Lys16]ShK (SEQ ID NO:16) in the rat. In contrast, very high exposures in the cynomolgus monkey showed no adverse effects and no concrete evidence of the biological consequences of mast cell degranulation. It is unknown whether humans will be sensitive to ShK-induced mast cell degranulation; however, the intradermal studies demonstrate an opportunity to explore this with low risk of a systemic response in dosed humans.

[00507] To examine further the impacts of PEG-[Lys16]ShK (SEQ ID NO:16) on mast cells, isolated peritoneal mast cells from rat and mice, as well as, human CD34-derived mast cells were tested to assess mast cell degranulation and histamine release in vitro. PEG-[Lys16]ShK (SEQ ID NO:16), monovalent aKLH HC-ShK(1-35, Q16K) Ab and monovalent Fc-L10-ShK(1-35, Q16K) caused degranulation of rat peritoneal mast cells (Figure 39), similar to positive control basic secretagogues MCDP, compound 48/80 and substance P. Importantly, PEG-[Lys16]ShK (SEQ ID NO:16) and monovalent Fc-L10-ShK(1-35, Q16K) caused little to no degranulation of human mast cells (Figure 40), whereas the basic secretagogues MCDP, compound 48/80 and substance P were highly active. To further assess the species specificity of PEG-[Lys16]ShK-induced mast cell degranulation, it was also tested for activity against mouse peritoneal mast cells and mast cells from a second strain of rat. PEG-[Lys16]ShK (SEQ ID NO:16) caused degranulation of peritoneal mast cells from both strains of rats, but was inactive against mouse peritoneal mast cells and human mast cells (Figure 41). To confirm that PEG-[Lys16]ShK degranulation of rat peritoneal mast cells was a result of an off-target impact and not due to Kv1.3 or another ShK-sensitive potassium channel, electrophysiology studies were performed on rat peritoneal mast cells. As shown in Figure 54A-B, rat peritoneal mast cells did not show an outward current resembling Kv1.3, and the observed current was insensitive to high concentrations of the potent Kv1.3 inhibitors PEG-[Lys16]ShK and charybdotoxin (ChTx). The ShK analog, ShK-192 (SEQ ID NO:438), which is a selective Kv1.3 inhibitor (Pennington et al. Molecular Pharmacology 75, 762-773 (2009)) also caused degranulation of rat mast cells through this off-target pathway, but did not degranulate mouse or human mast cells. ShK-192 (SEQ ID NO:438) exhibited greater potency in inducing rat mast cell degranulation (EC_{50} about 0.25 uM) than the larger 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16) molecule (EC_{50} about 1.63 uM), however, neither molecule showed any significant degranulation of human or mouse mast cells. The micromolar EC_{50} of PEG-[Lys16]ShK (SEQ ID NO:16) for degranulation of rat peritoneal mast cells implies that high concentrations of drug would be necessary to cause 50% or more

of the mast cells to degranulate in vivo. Based on pharmacokinetic studies in rats, this level of drug would occur only after large bolus doses. Indeed, severe overt anaphylactoid shock and vascular collapse were only observed when high serum drug concentrations were achieved. Due to the fact that human mast cells appear orders of magnitude less sensitive than rat, even higher drug levels might be necessary in human, making this unlikely to occur. Irrespective, the 0.1 nM IC_{50} of PEG-[Lys16]ShK (SEQ ID NO:16) in blocking Kv1.3-dependent human T cell responses is at least 10,000 times lower than its EC_{50} in activating rat mast cells (1000 – 2000 nM), making it conceivable that there would be a good safety margin against mast cells from a less sensitive species, such as human.

[00508] The Mas-related gene receptors (Mrg), were originally identified as a family of orphan GPCRs expressed in a specific subset of nociceptive sensory neurons that may serve a role in modulating sensation and pain (Dong et al., Cell **106**, 619-632 (2001)). Recently, rat and human mast cells were also reportedto express members of the Mas-related GPCR family which allowed for non-IgE mediated signaling by basic secretagogues via the peptidonergic pathway (Tatemoto et al Biochem. Biophys. Res. Commun. **349**, 1322-1328 (2006)). Whereas rat mast cells expressed the family member MrgB3 to elicit mast cell degranulation, human mast cells expressed the family member MrgX2. The basic secretagogues MCDP and substance P were shown, not only, to induce human and rat mast cell degranulation, but also to activate cells transfected with human MrgX2 and rat MrgB3. Consistent with earlier observations that basic secretagogues signal through a pertussis toxin (PTX) sensitive pathway in mast cells, substance P activation of cell lines expressing human MrgX2 was also shown to be PTX-sensitive. We have similarly found that PEG-[Lys16]ShK (SEQ ID NO:16) mediated degranulation of rat peritoneal mast cells is PTX-sensitive. Thus, the family of Mas-related GPCRs (Mrg) may represent the off-target responsible for the species specific effect of ShK and PEG-[Lys16]ShK on rat mast cells. Indeed, members of the Mas-related GPCR family members exhibit poor conservation across species. Humans express just four members (MrgX1, MrgX2, MrgX3, MrgX4), whereas rats express one each of the MrgA, MrgC, and

MrgD genes and ten MrgB genes (Tatemoto et al. (ibid); Dong (ibid); Zylka et al., PNAS **100**, 10043-10048 (2003)). Despite MrgB3 originally being described as a pseudo-gene (Zylka, ibid), Tatemoto et al. (ibid) find this gene is expressed. The impact of PEG-[Lys16]ShK (SEQ ID NO:16) on rat mast cells and the apparent lack of effects on human mast cells, may reflect the unique Mrg expression pattern in rat mast cells and the fact that the human genome is without a homolog to rat MrgB3. Monkey, however, expresses the same four MrgX family members as human (Burstein et al. Br. J. Pharmacol. 147, 73-82 (2006); Zhang et al., Molecular Brain Res. 133, 187-197 (2005)), making it a more relevant species to humans. Importantly, toxicology studies in cynomolgus monkeys indicate PEG-[Lys16]ShK (SEQ ID NO:16) is well tolerated at doses providing a margin of coverage 54-197 times the predicted human efficacious dose (data not shown).

[00509] Materials and methods for mast cell degranualtion studies.

[00510] Reagents. MCDP was obtained from Alomone Labs (Israel). Compound 48/80, substance P, A23187 were obtained from Sigma (Saint Louis, MI). STEMPRO-34 SFM complete medium was obtained from Invitrogen (Carlsbad, CA). Recombinant human SCF was prepared in-house. Human IL-6 was obtained from R&D Systems (Minneapolis, MN). Human IL-3 was obtained from Invitrogen (Camarillo, CA). Histamine Elisa kit was obtained from NEOGEN (Lexington, KY). Tyrode's buffer was made in-house (10 mM Hepes, pH7.4), 130 mM NaCl, 5 mM KCl, 5.6 mM glucose, 0.1% BSA, 1 mM $CaCl_2$, 0.6 mM $MgCl_2$). Diff Quick Stain was obtained from Dade Behring (NEWARK, DE)

[00511] Cells. Human peripheral blood CD34+ derived mast cells were obtained by long-term culture of peripheral blood progenitor CD34+ cells (Allcells) in vitro in STEMPRO-34 serum- free medium containing 100 ng/ml of human SCF, 40 ng/ml of human IL-6 and 30 ng/ml of human IL-3 for one week, then terminally differentiated in the same serum-free medium containing only 100 ng/ml of human SCF and 40 ng/ml of human IL-6 for about 4 weeks. Terminally differentiated mast cells were determined by Flow Cytometry analysis for the surface c-Kit expression (> 95% of the cells expressed c-Kit, data not shown) and

IgE/anti-IgE induced histamine release (about 40% of histamine released of the total histamine content, data not shown).

[00512] Rat peritoneal fluid was collected in Tyrode's buffer from 8 weeks old of female Sprague Dawley or Lewis. (Gillespie et al., Histamine release from rat peritoneal mast cells: inhibition by colchicine and potentiation, 1968; 154-1). Mouse peritoneal fluid was collected in Tyrode's buffer from 8 weeks old of female C57B6. Percentage of rat and mouse peritoneal mast cells were determined by staining with Diff Quick Stain.

[00513] Measurement of rat serum histamine. Male Sprage Dawley Rats serum samples from intravenous single-dose or subcutaneous single-dose were diluted in Tyrode's buffer and seeded on 96-well plates. The histamine was quantified by ELISA (followed the manufacturer's instruction). The absorbance was measured at 450 nm and 650 nm in microplate reader. Molecular Devices, SPECTRA mac 340 pc). (Figure 37 and Figure 38).

[00514] Measurement of mast cell degranulation. Rat or mouse peritoneal fluid collected in Tyrode's buffer or human mast cells in Tyrode's buffer containing 200 ng/ml of human SCF and 80 ng/ml of human IL-6 was seeded on 96-well plates (rat or mouse peritoneal mast cells, 4-5000 cells/well; human mast cells, 20,000 cells/well) with half log diluted control molecules: MCDP, Compound 48/80, Substance P and A23187 and test peptides: 20kDa-PEG-[Lys16]ShK (SEQ ID NO:16), monovalent aKLH Ab-[Lys16]ShK (tetramer of SEQ ID NOS:338, 339, 338, 342) and monovalent Fc/Fc-L10-[Lys16]ShK (heterodimer of SEQ ID NOS:337, 348) in Tyrode's buffer at 37C for 1 h. Cells were centrifuged at 800 rpm, 4C for 15 min. Supernatant was collected, released histamine was quantified by ELISA.The absorbance was measured at 450 nm and 650 nm in microplate reader. Total histamine content of the cells (0.1% Triton X-100) and spontaneous release were also measured to determine the percent of histamine release (Figures 39-41). Percent histamine release was calculated by the following formulam (III):

(III)

% histamine release = (ng/ml histamine release by test article – ng/ml spontaneous histamine release in Tyrode's buffer)/(ng/ml total histamine content of cells lysed in 0.1% Triton X-100 – ng/ml spontaneous histamine release in Tyrode's buffer) X 100.

Table 4F. Design of repeat-dose pharmacology study on PEG-[Lys16]ShK (SEQ ID NO:16) in cynomolgus monkeys.

Activity	Week											
	1	2	3	4	5	6	7	8	9	10	11	12
0.5 mg/kg 20kDa-PEG-ShK[Lys16] Weekly SC Doses (n=6, male cynos)			X	X	X	X						
Whole Blood Sample (1mL) for CBC Analysis	X	X	X	X	X	X	X	X	X	X	X	X
Whole Blood Sample (3mL) for FACS & PD Analysis	X	X	X	X	X	X	X	X	X	X	X	X
Serum Sample for Clinical Chemistry and PK Analysis a. 1.0 mL for clinical pathology b. ~0.5 mL for PK Analysis	X	X	X	X	X	X	X	X	X	X	X	X

Table 4G. Average pre-dose baseline CBCs and clinical pathology measures of monkeys (n= 6 male cynomolgus monkeys; 3 pre-dose samples collected for each cyno over two weeks).

Hematology

		Pre-Dose (Average)	Pre-Dose (Stdev)
WBC	10^3/µL	10.944	1.898
RBC	10^6/µL	4.886	0.289
HGB	g/dL	12.011	0.610
HCT	%	36.961	1.334
MCV	fL	75.733	2.828
MCH	pg	24.594	0.727
MCHC	g/dL	32.500	0.849
RDW	%	12.556	0.315
PLT	10^3/µL	381.278	27.296
MPV	fL	8.294	0.538
NEUT	%	35.611	12.571
LYMPHS	%	59.833	11.994
MONO	%	3.611	0.574
EOS	%	1.333	0.667
BASO	%	0.000	0.000
BANDS	%	0.000	0.000
Neut	10^3/µL	4.080	1.925
Lymphs	10^3/µL	6.369	1.447
Mono	10^3/µL	0.403	0.083
Eos	10^3/µL	0.139	0.068
Baso	10^3/µL	0.000	0.000
Bands	10^3/µL	0.000	0.000
Retic	%	1.233	0.250
Retic count	10^3/µL	60.026	10.687

Blood Chemistry

		Pre-Dose (Average)	Pre-Dose (Stdev)
Alb	g/dL	4.417	0.278
Total Pro	g/dL	7.072	0.226
ALP	U/L	349.389	145.717
ALT	U/L	30.444	3.250
AST	U/L	32.389	8.747
CPK	U/L	632.389	322.364
T bili	mg/dL	0.161	0.033
Dir Bili	mg/dL	0.011	0.017
BUN	mg/dL	20.611	2.195
Creatinine	mg/dL	0.856	0.054
Calcium	mg/dL	10.206	0.406
Choles	mg/dL	122.833	23.122
Glucose	mg/dL	69.278	4.041
Phosphor	mg/dL	5.344	0.449
GGT	U/L	71.278	11.275
CO2	mEq/L	22.944	1.163
Na	mEq/L	146.500	1.670
K	mEq/L	4.339	0.272
Cl	mEq/L	106.778	1.129
Globulin	mg/dL	2.656	0.154
A/G	Ratio	1.678	0.185
B/C	Ratio	24.167	1.964
Ind Bili	mg/dL	0.150	0.028
ANION	mEq/L	21.056	1.782

Table 4G(a). PEG-[Lys16]ShK (SEQ ID NO:16) Cerebral Spinal Fluid and Serum Concentrations at 24 and 48 hours in Three Cynomolgus Monkeys After a 0.5 mg/kg Subcutaneous Dose of PEG-[Lys16]ShK (SEQ ID NO:16).

Cyno - sample time	Serum Conc. (ng/mL)	CSF Conc. (ng/mL	Serum/CSF Ratio
4 - 24h	1410	1.68	839
4 - 48h	1360	1.72	791
5 - 24h	1270	2.55	498
5 - 48h	910	2.14	425
6 - 24h	1420	2.30	617
6 - 48h*	1080	7.62	142

* Sample visually contaminated with blood.

Table 4G(b). PEG-[Lys16]ShK (SEQ ID NO:16) Cerebral Spinal Fluid and Serum Concentrations at 6 and 48 hours in Male Sprague-Dawley Rats Receiving Various Intravenous Doses of PEG-[Lys16]ShK (SEQ ID NO:16).

Dose(mg/kg) - timepoint	Serum Conc. (ng/mL)	CSF Conc. (ng/mL)	Serum/CSF Ratio
0.04 - 48h*	6.46	5.7	1.13
0.04 - 48h	8.68	BQL	> 8.68
0.04 - 48h	4.75	BQL	> 4.75
0.12 - 48h	31.2	BQL	> 31.2
0.20 - 48h	40.6	BQL	> 40.6
0.20 - 48h	69.2	BQL	> 69.2
0.40 - 48h	173	BQL	> 173
0.40 - 48h	151	BQL	> 151
1.20 - 6h**	5970	12.7	470
4.00 - 6h**	2000	43	46.5

* Sample visually contaminated with blood.
**Animals sacrificed early due to adverse events.
BQL - Below the assay limit of quantitation of 1 ng/mL.

Table 4G(c). PEG-[Lys16]ShK (SEQ ID NO:16) was administered by subcutaneous injection to telemeterized male Sprague Dawley rats (n=3/dose group). Mean arterial pressure and heart rate were monitored continuously for 24 hours.

	Heart Rate (bpm)	**Mean Arterial Pressure (mm Hg)**
Vehicle	**336 ± 6**	**115 ± 10**
0.7 mg/kg PEG—[Lys16]ShK	**413 ± 22 (+23%)**	**90 ± 5 (-13%)**
2.5 mg/kg PEG—[Lys16]ShK	**464 ± 12 (+38%)**	**84 ± 8 (-26%)**

Example 11

Expression and Purification of Immunoglobulin- and/or Fc domain-Toxin Peptide Analog Fusions

[00515] An assortment of bivalent and monovalent structures were expressed and purified as exemplary embodiments of the invention. Those included IgG2 Fc/Fc-ShK variants (see Figure 12A), aKLH IgG2/Fc-ShK variants (see Figure 12E), and anti-KLH IgG2-ShK variants (see Figure 12F-L). For example, bivalent Fc-L10-ShK[1-35], bivalent Fc-L10-ShK[2-35], monovalent Fc/Fc-L10-ShK[2-35] fusions were made by recombinant methods as described in Sullivan et al., WO 2008/088422 A2, and in particular Examples 1, 2, and 56, incorporated by reference in its entirety, or as modified herein. Monovalent anti-Keyhole Limpet Hemocyanin (KLH) immunoglobulin heavy chain-[Lys16]ShK fusion antibody (designated "aKLH HC-[Lys16]ShK Ab"; see Figure 12F), and monovalent anti-KLH immunoglobulin light chain-[lys16]ShK antibody fusions (designated "aKLH LC-[Lys16]ShK Ab"; see Figure 12J) were made by similar recombinant methods and as further modified herein.

[00516] Transient expression system used to generate toxin peptide analog-Fc fusions ("peptibodies") or other immunoglobulin fusion embodiments. HEK 293-6E cells were maintained in 3L Fernbach Erlenmeyer Flasks between 2e5 and 1.2e6 cells/ml in F17 medium supplemented with L-Glutamine (6mM) and Geneticin (25 μg/ml) at 37°C, 5% CO2, and shaken at 65 RPM. At the time of transfection, cells were diluted to 1.1 x 10^6 cells/mL in the F17 medium mentioned above at 90% of the final culture volume. DNA complex was prepared in Freestyle293 medium at 10% of the final culture volume. DNA complex includes 500ug total DNA per liter of culture and 1.5ml PEImax per liter of culture. DNA complex is briefly shaken once ingredients are added and incubated at room temperature for 10 to 20 minutes before being added to the cell culture and placed back in the incubator. The day after transfection, Tryptone N1 (5g/L) was added to the culture from liquid 20% stock. Six days after transfection, culture was centrifuged at 4,000 RPM for 40 minutes to pellet the cells and the cultured medium was harvested through a 0.45um filter.

[00517] In preparing the DNA complex, the ratio of plasmids was proportional to the desired molar ratio of the peptides needed to generate the intended product. The components of the IgG2 Fc/Fc-ShK include IgG2 Fc and IgG2 Fc-ShK at a 1:1 ratio. During expression these assemble into IgG2 Fc homodimers, IgG2 Fc/Fc-ShK heterodimers, and IgG2 Fc-ShK homodimers. The IgG2 Fc/Fc-ShK heterodimer (monovalent form) was isolated during purification using cation exchange chromatography.

[00518] IgG2 Fc-ShK[2-35]; IgG2 Fc Shk[2-35, Q16K];IgG2 Fc-Shk[1-35]; IgG2 Fc-ShK[1-35, Q16K] mammalian expression. DNA sequences coding for the immunoglobulin Fc domain of human IgG2:
MEWSWVFLFFLSVTTGVHSERKVECPPCPAPPVAGPSVFLFPPKPKDTLMI
SRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRV
VSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLP
PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDG
SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK// (SEQ ID NO:337),

fused in-frame to a monomer of the Kv1.3 inhibitor peptide ShK[2-35] or a mutated ShK[2-35, Q16K] were constructed using standard PCR technology. The ShK[2-35] or ShK[2-35, Q16K] and the 10 amino acid linker portion of the molecule were generated in a PCR reaction using the original Fc-2xL-ShK[2-35] in pcDNA3.1(+)CMVi as a template (see Sullivan et al., WO 2008/088422 A2, Example 2, Figure 15A-B). The ShK[1-35] was generated in a PCR reaction using the original Fc-2xL-ShK[1-35] in pcDNA3.1(+)CMVi as a template (Sullivan et al., WO 2008/088422 A2, Example 1, Figure 14A-B).These ShK constructs have the following modified VH21 Signal peptide amino acid sequence of MEWSWVFLFFLSVTTGVHS// SEQ ID NO:318 generated from a pSelexis-Vh21-hIgG2-Fc template with the following oligos:
5'- CAT GAA TTC CCC ACC ATG GAA TGG AGC TGG -3' (SEQ ID NO:319); and
5'- CA CGG TGG GCA CTC GAC TTT GCG CTC GGA GTG GAC ACC -3' (SEQ ID NO:320).

[00519] Wild Type ShK[2-35] with N-terminal linker extension (amino acid sequence GGGGSGGGGSSCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC// SEQ ID NO:322) was encoded by the DNA sequence below:
GGAGGAGGAGGATCCGGAGGAGGAGGAAGCAGCTGCATCGACACCAT CCCCAAGAGCCGCTGCACCGCCTTCCAGTGCAAGCACAGCATGAAGTA CCGCCTGAGCTTCTGCCGCAAGACCTGCGGCACCTGC// (SEQ ID NO:321). A fragment containing this coding sequence (SEQ ID NO:321) was generated using the oligos below (SEQ ID NO:323 and SEQ ID NO:324)-and the original Fc-L10-ShK[2-35] in pcDNA3.1(+)CMVi as a template (Sullivan et al., WO 2008/088422 A2, Example 2, Figure 15A-B, incorporated by reference):
5'-GTC CAC TCC GAG CGC AAA GTC GAG TGC CCA CCG TGC C-3' (SEQ ID NO:323); and
5'- TCC TCC TCC TTT ACC CGG AGA CAG GGA GAG -3'// (SEQ ID NO:324).

[00520] Mutant ShK[2-35, Q16K] was generated using site directed mutagenesis with Stratagene's QuikChange Multi site-Directed Mutagenesis kit cat# 200531 per the manufacterer's instruction. Oligos used to generate the mutagenesis were:
5'-GCT GCA CCG CCT TCA AGT GCA AGC ACA GC 3' (SEQ ID NO:325); and
5'- GCT GTG CTT GCA CTT GAA GGC GGT GCA GC -3' (SEQ ID NO:326); and using the original Fc-L10-ShK[2-35] in pcDNA3.1(+)CMVi as a template (Sullivan et al., WO 2008/088422 A2, Example 2, Figure 15A-15B) resulting in the DNA coding sequence
GGAGGAGGAGGATCCGGAGGAGGAGGAAGCAGCTGCATCGACACCAT CCCCAAGAGCCGCTGCACCGCCTTCAAGTGCAAGCACAGCATGAAGTA CCGCCTGAGCTTCTGCCGCAAGACCTGCGGCACCTGC// (SEQ ID NO:327), which encodes the amino acid sequence
GGGGSGGGGSSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCGTC// SEQ ID NO:328).

[00521] ShK[1-35]WT fragment was generated using the original Fc-2xL-ShK[1-35] in pcDNA3.1(+)CMVi as a template (Sullivan et al., WO 2008/088422 A2, Example 1, Figure 14A-B) and oligos:
5'-GTC CAC TCC GAG CGC AAA GTC GAG TGC CCA CCG TGC C-3' (SEQ ID NO:323); and
5'- TCC TCC TCC TTT ACC CGG AGA CAG GGA GAG -3' (SEQ ID NO:324).

[00522] The IgG2Fc region was generated using oligos:
5'-CCG GGT AAA GGA GGA GGA GGA TCC GGA G-3' (SEQ ID NO:329); and
5'- CAT GCG GCC GCT CAT TAG CAG GTG -3' (SEQ ID NO:330), and the pSelexis Vh21-hIgG2-Fc template resulting in a fragment containing the following DNA coding sequence:
GCACCACCTGTGGCAGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCC AAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTG

GTGGACGTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTG
GACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCACGGGAGGAGCA
GTTCAACAGCACGTTCCGTGTGGTCAGCGTCCTCACCGTTGTGCACCA
GGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAG
GCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAACCAAAGGGCAGC
CCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGA
CCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCA
GCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAAC
TACAAGACCACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTCCTCT
ACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTC
TTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAG
AAGAGCCTCTCCCTGTCTCCGGGTAAA // SEQ ID NO:331, which encodes the amino acid sequence
APPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDG
VEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLP
APIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSLSLSPGK SEQ ID NO:332).

[00523] The PCR fragments were generated and the products were run out on a gel. After gel purification, the DNA fragments were put together in a PCR tube and sewn together with outside primers:

5'- CAT GAA TTC CCC ACC ATG GAA TGG AGC TGG -3' (SEQ ID NO:319); and

5'- CAT GCG GCC GCT CAT TAG CAG GTG -3' (SEQ ID NO:330).

[00524] The PCR products were digested with EcoRI and NotI (Roche) restriction enzymes and agarose gel purified by Gel Purification Kit. At the same time, the pTT14 vector (an Amgen vector containing a CMV promoter, Poly A tail and a Puromycin resistance gene) was digested with EcoRI and NotI restriction enzymes and the large fragment was purified by Gel Purification Kit. Each purified PCR product was ligated to the large fragment and transformed into OneShot Top10 bacteria. DNAs from transformed bacterial colonies were

isolated and subjected to EcoRI and NotI restriction enzyme digestions and resolved on a one percent agarose gel. DNAs resulting in an expected pattern were submitted for sequencing. Although, analysis of several sequences of clones yielded a 100% percent match with the above sequence, only one clone of each construct was selected for large scaled plasmid purification. The final pTT14-VH1SP-IgG2-Fc construct encoded IgG2-Fc-L10-ShK(2-35) fusion polypeptide having the following sequence:
MEWSWVFLFFLSVTTGVHSERKVECPPCPAPPVAGPSVFLFPPKPKDTLMI
SRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRV
VSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLP
PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDG
SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGG
SGGGGSSCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC// (SEQ ID NO:345).

[00525] The pTT14-VH21SP-IgG2-Fc-ShK2-35Q16K construct encoded a IgG2-Fc-L10-ShK(2-35, Q16K) fusion polypeptide sequence:
MEWSWVFLFFLSVTTGVHSERKVECPPCPAPPVAGPSVFLFPPKPKDTLMI
SRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRV
VSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLP
PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDG
SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGG
SGGGGSSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCGTC// SEQ ID NO:346;
and pTT14-VH21SP-IgG2-Fc ShK1-35 construct contained a coding sequence for IgG2 Fc-L10-ShK(1-35) fusion polypeptide having the following sequence:
MEWSWVFLFFLSVTTGVHSERKVECPPCPAPPVAGPSVFLFPPKPKDTLMI
SRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRV
VSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLP
PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDG
SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGG

SGGGGSRSCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC// (SEQ ID NO:334).

[00526] Generating the VH21SP-IgG2-Fc-only construct in pYD16 (an Amgen vector containing a CMV promoter, Poly A tail and a Hygromycin resistance gene) occurred as follows: The VH21 signal peptide was generated using the following oligos:

5'-CAT AAG CTT CCC ACC ATG GAA TGG AGC TGG-3' (SEQ ID NO:335); and

5'- CA CGG TGG GCA CTC GAC TTT GCG CTC GGA GTG GAC ACC -3' (SEQ ID NO:320), and using the pSelexis template as noted above.

[00527] The Fc region was generated using the pSelexis template described above and following oligos:

5'-GTC CAC TCC GAG CGC AAA GTC GAG TGC CCA CCG TGC C-3' (SEQ ID NO:323); and

5'- CAT GGA TCC TCA TTT ACC CGG AGA CAG GGA G -3' (SEQ ID NO:336).

[00528] The PCR fragments were gel purified and sewn together in single PCR reaction using outside primers SEQ ID NO:335 and SEQ ID NO:336. The resulting PCR fragment was gel purified, and digested by HindIII and BamHI. Concurrently, pYD16 vector (an Amgen vector containing a CMV promoter, Poly A tail and a Hygromycin resistance gene) was also cut by HindIII and BamHI and the large vector fragment was purified by Qiagen's Gel Purification Kit. The purified PCR product was ligated to the large fragment and transformed into OneShot Top10 bacteria. DNA from transformed bacterial colonies were isolated and subjected to HindIII and BamHI restriction enzyme digestions and resolved on a one percent agarose gel. DNAs resulting in an expected pattern were submitted for sequencing. Although, analysis of several sequences of clones yielded a 100% percent match with the above sequence, only one clone was selected for large scaled plasmid purification. The final pYD16-VH21SP-IgG2-Fc construct encoded human IgG2-Fc (SEQ ID NO:337 above).

[00529] Immunizations. Anti-DNP antibodies were generated by immunizing XenoMouse® mice with DNP-KLH, over a period of 4 weeks, and by screening for those antibodies that bind to DNP-lysine. More particularly, XenoMouse® XMG2 strain of mice were generated generally as described previously (Mendez et al., *Nat. Genet.* 15:146-156 (1997); published International Patent Application Nos. WO 98/24893, and WO 00/76310, the disclosures of which are hereby incorporated by reference) and immunized with 2,4-Dinitrophenyl-Keyhole Limpet Hemocyanin (DNP-KLH conjugate; BioSearch Technologies, Novato, CA), using a range of 10-30 μg/mouse of immunogen emulsified in TiterMax Gold adjuvant (Sigma-Aldrich, Oakville, Ontario) for the initial immunization of the XMG2 strain of XenoMouse™ according to the methods disclosed in International Patent Application Nos. WO 98/24893, and WO 00/76310, the disclosures of all of which are hereby incorporated by reference. Following the initial immunization, subsequent boost of immunogen (5 - 20 μg/mouse) were administered on a schedule and for the duration necessary to induce a suitable anti-DNP titer in the mice. Titers were determined by enzyme immunoassay using immobilized DNP-BSA (BioSearch Technologies, Novato, CA), this conjugate was prepared such that the final DNP:BSA molar ratio was 30:1.

[00530] Immunizations to raise anti-KLH antibodies were conducted, over a period of 4 weeks, using Imject® Mariculture Keyhole Limpet hemocyanin (mcKLH; Pierce Biotechnology, Rockford, IL; cat# 77600, lot#B144095B). Immunizations were conducted using 10μg of KLH per mouse in Aluminium Phosphate Gel Adjuvant (HCI Biosector, Frederikssund, Denmark; Catalog # 1452-250); delivered via footpad injection. The initial immunization of the XMG1K strain of XenoMouse® was according to methods previously disclosed (Mendez et al., *Nat. Genet.* 15:146-156 (1997); published International Patent Application Nos. WO 98/24893, and WO 00/76310, the disclosures of which are hereby incorporated by reference, which are all hereby incorporated by reference). Following the initial immunization, subsequent boosts of immunogen (5-10 μg/mouse) were administered on a schedule and for the duration necessary to

induce a suitable anti-KLH titer in the mice. Titers were determined by enzyme immunoassay using immobilized KLH (Pierce Biotechnology, Rockford, IL).

[00531] Preparation of monoclonal antibodies. Mice exhibiting suitable titers were identified, and lymphocytes and splenocytes were obtained from draining lymph nodes and spleen, then were pooled for each cohort. B cells were dissociated from the tissue by grinding in a suitable medium (for example, Dulbecco's Modified Eagle Medium; DMEM; Invitrogen, Carlsbad, CA) to release the cells from the tissues, and were suspended in DMEM. B cells were selected and/or expanded using standard methods, and fused with suitable fusion partner, for example, nonsecretory myeloma P3X63Ag8.653 cells (American Type Culture Collection CRL 1580; Kearney et al, *J. Immunol.* 123:1548-1550 (1979)), using techniques known in the art.

[00532] B cells were mixed with fusion partner cells at a ratio of 1:4. The cell mixture was gently pelleted by centrifugation at 400 x g for 4 minutes, the supernatant was decanted, and the cell mixture was gently mixed by using a 1 ml pipette. Fusion was induced with PEG/DMSO (polyethylene glycol/dimethyl sulfoxide; obtained from Sigma-Aldrich, St. Louis MO; 1 ml per million of lymphocytes). PEG/DMSO was slowly added with gentle agitation over one minute followed, by one minute of mixing. IDMEM (DMEM without glutamine; 2 ml per million of B cells), was then added over 2 minutes with gentle agitation, followed by additional IDMEM (8 ml per million B-cells) which was added over 3 minutes.

[00533] The fused cells were gently pelleted (400 x g 6 minutes) and resuspended in 20 ml Selection medium (for example, DMEM containing Azaserine and Hypoxanthine [HA] and other supplemental materials as necessary) per million B-cells. Cells were incubated for 20-30 minutes at 37°C and then were resuspended in 200 ml Selection medium and cultured for three to four days in T175 flasks prior to 96-well plating.

[00534] Cells were distributed into 96-well plates using standard techniques to maximize clonality of the resulting colonies. After several days of culture, the hybridoma supernatants were collected and subjected to screening assays as

detailed in the examples below, including confirmation of binding to KLH or DNP, respectively. Positive cells were further selected and subjected to standard cloning and subcloning techniques. Clonal lines were expanded in vitro, and the secreted human antibodies obtained for analysis. Several cell lines secreting DNP-specific antibodies were obtained, and the antibodies were further characterized. The sequences thereof are presented herein and in the Sequence Listing, and results of various tests using these antibodies are provided.

[00535] Transient expression to generate recombinant monoclonal antibodies. Transient transfections were carried out in HEK 293-6E cells as follows. The human embryonic kidney 293 cell line stably expressing Epstein Barr virus Nuclear Antigen-1 (293-6E cells) was obtained from the National Research Council (Montreal, Canada). Cells were maintained as serum-free suspension cultures using F17 medium (Invitrogen, Carlsbad, CA) supplemented with 6 mM L-glutamine (Invitrogen, Carlsbad, CA), 1.1% F-68 Pluronic (Invitrogen, Carlsbad, CA) and 250 μg/ul Geneticin (Invitrigen, Carlsbad, CA). The suspension cell cultures were maintained in Erlenmeyer shake flask cultures. The culture flasks were shaken at 65 rpm at 37 °C in a humidified, 5% CO_2 atmosphere. A stock solution (1mg/ml) of 25-kDa linear PEI (Polysciences, Warrington, PA) was prepared in water, acidified with HCl to pH 2.0 until dissolved, then neutralized with NaOH, sterilized by filtration (0.2 μm), aliquoted, and stored at -20°C until used. Tryptone N1 was obtained from OrganoTechni S.A. (TekniScience, QC, Canada). A stock solution (20%, w/v) was prepared in Freestyle medium (Invitrogem, Carlsbad, CA), sterilized by filtration through 0.2 μm filters, and stored at 4°C until use. Typically, transfections were performed at the 1L scale. Cells (293-6E) were grown too a viable cell density of 1.1 X 106 cells/ml then transfection complexes were prepared in 1/10th volume of the final culture volume. For a 1-L transfection culture, transfection complexes were prepared in 100 ml F17 basal medium, and 500 μg plasmid DNA (heavy chain and light chain DNA, 1:1 ratio) was first diluted in 100 ml F17 medium. After a 5-minute incubation at room temperature, 1.5 ml of PEI solution was added. The complexes were vortexed mildly, then incubated for 15 minutes at room

temperature. The cells were transfected by adding the transfection complex mix to the cells in the shale flask culture. 24 hours post-transfection, Tryptone N1 was added to the transfected culture to a final concentration of 0.5%, and the transfected cultures were maintained on a shaker at 65 rpm at 37°C in a humidified, 5% CO_2 atmosphere for another 5 days after which they were harvested. The conditioned medium was harvested by centrifugation at 4000 rpm, and then sterile filtered through 0.2 μm filter (Corning Inc.).

[00536] The stably expressed aKLH 120.6 control antibody pool was created by transfecting CHO d- host cells with expression plasmids pDC323 anti-KLH 120.6 kappa LC and pDC324 anti-KLH 120.6-IgG2 HC using a standard electroporation procedure. After transfection, the cells were grown as a pool in a serum free -GHT selective growth media to allow for selection and recovery of the plasmid containing cells. Cell pools grown in –GHT selective media were cultured until they reached > 85% viability. The selected cell pools were amplified with 150 nm and 300 nM methotrexate (MTX). Upon reaching >85% viability the 150 nM pools were then further re amplified in 500 nm MTX. When the viability of the MTX amplified pools reached >85% viability, the pools were screened using an abbreviated six day batch production assay with an enriched production media to assess expression. The expression of the amplified pools ranged from 120-400 μg/mL. The best pool was chosen based on the six-day assay and scaled-up using a ten-day fed batch process. The conditioned media was harvested and purified to provide protein for analysis.

[00537] The stably expressed aKLH 120.6 antibody pool was created by transfecting CHO d- host cells with expression plasmids pDC323 anti-KLH 120.6 kappa LC and pDC324 anti-KLH 120.6-IgG2 HC using a standard electroporation procedure. After transfection, the cells were grown as a pool in a serum free -GHT selective growth media to allow for selection and recovery of the plasmid containing cells. Cell pools grown in –GHT selective media were cultured until they reached > 85% viability. The selected cell pools were amplified with 150 nm and 300nM MTX. Upon reaching >85% viability the 150nM pools were then further re amplified in 500 nm MTX. When the viability of the MTX amplified

pools reached >85% viability, the pools were screened using an abbreviated six day batch production assay with an enriched production media to assess expression. The expression of the amplified pools ranged from 120-400 μg/mL. The best pool was chosen based on the six day assay and scaled up using a ten day fed batch process. The conditioned media was harvested and purified to provide protein for analysis.

[00538] The aDNP 3A4 antibody stable expression pools were created by transfecting CHO DHFR(-) host cells with corresponding heavy chain and light chain expression plasmid sets using a standard electroporation procedure. Per each antibody molecule, 3-4 different transfections were performed to generate multiple pools. After transfection the cells were grown as a pool in a serum free -GHT selective growth media to allow for selection and recovery of the plasmid containing cells. Cell pools grown in –GHT selective media were cultured until they reached >85% viability. The selected cell pools were amplified with 150 nm methotrexate. When the viability of the methotrexate amplified pools reached >85% viability, the pools were screened using an abbreviated six day batch production assay with an enriched production media to assess expression. The best pool was chosen based on the six day assay titer and correct mass confirmation.

[00539] Antibody purification and selections. The antibodies were purified by Mab Select Sure chromatography (GE Life Sciences) using 8 column volumes of Dulbecco's PBS without divalent cations as the wash buffer and 100 mM acetic acid, pH 3.5, as the elution buffer at 7°C. The elution peak was pooled based on the chromatogram and the pH was raised to about 5.0 using 2 M Tris base. The pool was then diluted with at least 3 volumes of water, filtered through a 0.22-μm cellulose acetate filter and then loaded on to an SP-HP sepharose column (GE Life Sciences) and washed with 10 column volumes of S-Buffer A (20 mM acetic acid, pH 5.0) followed by elution using a 20 column volume gradient to 50% S-Buffer B (20 mM acetic acid, 1 M NaCl, pH 5.0) at 7 °C. A pool was made based on the chromatogram and SDS-PAGE analysis, then the material was concentrated about 7-fold and diafiltered against about 5 volumes of 10 mM acetic acid, 9% sucrose,

pH 5.0 using a VivaFlow TFF cassette with a 30 kDa membrane. The dialyzed material was then filtered through a 0.22-μm cellulose acetate filter and the concentration was determined by the absorbance at 280 nm.

[00540] IgG2-Fc ShK[1-35, Q16K] mammalian expression. Using the DNA pTT5-aKLH120.6-VK1SP-IgG2-HC-L10-ShK[1-35, Q16K] construct, the fragment containing the DNA coding sequence

GGATCCGGAGGAGGAGGAAGCCGCAGCTGCATCGACACCATCCCCAA
GAGCCGCTGCACCGCCTTCAAGTGCAAGCACAGCATGAAGTACCGCCT
GAGCTTCTGCCGCAAGACCTGCGGCACCTGCTAATGAGCGGCCGCTCG
AGGCCGGCAAGGCCGGATCC// (SEQ ID NO:436)

was cut out using BamHI/BamHI. This coding sequence (SEQ ID NO:436) encodes ShK(1-35, Q16K) with an N-terminal linker sequence:

GSGGGGSRSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCGTC// (SEQ ID NO:437).

[00541] At the same time, pTT14-hIgG2-Fc-ShK[1-35]WT construct, was also digested by BamHI/BamHI, thereby removing the Shk[1-35] coding region to yield the coding sequence

ATGGAATGGAGCTGGGTCTTTCTCTTCTTCCTGTCAGTAACGACTGGTG
TCCACTCCGAGCGCAAAGTCGAGTGCCCACCGTGCCCAGCACCACCTG
TGGCAGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCC
TCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGACGTGA
GCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCGTGG
AGGTGCATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGC
ACGTTCCGTGTGGTCAGCGTCCTCACCGTTGTGCACCAGGACTGGCTG
AACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCAGC
CCCCATCGAGAAAACCATCTCCAAAACCAAAGGGCAGCCCCGAGAAC
CACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACC
AGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCG
CCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACC
ACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGC
TCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCT

CCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCT CCCTGTCTCCGGGTAAAGGAGGAGGA // (SEQ ID NO:391, encoding the amino acid sequence MEWSWVFLFFLSVTTGVHSERKVECPPCPAPPVAGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRV VSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLP PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGG// (SEQ ID NO:392).

[00542] The pTT14-hIgG2-Fc vector with the ShK removed was treated with Calf Intestine Phosphatase (CIP) to remove the 5' Phosphate group and Phenol/Chloroform extracted to prevent religation of the vector upon itself. The insert ShK[1-35, Q16K] fragment was gel purified away from its vector and cleaned up with Qiagen Gel Purification Kit. . The purified insert was ligated to the large vector fragment and transformed into OneShot Top10 bacteria. DNAs from transformed bacterial colonies were isolated and subjected to BamHI restriction enzyme digestion and resolved on a one percent agarose gel. DNAs resulting in an expected pattern were submitted for sequencing. Although, analysis of several sequences of clones yielded a 100% percent match with the above sequence, only one clone was selected for large scaled plasmid purification. The final pTT14-IgG2-Fc-ShK[1-35, Q16K] construct encoded the following IgG2 Fc-ShK(1-35, Q16K) fusion protein sequence: MEWSWVFLFFLSVTTGVHSERKVECPPCPAPPVAGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRV VSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLP PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGG SGGGGSRSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCGTC// (SEQ ID NO:348).

[00543] Mammalian expression of anti-KLH immunoglobulin heavy chain (HC) and light chain (LC) toxin peptide (and toxin peptide analog) fusions. The

components of the aKLH IgG2/Fc-ShK (schematically represented in Figure 12E) included:

[00544] (a) aKLH 120.6 kappa LC:
MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSLSASVGDRVTITCRASQG
IRNDLGWYQQKPGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQP
EDFATYYCLQHNSYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS
VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC// (SEQ ID NO:338);

[00545] (b) aKLH 120.6 IgG2 HC:
MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASG
YTFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRD
TSISTAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSAST
KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCV
ECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFN
WYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS
NKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK// SEQ ID NO:339;

[00546] and

[00547] (c) IgG2 Fc-L10-ShK(1-35):
MEWSWVFLFFLSVTTGVHSERKVECPPCPAPPVAGPSVFLFPPKPKDTLMI
SRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRV
VSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLP
PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDG
SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGG
SGGGGSRSCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC// (SEQ ID NO:340).

[00548] The desired aKLH IgG2/Fc-ShK product contained one copy of each of components (a)-(c), immediately above, configured as in Figure 12E. Because of this, the ratio was 1:1:1. This product can be described as half

antibody and half Fc fusion ("hemibody"), coupled together at the Fc domain. Additional peptide assemblies that had to be removed from the culture were the aKLH Ab and the Fc-ShK homodimer.

[00549] The components of the aKLH 120.6 IgG2-ShK fusion antibody (schematically represented in Figure 12F) include:

[00550] (a) aKLH 120.6 kappa LC (SEQ ID NO:338, above);

[00551] (b) aKLH 120.6 IgG2 HC (SEQ ID NO:339, above); and

[00552] (c) aKLH 120.6 IgG2 HC-ShK fusion having the following sequence:

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASG
YTFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRD
TSISTAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSAST
KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCV
ECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFN
WYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS
NKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSRSCIDTIPKSRCTAFQCK
HSMKYRLSFCRKTCGTC// (SEQ ID NO:341).

[00553] The components of the aKLH 120.6 IgG2 HC-ShK[1-35, Q16K] fusion Ab (schematically represented in Figure 12F) include:

[00554] (a) aKLH 120.6 kappa LC (SEQ ID NO:338, above);

[00555] (b) aKLH 120.6 IgG2 HC (SEQ ID NO:339, above); and

[00556] (c) aKLH 120.6 IgG2-ShK[1-35, Q16K] fusion having the following sequence:

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASG
YTFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRD
TSISTAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSAST
KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCV

ECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFN
WYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS
NKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSRSCIDTIPKSRCTAFKCK
HSMKYRLSFCRKTCGTC// (SEQ ID NO:342).

[00557] The components of the monovalent aKLH 120.6 HC-ShK[1-35, R1A, I4A, Q16K] fusion antibody (schematically represented in Figure 12F) include the following monomers:

[00558] (a) aKLH 120.6 kappa LC (SEQ ID NO:338);

[00559] (b) aKLH 120.6 IgG2 HC (SEQ ID NO:339); and

[00560] (c) aKLH 120.6 IgG2 HC-ShK[1-35, R1A, I4A, Q16K] fusion having the following amino acid sequence:

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASG
YTFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRD
TSISTAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSAST
KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCV
ECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFN
WYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS
NKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSASCADTIPKSRCTAFKCK
HSMKYRLSFCRKTCGTC// (SEQ ID NO:462).

[00561] The desired monovalent aKLH 120.6 IgG2 HC-ShK analogue product was a full antibody with the ShK peptide fused to the C-terminus of one heavy chain. With two different heavy chains sharing one variety of light chain, the ratio of heavy chain: chain:light chain:heavychain-ShK was 1:2:1. The expected expression products are aKLH 120.6 IgG2 antibody, monovalent aKLH 120.6 IgG2 HC-ShK peptide analog, and bivalent KLH 120.6 IgG2 HC-ShK peptide analog. The monovalent aKLH 120.6 IgG2 HC-toxin peptide fusion-

containing antibody was isolated from the mix using cation exchange chromatography, as described herein.

[00562] The components of the monovalent aKLH 120.6 HC-ShK[1-35, R1A, Q16K, K30E] fusion antibody (schematically represented in Figure 12F) included the following monomers:

[00563] (a) aKLH 120.6 kappa LC (SEQ ID NO:338);

[00564] (b) aKLH 120.6 IgG2 HC (SEQ ID NO:339); and

[00565] (c) aKLH 120.6 IgG2-ShK[1-35, R1A, Q16K, K30E] fusion having the following sequence:

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASG
YTFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRD
TSISTAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSAST
KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCV
ECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFN
WYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS
NKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSASCIDTIPKSRCTAFKCK
HSMKYRLSFCRETCGTC// (SEQ ID NO:463).

[00566] The desired monovalent aKLH 120.6 IgG2 HC-ShK analogue Ab product was a full antibody with the ShK peptide fused to the C-terminus of one heavy chain. With two different heavy chains sharing one variety of light chain, the ratio of heavy chain: chain:light chain:heavychain-ShK was 1:2:1. The expected expression products are aKLH 120.6 IgG2 antibody, monovalent aKLH 120.6 IgG2 HC-ShK peptide analog, and bivalent aKLH 120.6 IgG2 HC-ShK peptide analog. The monovalent aKLH 120.6 IgG2 HC-toxin peptide fusion-containing antibody protein was isolated from the mix using cation exchange chromatography, as described herein.

[00567] The components of the monovalent aKLH 120.6 HC (IgG2)-ShK[1-35, R1H, I4A, Q16K] fusion Ab (schematically represented in Figure 12F) include monomers:

[00568] (a) aKLH 120.6 kappa LC (SEQ ID NO:338);

[00569] (b) aKLH 120.6 IgG2 HC (SEQ ID NO:339); and

[00570] (c) aKLH 120.6 HC IgG2-ShK[1-35, R1H, I4A, Q16K] fusion having the following amino acid sequence:

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASG
YTFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRD
TSISTAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSAST
KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCV
ECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFN
WYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS
NKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSHSCADTIPKSRCTAFKCK
HSMKYRLSFCRKTCGTC// (SEQ ID NO:464).

[00571] The desired monovalent aKLH 120.6 IgG2 HC-ShK analogue product was a full antibody with the ShK peptide fused to the C-terminus of one heavy chain. With two different heavy chains sharing one variety of light chain, the ratio of heavy chain: chain:light chain:heavychain-ShK peptide analog was 1:2:1. The expected expression products are aKLH 120.6 IgG2 antibody, monovalent aKLH 120.6 IgG2 HC-ShK peptide analog Ab, and bivalent KLH 120.6 IgG2 HC-ShK peptide analog Ab. The monovalent aKLH 120.6 IgG2 HC-toxin peptide fusion-containing Ab protein was isolated from the mix using cation exchange chromatography, as described herein.

[00572] The components of the monovalent aKLH 120.6 HC-ShK[1-35, R1H, Q16K, K30E] fusion Ab (schematically represented in Figure 12F) included the monomers:

[00573] (a) aKLH 120.6 kappa LC (SEQ ID NO:338);

[00574] (b) aKLH 120.6 IgG2 HC (SEQ ID NO:339); and

[00575] (c) aKLH 120.6 IgG2-ShK[1-35, R1H, Q16K, K30E] fusion having the following sequence:

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASG
YTFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRD
TSISTAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSAST
KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCV
ECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFN
WYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS
NKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSHSCIDTIPKSRCTAFKCK
HSMKYRLSFCRETCGTC// (SEQ ID NO:465).

[00576] The desired monovalent aKLH 120.6 IgG2 HC-ShK analogue Ab product was a full antibody with the ShK peptide fused to the C-terminus of one heavy chain. With two different heavy chains sharing one variety of light chain, the ratio of heavy chain: chain:light chain:heavychain-ShK peptide analog was 1:2:1. The expected expression products are aKLH 120.6 IgG2 antibody, monovalent aKLH 120.6 IgG2 HC-ShK peptide analog Ab, and bivalent KLH 120.6 IgG2 HC-ShK peptide analog Ab. The monovalent aKLH 120.6 IgG2 HC-toxin peptide fusion-containing Ab protein was isolated from the mix using cation exchange chromatography, as described herein.

[00577] The components of the monovalent aKLH 120.6 HC-ShK[1-35, R1K, I4A, Q16K] fusion Ab (schematically represented in Figure 12F) included the monomers:

[00578] (a) aKLH 120.6 kappa LC (SEQ ID NO:338);

[00579] (b) aKLH 120.6 IgG2 HC (SEQ ID NO:339); and

[00580] (c) aKLH 120.6 HC (IgG2)-ShK[1-35, R1K, I4A, Q16K] fusion having the following sequence:

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASG
YTFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRD
TSISTAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSAST
KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCV
ECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFN
WYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS
NKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSKSCADTIPKSRCTAFKCK
HSMKYRLSFCRKTCGTC// (SEQ ID NO:466).

[00581] The desired monovalent aKLH 120.6 IgG2 HC-ShK analogue Ab product was a full antibody with the ShK peptide fused to the C-terminus of one heavy chain. With two different heavy chains sharing one variety of light chain, the ratio of heavy chain: chain:light chain:heavychain-ShK peptide analog was 1:2:1. The expected expression products are aKLH 120.6 IgG2 antibody, monovalent aKLH 120.6 IgG2 HC-ShK peptide analog Ab, and bivalent KLH 120.6 IgG2 HC-ShK peptide analog Ab. The monovalent aKLH 120.6 IgG2 HC-toxin peptide fusion-containing Ab protein was isolated from the mix using cation exchange chromatography, as described herein.

[00582] The components of the monovalent aKLH 120.6 HC-ShK[1-35, R1K, Q16K, K30E] fusion Ab (schematically represented in Figure 12F) included the monomers:

[00583] (a) aKLH 120.6 kappa LC (SEQ ID NO:338);

[00584] (b) aKLH 120.6 IgG2 HC (SEQ ID NO:339); and

[00585] (c) aKLH 120.6 IgG2-ShK[1-35, R1K, Q16K, K30E] fusion having the following amino acid sequence:

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASG
YTFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRD
TSISTAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSAST
KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP

AVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCV
ECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFN
WYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS
NKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSKSCIDTIPKSRCTAFKCK
HSMKYRLSFCRETCGTC// (SEQ ID NO:467).

[00586] The desired monovalent aKLH 120.6 IgG2 HC-ShK analogue Ab product was a full antibody with the ShK peptide fused to the C-terminus of one heavy chain. With two different heavy chains sharing one variety of light chain, the ratio of heavy chain: chain:light chain:heavychain-ShK peptide analog was 1:2:1. The expected expression products are aKLH 120.6 IgG2 antibody, monovalent aKLH 120.6 IgG2 HC-ShK peptide analog Ab, and bivalent KLH 120.6 IgG2 HC-ShK peptide analog Ab. The monovalent aKLH 120.6 IgG2 HC-toxin peptide fusion-containing Ab protein was isolated from the mix using cation exchange chromatography, as described herein.

[00587] The components of the aKLH 120.6 IgG2-ShK[2-35, Q16K] fusion Ab (schematically represented in Figure 12F) include :

[00588] (a) aKLH 120.6 kappa LC (SEQ ID NO:338, above);

[00589] (b) aKLH 120.6 IgG2 HC (SEQ ID NO:339, above); and

[00590] (c) aKLH 120.6 IgG2-ShK[2-35, Q16K] fusion having the following sequence:

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASG
YTFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRD
TSISTAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSAST
KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCV
ECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFN
WYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS
NKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS

CSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSSCIDTIPKSRCTAFKCKH SMKYRLSFCRKTCGTC// (SEQ ID NO:387).

[00591] The desired aKLH 120.6 IgG2-ShK Ab product was a full antibody with the ShK peptide fused to the C-terminus of one heavy chain, configured as in Figure 12F. With two different heavy chains sharing one variety of light chain, the ratio of heavy chain:light chain:heavy chain-ShK was 1:2:1. The expected expression products are aKLH 120.6 IgG2, monovalent aKLH 120.6 IgG2-ShK Ab, and bivalent aKLH 120.6 IgG2-ShK Ab. The monovalent aKLH 120.6 IgG2-toxin peptide (or toxin peptide analog) fusion-containing Ab was isolated from the mix using cation exchange chromatography, as described herein.

[00592] The aKLH IgG1-loop-ShK Ab also had a single copy of the ShK peptide sequence inserted into one of the heavy chains, but in this case it was inserted into an internal conjugation site in the Fc domain instead of at the C-terminus. (See, e.g., Gegg et al., U.S. Patent No. 7,442,778; U.S. Patent No. 7,655,765; U.S. Patent No. 7,655,764; U.S. Patent No. 7,662,931; U.S. Patent No. 7,645,861; published U.S. Patent Applications US 2009/0281286; and US 2009/0286964, each of which are incorporated herein by reference in their entireties). The components of the aKLH IgG1-loop-ShK Ab included

[00593] (a) aKLH 120.6 kappa LC (SEQ ID NO:338, above);

[00594] (b) aKLH 120.6 IgG1 HC:
MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASG YTFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRD TSISTAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK// (SEQ ID NO:343);

[00595] and

[00596] (c) aKLH 120.6 IgG1-loop-ShK:
MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASG
YTFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRD
TSISTAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSAST
KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELGGRSCIDTIPKSRC
TAFKCKHSMKYRLSFCRKTCGTCGGTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL
HNHYTQKSLSLSPGK// (SEQ ID NO:344).

[00597] With two different heavy chains sharing one light chain, the ratio of heavy chain:light chain:heavy chain-ShK is 1:2:1. The expected expression products are aKLH 120.6 IgG1, monovalent aKLH 120.6 IgG1-loop-ShK Ab, and bivalent aKLH 120.6 IgG1-loop-ShK Ab. The monovalent aKLH 120.6 IgG1-loop-ShK Ab (represented schematically by Figure 12N) was isolated from the mix using cation exchange chromatography as described herein.

[00598] Monovalent aKLH 120.6 kappa LC-ShK[1-35, Q16K] fusion. The components of the monovalent aKLH 120.6 kappa LC-ShK[1-35, Q16K] fusion Ab (schematically represented in Figure 12J) included the monomers:

[00599] (a) aKLH 120.6 IgG2 HC (SEQ ID NO:339);

[00600] (b) aKLH 120.6 kappa LC (SEQ ID NO:338); and

[00601] (c) aKLH 120.6 kappa LC-ShK[1-35, Q16K] fusion having the following sequence:
MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSLSASVGDRVTITCRASQG
IRNDLGWYQQKPGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQP
EDFATYYCLQHNSYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS
VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSGGGGSRSCIDTI
PKSRCTAFKCKHSMKYRLSFCRKTCGTC// (SEQ ID NO:439).

This embodiment of monovalent aKLH 120.6 IgG2 LC-ShK[1-35, Q16K] Ab product was a full antibody with the ShK peptide fused to the C-terminus of one light chain as shown in Figure 12J. With two different light chains sharing one variety of heavy chain, the ratio of light chain:heavy chain:light chain-ShK[1-35, Q16K] was 1:2:1. The expected expression products are aKLH 120.6 IgG2, monovalent KLH 120.6 IgG2 LC-ShK[1-35, Q16K] Ab, and bivalent KLH 120.6 IgG2 LC-ShK[1-35, Q16K] Ab. The monovalent KLH 120.6 IgG2 LC-toxin peptide fusion-containing Ab was isolated from the mix using cation exchange chromatography, as described herein.

[00602] Monovalent aKLH 120.6 kappa LC-ShK[2-35, Q16K] fusion. The components of the monovalent aKLH 120.6 kappa LC-ShK[2-35, Q16K] fusion Ab (schematically represented in Figure 12J) included the monomers:

[00603] (a) aKLH 120.6 IgG2 HC (SEQ ID NO:339);

[00604] (b) aKLH 120.6 kappa LC (SEQ ID NO:338); and

[00605] (c) aKLH 120.6 kappa LC-ShK[2-35, Q16K] fusion having the following sequence:

[00606] MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSLSASVGDR VTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPSRFSGSGSGT EFTLTISSLQPEDFATYYCLQHNSYPLTFGGGTKVEIKRTVAAPSVFIFPPSD EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSGG GGSSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCGTC// (SEQ ID NO:440). This embodiment of monovalent aKLH 120.6 IgG2 LC-ShK[2-35, Q16K] Ab product was a full antibody with the ShK peptide fused to the C-terminus of one light chain as shown in Figure 12J. With two different light chains sharing one variety of heavy chain, the ratio of light chain:heavy chain:light chain-ShK[2-35, Q16K] was 1:2:1. The expected expression products are KLH 120.6 IgG2, monovalent KLH 120.6 IgG2 LC-ShK[2-35, Q16K] Ab, and bivalent KLH 120.6 IgG2 LC-ShK[2-35, Q16K] Ab. The monovalent KLH 120.6 IgG2 LC-toxin peptide fusion-containing Ab was isolated from the mix using cation exchange chromatography, as described herein.

[00607] Bivalent aKLH 120.6 kappa LC-ShK[1-35, Q16K] fusion. The components of the bivalent aKLH 120.6 kappa LC-ShK[1-35, Q16K] fusion Ab (schematically represented in Figure 12K) included the monomers:

[00608] (a) aKLH 120.6 IgG2 HC (SEQ ID NO:339); and

[00609] (b) aKLH 120.6 kappa LC-ShK[1-35, Q16K] fusion (SEQ ID NO:439), above.

This embodiment of bivalent KLH 120.6 IgG2 LC-ShK[1-35, Q16K] Ab product was a full antibody with the ShK peptide fused to the C-terminus of both light chains as shown in Figure 12K. The ratio of heavy chain:light chain-ShK[1-35, Q16K] was 1:1. The expected expression product is bivalent aKLH 120.6 IgG2 LC-ShK[1-35, Q16K] Ab. The bivalent KLH 120.6 IgG2 LC-ShK[1-35, Q16K] peptide fusion-containing Ab was isolated from the mix using cation exchange chromatography, as described herein.

[00610] Bivalent aKLH 120.6 kappa LC-ShK[2-35, Q16K] fusion. The components of the bivalent aKLH 120.6 kappa LC-ShK[2-35, Q16K] fusion Ab (schematically represented in Figure 12K) included the monomers:

[00611] (a) aKLH 120.6 IgG2 HC (SEQ ID NO:339); and

[00612] (b) aKLH 120.6 kappa LC-ShK[2-35, Q16K] fusion (SEQ ID NO:440), above.

This embodiment of bivalent aKLH 120.6 IgG2 LC-ShK[2-35, Q16K] Ab product was a full antibody with the ShK peptide fused to the C-terminus of both light chains as shown in Figure 12K. The ratio of heavy chain:light chain-ShK[2-35, Q16K] was 1:1. The expected expression product is bivalent aKLH 120.6 IgG2 LC-ShK[2-35, Q16K] Ab. The bivalent KLH 120.6 IgG2 LC-ShK[2-35, Q16K] peptide fusion-containing Ab was isolated from the mix using cation exchange chromatography, as described herein.

[00613] Trivalent aKLH 120.6 kappa LC-ShK[1-35, Q16K] fusion. The components of the trivalent aKLH 120.6 kappa LC-ShK[1-35, Q16K] fusion Ab (schematically represented in Figure 12L) included the monomers:

[00614] a) aKLH 120.6 IgG2 HC (SEQ ID NO:339, above);

[00615] (b) aKLH 120.6 IgG2 HC-Shk[1-35, Q16K] fusion having the amino acid of SEQ ID NO:342, above; and

[00616] (c) aKLH 120.6 kappa LC-ShK[1-35, Q16K] fusion having the amino acid sequence of SEQ ID NO:439, above.

This embodiment of trivalent aKLH 120.6 IgG2 LC-ShK Ab product was a full antibody with the ShK[1-35, Q16K] peptide fused to the C-terminus of both light chains and one heavy chain as shown in Figure 12L. With two different heavy chains sharing one variety of light chain, the ratio of heavy chain:light chain-ShK[1-35, Q16K]:heavy chain-ShK[1-35, Q16K] was 1:2:1. The expected expression products were a bivalent aKLH 120.6 IgG2 LC-ShK[1-35, Q16K] Ab, trivalent aKLH 120.6 IgG2 LC-ShK[1-35, Q16K] Ab, and tetravalent aKLH 120.6 IgG2 LC-ShK[1-35, Q16K] Ab. The trivalent KLH 120.6 IgG2 LC-toxin peptide fusion-containing Ab was isolated from the mix using cation exchange chromatography, as described herein.

[00617] Trivalent aKLH 120.6 kappa LC-ShK[2-35, Q16K] fusion. The components of the trivalent KLH 120.6 kappa LC-ShK[2-35, Q16K] fusion Ab (schematically represented in Figure 12L) included the monomers:

[00618] a) aKLH 120.6 IgG2 HC (SEQ ID NO:339);

[00619] (b) aKLH 120.6 IgG2 HC-Shk[2-35, Q16K] fusion (SEQ ID NO:387), above; and

[00620] (c) aKLH 120.6 kappa LC-ShK[2-35, Q16K] fusion (SEQ ID NO:440), above.

This embodiment of trivalent aKLH 120.6 IgG2 LC-ShK[2-35, Q16K] Ab product was a full antibody with the ShK[2-35, Q16K] peptide fused to the C-terminus of both light chains and one heavy chain as shown in Figure 12L. With two different heavy chains sharing one variety of light chain, the ratio of heavy chain:light chain-ShK[2-35, Q16K]:heavy chain-ShK[2-35, Q16K] was 1:2:1. The expected expression products were a bivalent aKLH 120.6 IgG2 LC-ShK[2-35, Q16K] Ab, trivalent aKLH 120.6 IgG2 LC-ShK[2-35, Q16K] Ab, and tetravalent aKLH 120.6 IgG2 LC-ShK[2-35, Q16K] Ab. The trivalent aKLH 120.6 IgG2 LC-toxin

peptide fusion-containing Ab protein was isolated from the mix using cation exchange chromatography, as described herein.

[00621] Anti-KLH 120.6 Antibody Light Chain mammalian expression. The XenoMouse® hybridoma expressing aKLH monoclonal antibody 120.6 was used as a source to isolate total RNA using TRIzol® reagent (Invitrogen). First strand cDNA was synthesized using a random primer with an extension adapter 5'-GGC CGG ATA GGC CTC CAN NNN NNT–3'(SEQ ID NO:349) and a 5' RACE (rapid amplification of cDNA ends) was performed using the GeneRacer™ Kit (Invitrogen). For the light chain sequence determination, the forward primer was 5'-GTG GTT GAG AGG TGC CAG ATG TGA CAT TGT GAT GAC TCA GTC TCC -3' (SEQ ID NO:350) and the reverse primer was 5'- AAC CGT TTA AAC GCG GCC GCT CAA CAC TCT CCC CTG TTG AA -3' (SEQ ID NO:351). The RACE product was cloned into pCR4-TOPO (Invitrogen) and the sequences determined. Consensus sequences were used to determine probable framework and signal peptide sequence and design primers for full-length antibody chain PCR amplification.

[00622] The expression clone for the anti-KLH 120.6 kappa light chain was prepared by PCR. The 5' PCR primer encoded the amino terminus of the signal sequence, an *Sal*I restriction enzyme site, and an optimized Kozak sequence 5'-AAG CTC GAG GTC GAC TAG ACC ACC ATG GAC ATG AGG GTC CCC G -3' (SEQ ID NO:352). The 3' primer encoded the carboxyl terminus and termination codon, as well as a *Not*I restriction site 5'-AAC CGT TTA AAC GCG GCC GCT CAA CAC TCT CCC CTG TTG AA -3' (SEQ ID NO:351). The resulting product was cloned into pCR4-TOPO (Invitrogen) and the sequences determined. After the insert was confirmed, the pCR4-TOPO product was cut with *Sal*I and *Not*I, the insert gel isolated and Qiagen purified, and then ligated into the mammalian expression vector pTT5.

[00623] A PCR was done to change the signal peptide from the native peptide derived from the hybridoma to the VK1/O12 peptide. The primers used for the VK1/O12 fragment were 5' AAG CTC GAG GTC GAC TAG ACC ACC

ATG GAC ATG AGG GTG CCC GCT 3' (SEQ ID NO:353) and 5'-TCA TCT GGA TGT CAC ATC TGG CAC C –3' (SEQ ID NO:354). The primers used for the mature light chain peptide were 5'-GGT GCC AGA TGT GAC ATC CAG ATG A –3' (SEQ ID NO:355) and (SEQ ID NO:351). The resulting fragments were joined by overlap PCR using primers SEQ ID NO:353 and SEQ ID NO:351. The sequence of the resulting clone encodes the following immunoglobulin kappa LC sequence:
MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSLSASVGDRVTITCRASQG IRNDLGWYQQKPGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQP EDFATYYCLQHNSYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC// (SEQ ID NO:338).

[00624] Anti-KLH 120.6 Antibody Light Chain-ShK peptide analog mammalian expression. The Shk[1-35, Q16K] fragment was generated by PCR using the pTT14-huIgG2-Fc ShK[1-35, Q16K] encoding (SEQ ID NO:348), described above, as a template and the oligos:

[00625] 5'- AAC AGG GGA GAG TGT GGA GGA GGA GGA TCC GGA G -3' (SEQ ID NO:441); and

[00626] 5'- CAT GCG GCC GCT CAT TAG CAG G -3' (SEQ ID NO:442).

[00627] The light chain fragment and ShK PCR product were then amplified by PCR using the outside primers SEQ ID NO: CAT TCT AGA ACC ACC ATG GAC ATG AGG GTG//(SEQ ID NO:482) and SEQ ID NO:442. The PCR product was then digested by XbaI and NotI and PCR clean up kit (Qiagen) purified. At the same time, pYD16 was cut by XbaI and NotI. The pYD16 vector was run out on a 1% agarose gel and the larger fragment was cut out and gel purified by Qiagen's Gel Purification Kit. The purified PCR product was ligated to the large vector fragment and transformed into OneShot Top10 bacteria. DNAs from transformed bacterial colonies were isolated and subjected to XbaI and NotI restriction enzyme digestions and resolved on a one percent agarose gel. DNAs resulting in an expected pattern were submitted for sequencing. Although,

analysis of several sequences of clones yielded a 100% percent match with the above sequence, only one clone was selected for large scaled plasmid purification. The final pYD16-aKLH120.6-VK1SP-LC-L10-ShK[1-35, Q16K] construct encoded an aKLH 120.6 LC-L10-ShK[1-35, Q16K] fusion polypeptide (SEQ ID NO:439).

[00628] The Shk[2-35, Q16K] fragment was generated as described above using pTT5-aKLH120.6 HC-ShK[2-35, Q16K] as a template and the oligonucleotide primers SEQ ID NO:441 and SEQ ID NO:442.

[00629] The light chain and ShK PCR products were amplified by PCR using the outside primers SEQ ID NO:482 and SEQ ID NO:442. The PCR product was then digested by XbaI and NotI and PCR clean up kit (Qiagen) purified. At the same time, pYD16 was cut by XbaI and NotI. The pYD16 vector was run out on a 1% agarose gel and the larger fragment was cut out and gel purified by Qiagen's Gel Purification Kit. The purified PCR product was ligated to the large vector fragment and transformed into OneShot Top10 bacteria. DNAs from transformed bacterial colonies were isolated and subjected to XbaI and NotI restriction enzyme digestions and resolved on a one percent agarose gel. DNAs resulting in an expected pattern were submitted for sequencing. Although, analysis of several sequences of clones yielded a 100% percent match with the above sequence, only one clone was selected for large scaled plasmid purification. The final pYD16-aKLH120.6-VK1SP-LC-L10-ShK[2-35, Q16K] construct encoded an aKLH LC-L10-ShK[2-35, Q16K] fusion polypeptide (SEQ ID NO:440).

[00630] aKLH -IgG2 Heavy Chain-L10-ShK[1-35] and aKLH-IgG2 Heavy Chain-L10-ShK[1-35, Q16K] mammalian expression.

Using oligos

5'-CAT TCT AGA CCC ACC ATG GAC ATG AGG GTG-3' (SEQ ID NO:393); and

5'-GGA TCC TCC TCC TCC ACC CGG AGA CAG GGA GAG G-3' (SEQ ID NO:358),

the a-KLH-IgG2-Heavy Chain region was amplified by PCR from a pTT5-aKLH 120.6-VK1SP-IgG2 Heavy Chain(HC) construct containing the coding sequence (SEQ ID NO:356; below), encoding aKLH 120.6-VK1SP-IgG2 Heavy Chain (SEQ ID NO:357; below):

ATGGACATGAGGGTGCCCGCTCAGCTCCTGGGGCTCCTGCTGCTGTGG
CTGAGAGGTGCCAGATGTCAGGTGCAGCTGGTGCAGTCTGGGGCTGAG
GTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTCCTGCAAGGCTTCTGGA
TACACCTTCACCGGCTACCACATGCACTGGGTGCGACAGGCCCCTGGA
CAAGGGCTTGAGTGGATGGGATGGATCAACCCTAACAGTGGTGGCAC
AAACTATGCACAGAAGTTTCAGGGCAGGGTCACCATGACCAGGGACA
CGTCCATCAGCACAGCCTACATGGAGCTGAGCAGGCTGAGATCTGACG
ACACGGCCGTGTATTACTGTGCGAGAGATCGTGGGAGCTACTACTGGT
TCGACCCCTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGCCTCCA
CCAAGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCT
CCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCG
AACCGGTGACGGTGTCGTGGAACTCAGGCGCTCTGACCAGCGGCGTGC
ACACCTTCCCAGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAG
CGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCCAGACCTACACCTG
CAACGTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGACAGTTG
AGCGCAAATGTTGTGTCGAGTGCCCACCGTGCCCAGCACCACCTGTGG
CAGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCA
TGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGACGTGAGCC
ACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCGTGGAG
GTGCATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGCAC
GTTCCGTGTGGTCAGCGTCCTCACCGTTGTGCACCAGGACTGGCTGAA
CGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCAGCCC
CCATCGAGAAAACCATCTCCAAAACCAAAGGGCAGCCCCGAGAACCA
CAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCA
GGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGC
CGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCA
CACCTCCCATGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCT

CACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTC
CGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTC
CCTGTCTCCGGGT// (SEQ ID NO:356),
encoding the amino acid sequence
MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASG
YTFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRD
TSISTAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSAST
KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCV
ECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFN
WYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS
NKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPG// (SEQ ID NO:357).

[00631] The ShK[1-35]WT fragment was generated using the original Fc-L10-ShK[1-35] in pcDNA3.1(+)CMVi as a template (described in Example 1, Figure 14A-14B in Sullivan et al., Toxin Peptide Therapeutic Agents, PCT/US2007/022831, published as WO 2008/088422, which is incorporated herein by reference in its entirety) and the oligos:
5'-TCC CTG TCT CCG GGT GGA GGA GGA GGA TCC GGA G-3' (SEQ ID NO:359); and 5'- CAT GCG GCC GCT CAT TAG CAG GTG -3' (SEQ ID NO:330)
The PCR products were run on a 1% agarose gel. The bands were punched for an agarose plug and the plugs were placed in a fresh PCR reaction tube. The agarose plugs were then amplified by PCR using the outside primers SEQ ID NO:357 and SEQ ID NO:330. The PCR product was then digested by XbaI and NotI and PCR clean up kit (Qiagen) purified. At the same time, pTT5 Vector (an Amgen vector containing a CMV promoter and Poly A tail) was cut by XbaI and NotI. The pTT5 vector was run out on a 1% agarose gel and the larger fragment was cut out and gel purified by Qiagen's Gel Purification Kit. The purified PCR product was ligated to the large vector fragment and transformed into OneShot Top10 bacteria.

DNAs from transformed bacterial colonies were isolated and subjected to XbaI and NotI restriction enzyme digestions and resolved on a one percent agarose gel. DNAs resulting in an expected pattern were submitted for sequencing. Although, analysis of several sequences of clones yielded a 100% percent match with the above sequence, only one clone was selected for large scaled plasmid purification. The final pTT5-aKLH 120.6-VK1SP-IgG2-HC-L10-ShK[1-35] construct encoded an IgG2-HC-L10-ShK[1-35] fusion polypeptide with the amino acid sequence:
MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASG
YTFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRD
TSISTAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSAST
KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCV
ECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFN
WYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS
NKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSRSCIDTIPKSRCTAFQCK
HSMKYRLSFCRKTCGTC// (SEQ ID NO:394).

[00632] To generate the ShK[1-35, Q16K] mutant version of this construct, site-directed mutagenesis was performed using the Stratagene Quikchange Multi site Directed Mutagenesis Kit (Cat#200531), per manufacturer's instructions, and oligos:
5'-GCT GCA CCG CCT TCA AGT GCA AGC ACA GC 3' (SEQ ID NO:325); and
5'- GCT GTG CTT GCA CTT GAA GGC GGT GCA GC -3' (SEQ ID NO:326),.
The final construct pTT5-aKLH120.6-VK1SP-IgG2-HC-L10-ShK[1-35, Q16K] encoded IgG2-HC-L10-ShK[1-35, Q16K] fusion polypeptide with the following amino acid sequence:
MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASG
YTFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRD
TSISTAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSAST

KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCV
ECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFN
WYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS
NKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSRSCIDTIPKSRCTAFKCK
HSMKYRLSFCRKTCGTC// (SEQ ID NO:333).

[00633] aKLH-IgG2 Heavy Chain-L10-ShK[2-35, Q16K] mammalian expression. Using DNA construct pTT5-aKLH 120.6-VK1SP-IgG2-HC-L10-ShK[1-35] as the vector, the ShK[1-35] was cut out using BamHI/BamHI. The vector fragment from pTT5-aKLH 120.6-VK1SP-IgG2-HC without ShK[1-35] contained the coding sequence:

ATGGACATGAGGGTGCCCGCTCAGCTCCTGGGGCTCCTGCTGCTGTGG
CTGAGAGGTGCCAGATGTCAGGTGCAGCTGGTGCAGTCTGGGGCTGAG
GTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTCCTGCAAGGCTTCTGGA
TACACCTTCACCGGCTACCACATGCACTGGGTGCGACAGGCCCCTGGA
CAAGGGCTTGAGTGGATGGGATGGATCAACCCTAACAGTGGTGGCAC
AAACTATGCACAGAAGTTTCAGGGCAGGGTCACCATGACCAGGGACA
CGTCCATCAGCACAGCCTACATGGAGCTGAGCAGGCTGAGATCTGACG
ACACGGCCGTGTATTACTGTGCGAGAGATCGTGGGAGCTACTACTGGT
TCGACCCCTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGCCTCCA
CCAAGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCT
CCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCG
AACCGGTGACGGTGTCGTGGAACTCAGGCGCTCTGACCAGCGGCGTGC
ACACCTTCCCAGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAG
CGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCCAGACCTACACCTG
CAACGTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGACAGTTG
AGCGCAAATGTTGTGTCGAGTGCCCACCGTGCCCAGCACCACCTGTGG
CAGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCA
TGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGACGTGAGCC

ACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCGTGGAG
GTGCATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGCAC
GTTCCGTGTGGTCAGCGTCCTCACCGTTGTGCACCAGGACTGGCTGAA
CGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCAGCCC
CCATCGAGAAAACCATCTCCAAAACCAAAGGGCAGCCCCGAGAACCA
CAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCA
GGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGC
CGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCA
CACCTCCCATGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCT
CACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTC
CGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTC
CCTGTCTCCGGGTGGAGGAGGA // (SEQ ID NO:399),

encoding the amino acid sequence

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASG
YTFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRD
TSISTAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSAST
KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCV
ECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFN
WYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS
NKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGGGG// (SEQ ID NO:400).

The vector fragment was then treated with Calf Intestine Phosphatase (CIP) to remove the 5' Phosphate group and Phenol/Chloroform extracted to prevent religation of the vector upon itself. The insert came from pTT14-VH21SP-IgG2-Fc-ShK[2-35, Q16K] encoding IgG2 Fc-L10-ShK(2-35, Q16K):

MEWSWVFLFFLSVTTGVHSERKVECPPCPAPPVAGPSVFLFPPKPKDTLMI
SRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRV
VSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLP
PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDG

SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGG SGGGGSSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCGTC// (SEQ ID NO:346),

and the insert was also digested out using BamHI/BamHI. The insert ShK[2-35, Q16K] fragment was gel purified away from its vector and cleaned up with Qiagen Gel Purification Kit. A purified DNA insert containing the coding sequence

GGA TCC GGA GGA GGA GGA AGC AGC TGC ATC GAC ACC ATC CCC AAG AGC CGC TGC ACC GCC TTC AAG TGC AAG CAC AGC ATG AAG TAC CGC CTG AGC TTC TGC CGC AAG ACC TGC GGC ACC TGC TAA TGA // (SEQ ID NO:397),

encoding the amino acid sequence

GSGGGGSSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCGTC (SEQ ID NO:398), was ligated to the large vector fragment and transformed into OneShot Top10 bacteria. DNAs from transformed bacterial colonies were isolated and subjected to BamHI restriction enzyme digestion and resolved on a one percent agarose gel. DNAs resulting in an expected pattern were submitted for sequencing. Although, analysis of several sequences of clones yielded a 100% percent match with the above sequence, only one clone was selected for large scaled plasmid purification. The final construct pTT5-aKLH-IgG2 HC-L10-ShK[2-35,Q16K] encoded an IgG2 HC-L10-ShK[2-35,Q16K] fusion polypeptide:

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASG YTFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRD TSISTAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSAST KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCV ECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFN WYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS NKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS

CSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSSCIDTIPKSRCTAFKCKH SMKYRLSFCRKTCGTC// (SEQ ID NO:401).

[00634] The Shk[1-35, R1A, I4A, Q16K] fragment was generated using pTT5-aKLH 120.6-VK1SP-IgG2-HC-L10-ShK[1-35 Q16K] as a template and the oligos:

[00635] 5'- AGG AGG AGG AAG CGC CAG CTG CGC CGA CAC CAT CCC C -3'// (SEQ ID NO:468); and

[00636] 5'- GGG GAT GGT GTC GGC GCA GCT GGC GCT TCC TCC TCC T -3'// (SEQ ID NO:469).

Site-directed mutagenesis was performed using the Stratagene Quikchange Multi site Directed Mutagenesis Kit, per manufacturer's instructions. The final pTT5-aKLH120.6-VK1SP-IgG2-HC-L10-ShK[1-35 R1A, I4A, Q16K] construct encoded an IgG2-HC-L10-ShK[1-35, R1A, I4A, Q16K] fusion polypeptide (SEQ ID NO:462).

[00637] The Shk[1-35, R1A, Q16K, K30E] fragment was generated as described above using the following four oligos:

[00638] 5'- GAG GAG GAG GAA GCG CCA GCT GCA TCG ACA -3'// (SEQ ID NO:470);

[00639] 5'- GAG CTT CTG CCG CGA GAC CTG CGG CAC -3'// (SEQ ID NO:471);

[00640] 5'- CGA TGC AGC TGG CGC TTC CTC CTC CTC -3'// (SEQ ID NO:472); and

[00641] 5'- GTG CCG CAG GTC TCG CGG CAG AAG CTC -3'// (SEQ ID NO:473).

The final pTT5-aKLH120.6-VK1SP-IgG2-HC-L10-ShK[1-35 R1A, Q16K, K30E] construct encoded an IgG2-HC-L10-ShK[1-35, R1A, Q16K, K30E] fusion polypeptide (SEQ ID NO:463).

[00642] The ShK[1-35, R1H, I4A, Q16K] fragment was generated using pTT5-aKLH120.6-VK1SP-IgG2-HC-L10-ShK[1-35 Q16K] as a template and the oligos:

[00643] 5'- GGA GGA GGA AGC CAC AGC TGC GCC GAC ACC ATC CCC -3'// (SEQ ID NO:474); and

[00644] 5'- GGG GAT GGT GTC GGC GCA GCT GTG GCT TCC TCC TCC -3'// (SEQ ID NO:475).

Site-directed mutagenesis was performed using the Stratagene Quikchange Multi site Directed Mutagenesis Kit (Cat#200531), per manufacturer's instructions. The final pTT5-aKLH120.6-VK1SP-IgG2-HC-L10-ShK[1-35 R1H, I4A, Q16K] construct encoded an IgG2-HC-L10-ShK[1-35, R1H, I4A, Q16K] fusion polypeptide (SEQ ID NO:464).

[00645] The Shk[1-35, R1H, Q16K, K30E] fragment was generated as described above using the following four oligos:

[00646] 5'- GGA GGA GGA AGC CAC AGC TGC ATC GAC -3'// (SEQ ID NO:476) and SEQ ID NO:471;

[00647] 5'- GTC GAT GCA GCT GTG GCT TCC TCC TCC -3'// (SEQ ID NO:477) and SEQ ID NO:473.

The final pTT5-aKLH120.6-VK1SP-IgG2-HC-L10-ShK[1-35 R1H, Q16K, K30E] construct encoded an IgG2-HC-L10-ShK[1-35, R1H, Q16K, K30E] fusion polypeptide (SEQ ID NO:465).

[00648] The Shk[1-35, R1K, I4A, Q16K] fragment was generated using pTT5-aKLH 120.6-VK1SP-IgG2-HC-L10-ShK[1-35 Q16K] as a template and the oligos:

[00649] 5'- CCG GAG GAG GAG GAA GCA AGA GCT GCG CCG ACA CCA TCC CCA AGA -3'// (SEQ ID NO:478); and

[00650] 5'- TCT TGG GGA TGG TGT CGG CGC AGC TCT TGC TTC CTC CTC CTC CGG -3'// (SEQ ID NO:479).

Site-directed mutagenesis was performed using the Stratagene Quikchange Multi site Directed Mutagenesis Kit (Cat#200531), per manufacturer's instructions. The final pTT5-aKLH120.6-VK1SP-IgG2-HC-L10-ShK[1-35 R1K, I4A, Q16K] construct encoded an IgG2-HC-L10-ShK[1-35, R1K, I4A, Q16K] fusion polypeptide (SEQ ID NO:466).

[00651] The Shk[1-35, R1K, Q16K, K30E] fragment was generated as described above using the following four oligos:

[00652] 5'- CGG AGG AGG AGG AAG CAA GAG CTG CAT CGA CAC CA -3'// (SEQ ID NO:480) and SEQ ID NO:471;

[00653] 5'- TGG TGT CGA TGC AGC TCT TGC TTC CTC CTC CTC CG -3'// (SEQ ID NO:481) and SEQ ID NO:473.

The final pTT5-aKLH 120.6-VK1SP-IgG2-HC-L10-ShK[1-35 R1H, Q16K, K30E] construct encoded an IgG2-HC-L10-ShK[1-35, R1K, Q16K, K30E] fusion polypeptide (SEQ ID NO:467).

[00654] Method for Isolating Monovalent Ab HC- and Monovalent, Bivalent, and Trivalent Ab LC-Toxin Peptide Analog Fusions. Initial purification of the conditioned media was done by affinity fast protein liquid chromatography (FPLC) capture of the Fc region using Protein A Sepharose (GE Healthcare) followed by a column wash with Dulbecco's PBS without divalent cations (Invitrogen) and step elution with 100 mM acetic acid, pH 3.5 at a flow rate of 2.5 cm/min. Protein containing fractions were pooled, and the pH was adjusted to 5.0 using 10 N NaOH and further diluted with 5 volumes of water. The material was filtered through a 0.45 μm cellulose acetate filter (Corning) and further purified by cation exchange FPLC (SP Sepharose High Performance; GE Healthcare). Samples were loaded onto a column equilibrated with 100% buffer A (50 mM acetic acid, pH 5.0) and eluted with a gradient of 0 to 80% buffer B (50mM acetic acid, 1 M NaCl, pH 5.0) over 30 column volumes at a flowrate of 1.5 cm/min. Peaks containing target species were pooled and formulated into 10 mM sodium acetate, 9% sucrose, pH 5.0. Exemplary purifications of monovalent, bivalent and trivalent immunoglobulin-toxin peptide analog fusion proteins are shown in Figure 42-44A-B, 45-47A-B, 48-50A-B, and 51-53. The non-reducing SDS-PAGE analysis (Figures 42, 46, 48 and 51) demonstrate that the fully assembled antibody can be formed, and the reducing SDS-PAGE analysis demonstrates that the desired components are present. The size exclusion chromatograms (Figures 43, 46, 49 and 52) show that the majority of the purified product is in the desired

non-aggregated state. Finally, the mass spectral analysis (Figures 44A-B, 47A-B, 50A-B and 53) demonstrates that the desired fusion products are present. Taken together these examples demonstrate that the aKLH 120.6 antibody can accept fusions in a wide variety of configurations including species containing an even- or odd-numbered valence of at least one to eight pharmacologivcally active polypeptide moieties.

[00655] VH21SP-N-terminus ShK[1-35] Wild Type-IgG1-Fc mammalian expression. A DNA sequence coding for a monomer of the Kv1.3 inhibitor peptide ShK[1-35] fused in-frame to the N-terminal Fc region of human IgG1 was constructed as described below.

[00656] For construction of VH21 SP-ShK(1-35)-L10-IgG1 Fc expression vector, a PCR strategy was employed to generate the VH21 signal peptide ShK(1-35) gene linked to a four glycine and one serine amino acid flanked by HindIII and BamHI restriction sites and a four glycine and one serine amino acid linked to IgG1 Fc fragment flanked by BamHI and NotI restriction sites was generated in a PCR reaction using the Fc-L10-OSK1in pcDNA3.1(+)CMVi as a template (described in Example 41 and Figure 42A-B of Sullivan et al., WO 2008/088422A2, incorporated by reference).

[00657] To generate VH21 SP-ShK(1-35)-G_4S, two oligos with the sequence as depicted below were used in a PCR reaction with PfuTurbo HotStart DNA polymerase (Stratagene) at 95°C-30sec, 55°C-30sec, 75°C-45sec for 35 cycles; HindIII (aagctt) and BamHI (ggatcc) restriction sites are underlined:

Forward primer:

TGCAGAAGCTTCTAGACCACCATGGAATGGAGCTGGGTCTTTCTCTTCT
TCCTGTCAGTAACGACTGGTGTCCACTCCCGCAGCTGCATCGACACCA
TCCCCAAGAGCCGCTGCACCGCCTTCCAGT// (SEQ ID NO:361); and

Reverse primer:

CTCCGGATCCTCCTCCTCCGCAGGTGCCGCAGGTCTTGCGGCAGAAGC
TCAGGCGGTACTTCATGCTGTGCTTGCACTGGAAGGCGGTGCAGCGGC
TCTTGGGGATGGTGTCGAT// (SEQ ID NO:362).

[00658] The resulting PCR products were resolved as the 202bp bands on a two percent agarose gel. The 202bp PCR product was purified using PCR Purification Kit (Qiagen), then digested with HindIII and BamHI (Roche) restriction enzymes, and agarose gel was purified by Gel Extraction Kit (Qiagen).

[00659] To generate G_4S-IgG1 Fc, two oligos with the sequence as depicted below were used in a PCR reaction with PfuTurbo HotStart DNA polymerase (Stratagene) at 95°C-30sec, 55°C-30sec, 75°C-1min for 30 cycles; BamHI (ggatcc) and NotI (gcggccgc) restriction sites are underlined:

Forward primer:

GTAGGATCCGGAGGAGGAGGAAGCGACAAAACTCACAC// (SEQ ID NO:363); and

Reverse primer:

CGAGCGGCCGCTTACTATTTACCCGGAGACAGGGA// (SEQ ID NO:364).

[00660] The resulting PCR products were resolved as the 721-bp bands on a one percent agarose gel. The 721-bp PCR product was purified using PCR Purification Kit (Qiagen), then digested with BamHI and NotI (Roche) restriction enzymes, and agarose gel was purified by Gel Extraction Kit (Qiagen).

[00661] The pcDNA3.1(+)CMVi-Fc-L10-OSK1 vector was digested with BamHI and NotI restriction enzymes and the large fragment was purified by Gel Extraction Kit. The gel purified 4GS-IgG1 Fc fragment was ligated to the purified large fragment and transformed into One Shot® Top10 (Invitrogen) to create a pCMVi-Fc-L10-IgG1 Fc vector. Subsequently, pCMVi-Fc-L10-IgG1 Fc vector was digested with HindIII and BamHI restriction enzymes and the large fragment was purified by Gel Extraction Kit. The gel purified VH21 SP-ShK(1-35)-4GS fragment was ligated to the purified large fragment and transformed into One Shot® Top10 (Invitrogen) resulting in a pCMVi-VH21 SP-ShK(1-35)-L10-IgG1 Fc construct. DNAs from transformed bacterial colonies were isolated and digested with BamHI and NotI restriction enzymes and resolved on a one percent agarose gel. DNAs resulting in an expected pattern were submitted for sequencing. Although, analysis of several sequences of clones yielded a 100% percent match with the above sequences, only one clone from each gene was

selected for large scaled plasmid purification. The DNA from VH21 SP-ShK(1-35)-L10-IgG1 Fc in pCMVi vector was resequenced to confirm the Fc and linker regions and the sequence was 100% identical to the above sequence. Fragment VH21 SP-ShK(1-35)-L10-IgG1 Fc contained the coding sequence

ATGGAATGGAGCTGGGTCTTTCTCTTCTTCCTGTCAGTAACGACTGGTG
TCCACTCCCGCAGCTGCATCGACACCATCCCCAAGAGCCGCTGCACCG
CCTTCCAGTGCAAGCACAGCATGAAGTACCGCCTGAGCTTCTGCCGCA
AGACCTGCGGCACCTGCGGAGGAGGAGGATCCGGAGGAGGAGGAAGC
GACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGG
GGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATG
ATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCAC
GAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGT
GCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGT
ACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATG
GCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCA
TCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAG
GTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTC
AGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTG
GAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCC
TCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACC
GTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTG
ATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTG
TCTCCGGGTAAATAGTAA// (SEQ ID NO:365),

encoding amino acid sequence

MEWSWVFLFFLSVTTGVHSRSCIDTIPKSRCTAFQCKHSMKYRLSFCRKT
CGTCGGGGSGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP
EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK// (SEQ ID NO:366).

[00662] Mammalian expression of N-terminus ShK[1-35, Q16K]-aKLH HC; and N-terminus ShK[1-35Q16K]-aKLH LC. Using a construct encoding N-terminus ShK[1-35]Wild Type-L10-IgG1-Fc, site directed mutagenesis was performed using the following oligos to produce a Q16K mutation in the ShK region:

5'-GCT GCA CCG CCT TCA AGT GCA AGC ACA GC-3'// (SEQ ID NO:325); and

5'- GCT GTG CTT GCA CTT GAA GGC GGT GCA GC -3' (SEQ ID NO:326). The Stratagene QuikChange Multi Site Directed Mutagenesis Kit was used according to the manufacturer's instructions. The final construct for pCMVi-N-terminus-ShK[1-35Q16K]-L10-IgG1-Fc encoded the following Signal peptide-ShK[1-35, Q16K]-L10-IgG1-Fc fusion polypeptide:

MEWSWVFLFFLSVTTGVHSRSCIDTIPKSRCTAFKCKHSMKYRLSFCRKT
CGTCGGGGSGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP
EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK// (SEQ ID NO:402).

[00663] To generate the N-terminus ShK[1-35, Q16K]-aKLH HC construct, a PCR product containing the Signal peptide-ShK[1-35Q16K]-L10 linker was produced using the following oligos:

5'-CAT TCT AGA CCA CCA TGG AAT GG-3' (SEQ ID NO:367);

5'- CAG CTG CAC CTG GCT TCC TCC TCC TCC GG -3' (SEQ ID NO:368); and template pCMVi-N-terminus-ShK[1-35Q16K]-L10-IgG1-Fc, resulted in a fragment containing the coding sequence

ATGGAATGGAGCTGGGTCTTTCTCTTCTTCCTGTCAGTAACGACTGGTG
TCCACTCCCGCAGCTGCATCGACACCATCCCCAAGAGCCGCTGCACCG
CCTTCAAGTGCAAGCACAGCATGAAGTACCGCCTGAGCTTCTGCCGCA
AGACCTGCGGCACCTGCGGAGGAGGAGGATCCGGAGGAGGAGGAAGC
// (SEQ ID NO:369),

encoding the amino acid sequence
MEWSWVFLFFLSVTTGVHSRSCIDTIPKSRCTAFKCKHSMKYRLSFCRKT
CGTCGGGGSGGGGS// (SEQ ID NO:370).

[00664] To generate the aKLH-HC fragment, a PCR product was created using oligos:

5'-GGA GGA GGA AGC CAG GTG CAG CTG GTG CAG-3' (SEQ ID NO:371);

5'- CAT GCG GCC GCT CAT TTA CCC -3' (SEQ ID NO:372);

and template pTT5-aKLH 120.6-HC, resulting in a DNA fragment containing the coding sequence
CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGC
CTCAGTGAAGGTCTCCTGCAAGGCTTCTGGATACACCTTCACCGGCTA
CCACATGCACTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGAT
GGGATGGATCAACCCTAACAGTGGTGGCACAAACTATGCACAGAAGTT
TCAGGGCAGGGTCACCATGACCAGGGACACGTCCATCAGCACAGCCTA
CATGGAGCTGAGCAGGCTGAGATCTGACGACACGGCCGTGTATTACTG
TGCGAGAGATCGTGGGAGCTACTACTGGTTCGACCCCTGGGGCCAGGG
AACCCTGGTCACCGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTC
CCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCGGCCCTG
GGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGG
AACTCAGGCGCTCTGACCAGCGGCGTGCACACCTTCCCAGCTGTCCTA
CAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCC
AGCAACTTCGGCACCCAGACCTACACCTGCAACGTAGATCACAAGCCC
AGCAACACCAAGGTGGACAAGACAGTTGAGCGCAAATGTTGTGTCGA
GTGCCCACCGTGCCCAGCACCACCTGTGGCAGGACCGTCAGTCTTCCT
CTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGA
GGTCACGTGCGTGGTGGTGGACGTGAGCCACGAAGACCCCGAGGTCC
AGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACA
AAGCCACGGGAGGAGCAGTTCAACAGCACGTTCCGTGTGGTCAGCGTC
CTCACCGTTGTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGC
AAGGTCTCCAACAAAGGCCTCCCAGCCCCCATCGAGAAAACCATCTCC

AAAACCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCC
ATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGT
CAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATG
GGCAGCCGGAGAACAACTACAAGACCACACCTCCCATGCTGGACTCCG
ACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGT
GGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGC
ACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA//
(SEQ ID NO:373),
encoding amino acid sequence
QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYHMHWVRQAPGQGLEW
MGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYC
ARDRGSYYWFDPWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGC
LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGT
QTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKD
TLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNS
TFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQV
YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPML
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK/
/ (SEQ ID NO:374).

[00665] The two PCR products were run out on a gel and the appropriate sized band was punched for an agarose plug. The agarose plugs were placed in a single new PCR reaction, and the fragments were sewn together using outer most primers (SEQ ID NO:367) and (SEQ ID NO:372). The PCR fragment was cut using XbaI and NotI and cleaned with Qiagen PCR Cleanup Kit. At the same time, pTT5 vector was also cut by XbaI and NotI and gel purified. The purified insert was ligated to the large vector fragment and transformed into OneShot Top10 bacteria. DNAs from transformed bacterial colonies were isolated and subjected to XbaI and NotI restriction enzyme digestions and resolved on a one percent agarose gel. DNAs resulting in an expected pattern were submitted for sequencing. Although, analysis of several sequences of clones yielded a 100% percent match with the above sequence, only one clone was selected for large

scaled plasmid purification. The final construct pTT5-N-terminus ShK[1-35Q16K]-L10-aKLH120.6-HC encoded a ShK[1-35, Q16K]-L10-aKLH120.6-HC fusion polypeptide:
MEWSWVFLFFLSVTTGVHSRSCIDTIPKSRCTAFKCKHSMKYRLSFCRKT
CGTCGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYTFTGYHM
HWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYME
LSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSASTKGPSVFPLA
PCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPP
VAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEV
HNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIE
KTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH
NHYTQKSLSLSPGK// (SEQ ID NO:403).

[00666] Lastly, the N-terminus-ShK[1-35Q16K]-L10-aKLH120.6 Light Chain (LC) was generated in the same manner as above. A PCR product containing the signal peptide-ShK[1-35, Q16K]-L10 was created using oligos:
5'-CAT TCT AGA CCA CCA TGG AAT GG-3' (SEQ ID NO:367); and
5'- CAT CTG GAT GTC GCT TCC TCC TCC TCC GG -3' (SEQ ID NO:375);
and template pCMVi-N-terminus-ShK[1-35Q16K]-L10-IgG1-Fc, resulting in a DNA fragment containing the coding sequence
ATGGAATGGAGCTGGGTCTTTCTCTTCTTCCTGTCAGTAACGACTGGTG
TCCACTCCCGCAGCTGCATCGACACCATCCCCAAGAGCCGCTGCACCG
CCTTCAAGTGCAAGCACAGCATGAAGTACCGCCTGAGCTTCTGCCGCA
AGACCTGCGGCACCTGCGGAGGAGGAGGATCCGGAGGAGGAGGAAGC
// (SEQ ID NO:369),
encoding the amino acid sequence for a signal peptide (VH21 SP)-ShK(1-35, Q16K)-L10 linker:
MEWSWVFLFFLSVTTGVHSRSCIDTIPKSRCTAFKCKHSMKYRLSFCRKT
CGTCGGGGSGGGGS// (SEQ ID NO:370).
Using template and oligos:

5'-GGA GGA GGA AGC GAC ATC CAG ATG ACC CAG TC-3' (SEQ ID NO:378); and

5'- CAT CTC GAG CGG CCG CTC AAC -3' (SEQ ID NO:379).

The resulting cloned PCR fragment contained the coding sequence ATGGAATGGAGCTGGGTCTTTCTCTTCTTCCTGTCAGTAACGACTGGTG TCCACTCCCGCAGCTGCATCGACACCATCCCCAAGAGCCGCTGCACCG CCTTCAAGTGCAAGCACAGCATGAAGTACCGCCTGAGCTTCTGCCGCA AGACCTGCGGCACCTGCGGAGGAGGAGGATCCGGAGGAGGAGGAAGC GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAG ACAGAGTCACCATCACTTGCCGGGCAAGTCAGGGCATTAGAAATGATT TAGGCTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAACGCCTGATCT ATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGCGGCA GTGGATCTGGGACAGAATTCACTCTCACAATCAGCAGCCTGCAGCCTG AAGATTTTGCAACTTATTACTGTCTACAGCATAATAGTTACCCGCTCAC TTTCGGCGGAGGGACCAAGGTGGAGATCAAACGAACTGTGGCTGCAC CATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAC TGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAA GTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAG AGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAG CACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACG CCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCT TCAACAGGGGAGAGTGTTGA// (SEQ ID NO:380) was generated, encoding the amino acid sequence for N-terminus-ShK[1-35Q16K]-L10-aKLH120.6 Light Chain (LC) with an N-terminal signal peptide: MEWSWVFLFFLSVTTGVHSRSCIDTIPKSRCTAFKCKHSMKYRLSFCRKT CGTCGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWY QQKPGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYC LQHNSYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC// (SEQ ID NO:381).

[00667] Both PCR fragments (DNA fragment containing the coding sequence SEQ ID NO:369 and aKLH 120.6 Light Chain LC fragment containing the coding sequence SEQ ID NO:380) were run out on a gel, and the appropriate sized band was punched for an agarose plug. The agarose plugs were placed in a single new PCR reaction, and the fragments were sewn together using outer most primers (SEQ ID NO:367) and (SEQ ID NO:379). The resulting PCR fragment was cut using XbaI and NotI and cleaned with Qiagen PCR Cleanup Kit.

[00668] At the same time, pTT14 vector (an Amgen vector containing a CMV promoter, Poly A tail and a Puromycin resistance gene) was also cut by XbaI and NotI and gel purified. The purified insert was ligated to the large vector fragment and transformed into OneShot Top10 bacteria. DNAs from transformed bacterial colonies were isolated and subjected to XbaI and NotI restriction enzyme digestions and resolved on a one percent agarose gel. DNAs resulting in an expected pattern were submitted for sequencing. The final construct pTT14-N-terminus ShK[1-35Q16K]-L10-aKLH120.6-LC encoding a Signal Peptide-ShK[1-35, Q16K]-L10- aKLH120.6-LC fusion polypeptide sequence (i.e., SEQ ID NO:381).

[00669] Mammalian expression of aDNP 3A4 (W101F) IgG2-Shk[1-35].

[00670] Making of Plasmid pTT5 – aDNP 3A4 (W101F) IgG2 -Shk[1-35Q16K]: DNA sequences coding for the heavy chain of human anti-2,4-dinitrophenyl (DNP) antibody fused in frame to a monomer of the Kv1.3 inhibitor toxin peptide analog ShK[1-35, Q16K] (SEQ ID NO:13) were constructed using standard cloning technology. Plasid pTT5 – aDNP 3A4 (W101F) IgG2 -Shk[1-35Q16K] was generated by 3 way ligation of the pTT5 vector with a portion of an anti-DNP 3A4 (W101F) IgG2 Heavy Chain (pDC324:aDNP 3A4 HC (W101F) having an amino acid sequence:

MDMRVPAQLLGLLLLWLRGARCQVQLVESGGGVVQPGRSLRLSCAASG
FTFSSYGMHWVRQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDN
SKNTLYLQMNSLRAEDTAVYYCARYNFNYGMDVWGQGTTVTVSSASTK
GPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVE

CPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNW
YVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSN
KGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDI
AVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPG// (SEQ ID NO:405);

and a portion from IgG2Fc-Shk[1-35, Q16K]. The pTT5 vector was cut with SalI/NotI releasing the multiple cloning site. The vector was then treated with Calf Intestine Phosphatase (CIP) to reduce background. The first insert came from pDC324:aDNP 3A4 HC (W101F) by cutting with SalI/StuI, resulting in the a DNA fragment containing the coding sequence

ATGGACATGAGGGTGCCCGCTCAGCTCCTGGGGCTCCTGCTGCTGTGG
CTGAGAGGTGCGCGCTGTCAGGTGCAGCTGGTGGAGTCTGGGGGAGG
CGTGGTCCAGCCTGGGAGGTCCCTGAGACTCTCCTGTGCAGCGTCTGG
ATTCACCTTCAGTAGCTATGGCATGCACTGGGTCCGCCAGGCTCCAGG
CAAGGGGCTGGAGTGGGTGGCAGTTATATGGTATGATGGAAGTAATA
AATACTATGCAGACTCCGTGAAGGGCCGATTCACTATCTCCAGAGACA
ATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGG
ACACGGCTGTGTATTACTGTGCGAGGTATAACTTCAACTACGGTATGG
ACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCTAGTGCCTCCACCA
AGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCG
AGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAAC
CGGTGACGGTGTCGTGGAACTCAGGCGCTCTGACCAGCGGCGTGCACA
CCTTCCCAGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGT
GGTGACCGTGCCCTCCAGCAACTTCGGCACCCAGACCTACACCTGCAA
CGTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGACAGTTGAGC
GCAAATGTTGTGTCGAGTGCCCACCGTGCCCAGCACCACCTGTGGCAG
GACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGAT
CTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGACGTGAGCCACGA
AGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCA
TAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGCACGTTCC
GTGTGGTCAGCGTCCTCACCGTTGTGCACCAGGACTGGCTGAACGGCA

AGGAGTACAAGTGCAAGGTCTCCAACAAAGGC// (SEQ ID NO:383),
encoding amino acid sequence
MDMRVPAQLLGLLLLWLRGARCQVQLVESGGGVVQPGRSLRLSCAASG
FTFSSYGMHWVRQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDN
SKNTLYLQMNSLRAEDTAVYYCARYNFNYGMDVWGQGTTVTVSSASTK
GPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVE
CPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNW
YVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSN
KG// (SEQ ID NO:384).
The second insert was digested out using StuI/NotI and contained the coding sequence
CTCCCAGCCCCCATCGAGAAAACCATCTCCAAAACCAAAGGGCAGCCC
CGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACC
AAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGC
GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTA
CAAGACCACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTCCTCTAC
AGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTT
CTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAA
GAGCCTCTCCCTGTCTCCGGGTAAAGGAGGAGGAGGATCCGGAGGAG
GAGGAAGCCGCAGCTGCATCGACACCATCCCCAAGAGCCGCTGCACC
GCCTTCAAGTGCAAGCACAGCATGAAGTACCGCCTGAGCTTCTGCCGC
AAGACCTGCGGCACCTGCTAATGA// (SEQ ID NO:395),
encoding the following truncated IgG2 Fc-L10-ShK(1-35, Q16K) amino acid sequence
LPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM
HEALHNHYTQKSLSLSPGKGGGGSGGGGSRSCIDTIPKSRCTAFKCKHSM
KYRLSFCRKTCGTC// (SEQ ID NO:396).

[00671] The vector and insert fragments were gel purified and cleaned up with Qiagen Gel Purification Kit. The purified inserts were ligated to the large vector fragment and transformed into OneShot Top10 bacteria. DNAs from transformed bacterial colonies were isolated and subjected to SalI/NotI restriction enzyme digestion and resolved on a one percent agarose gel. DNAs resulting in an expected pattern were submitted for sequencing. A clone yielding a 100% percent match with the above sequence was selected for large scale plasmid purification. The final pTT5 – aDNP 3A4 (W101F) IgG2 –Shk[1-35, Q16K] construct encoded a aDNP 3A4 (W101F) IgG2-L10-Shk[1-35, Q16K] having the following amino acid sequence:

MDMRVPAQLLGLLLLWLRGARCQVQLVESGGGVVQPGRSLRLSCAASG
FTFSSYGMHWVRQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDN
SKNTLYLQMNSLRAEDTAVYYCARYNFNYGMDVWGQGTTVTVSSASTK
GPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVE
CPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNW
YVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSN
KGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDI
AVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPGGGGGSGGGGSRSCIDTIPKSRCTAFKCKHS
MKYRLSFCRKTCGTC// (SEQ ID NO:406).

[00672] Mammalian expression of anti-DNP 3A4 Antibody Light Chain. The XenoMouse® hybridoma expressing aDNP monoclonal antibody 3A4 was used as a source to isolate total RNA. One step RT-PCR with multiplex gene-specific primers was done to obtain a variable region product. This product was reamplified with a forward primer to add a 5' BssHII restriction site 5' - TTT TTT TTG CGC GCT GTG ACA TCC AGA TGA CCC AGT C – 3' (SEQ ID NO:385) and a reverse primer to add a 3' BsiWI restriction site 5' - AAA AAA CGT ACG TTT GAT ATC CAC TTT GGT CC – 3' (SEQ ID NO:386). The resulting PCR product was cleaned by Qiagen PCR clean-up, digested with BssHII and BsiWI restriction enzymes, cleaned by Qiagen nucleotide removal, and ligated into a

mammalian expression vector pTT5 containing a 5' VK1/O12 signal peptide and a 3' human kappa constant region. The amino acid sequence of the resulting anti-DNP 3A4 Antibody Light Chain is the following:
MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSVSASVGDRVTITCRASQ
GISRRLAWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQ
PEDFATYYCQQANSFPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTAS
VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC// (SEQ ID NO:407).

[00673] Method for Isolating Monovalent Fc-Toxin Peptide Analog and Ab HC- or Ab LC-Toxin Peptide Analog Fusions. Initial purification of the conditioned media was done by affinity fast protein liquid chromatography (FPLC) capture of the Fc region using Protein A Sepharose (GE Healthcare) followed by a column wash with Dulbecco's PBS without divalent cations (Invitrogen) and step elution with 100 mM acetic acid, pH 3.5 at a flow rate of 2.5 cm/min. Protein containing fractions were pooled, and the pH was adjusted to 5.0 using 10 N NaOH and further diluted with 5 volumes of water. The material was filtered through a 0.45 μm cellulose acetate filter (Corning) and further purified by cation exchange FPLC (SP Sepharose High Performance; GE Healthcare). Samples were loaded onto a column equilibrated with 100% buffer A (50 mM acetic acid, pH 5.0) and eluted with a gradient of 0 to 80% buffer B (50mM acetic acid, 1 M NaCl, pH 5.0) over 30 column volumes at a flowrate of 1.5 cm/min. Peaks containing monovalent species were pooled and formulated into 10 mM sodium acetate, 9% sucrose, pH 5.0. Exemplary purifications of monovalent Fc-toxin peptide analog fusion proteins and immunoglobulin-toxin peptide analog fusion proteins are shown in Figure 13A-C, Figure 15A-C, Figure 16A-C, and Figure 18A-C.

[00674] Method for Isolating Bivalent Fc-ShK and Ab HC-or LC-toxin peptide analog fusions. Bivalent molecules were purified similar to the monovalent molecules with the addition of a final polishing step to remove higher molecular weight protein aggregates. After cation exchange FPLC, peaks containing bivalent species were polished by gel filtration FPLC (Superdex 200;

GE Healthcare). Samples were loaded onto a column (2.6 x 56.5 cm) equilibrated with 10mM sodium acetate, 0.5M NaCl, 9% Sucrose, pH 5.0 and eluted with an isocratic gradient of buffer at a flowrate of 0.4 cm/min. Peaks containing bivalent species were pooled and formulated into 10 mM sodium acetate, 9% sucrose, pH 5.0. Exemplary purifications of bivalent Fc-toxin peptide analog fusion proteins and immunoglobulin-toxin peptide analog fusion proteins are shown in Figure 14A-C and Figure 17A-C.

Example 12

Monovalent Fc/Fc-L10-ShK[2-35] Heterodimers and Monovalent or Bivalent Fc/Fc-ShK(1-35 Q16K)(IgG2) Heterodimers and Other Immunoglobulin Fusion Proteins

[00675] Monovalent or bivalent Fc-L10-ShK[2-35], monovalent or bivalent Fc-L10-ShK[1-35], monovalent or bivalent Fc-L10-ShK(1-35, Q16K), monovalent or bivalent anti-KLH HC-ShK(1-35, Q16K) Ab, monovalent or bivalent anti-KLH AbLoop-[Lys16]ShK fusion Ab proteins, monovalent Fc-ShK(1-35 Q16K)/KLH Ab heterotrimer, and other exemplary embodiments listed in Table 4H, were expressed, isolated and purified by methods described in Example 11.

[00676] Generally, Figure 12A and Figure 12B show a schematic representation of monovalent and bivalent Fc-toxin peptide (or toxin peptide analog) fusion proteins (or "peptibodies"), respectively. The bivalent Fc-ShK molecule is a homodimer containing two Fc-ShK chains. The monovalent Fc-ShK toxin peptide (or toxin peptide analog) molecule is a heterodimer containing one Fc chain and one Fc-ShK (or analog) chain. Since the monovalent Fc-ShK molecule contains just a single ShK peptide per dimer, it is considered monovalent. Constructs or chains referred to as Fc-(toxin peptide analog), contain an N-terminal Fc region and an optional flexible linker sequence (e.g., L10 peptidyl linker GGGGSGGGGS; SEQ ID NO:292) covalently attached to the toxin peptide or toxin peptide analog, such that the orientation from N- to C-terminus would be: Fc-linker-toxin peptide or toxin peptide analog.

[00677] In Examples 1 and 2 of Sullivan et al., WO 2008/088422A2, we described earlier the activity of bivalent Fc-ShK peptibodies, Fc-L10-ShK(1-35) and Fc-L10-ShK(2-35) expressed from mammalian cells. In Example 1 of WO 2008/088422A2, we also described isolation of a monovalent Fc-L10-ShK(1-35) molecule, formed as a small by-product during expression. The bivalent Fc-L10-ShK(1-35) and Fc-L10-ShK(2-35) conjugates provided potent blockade of Kv1.3 and T cell cytokine secretion in human whole blood (see, Table 4H). By whole cell patch clamp electrophysiology, the bivalent Fc-L10-ShK(1-35) molecule had about 8-fold greater Kv1.3 activity compared to the bivalent Fc-L10-ShK(2-35) molecule that is devoid of Arg1 of ShK. Like N-terminal PEG conjugates of native ShK (see, Example 5), both bivalent Fc-ShK conjugates showed little selectivity for Kv1.3 versus Kv1.1. Thus, N-terminal conjugation of native ShK alone (with either PEG or Fc-linker) does not significantly improve its Kv1.3 versus Kv1.1 selectivity. Pharmacokinetic (PK) studies in rats were performed on bivalent Fc-L10-ShK(1-35) and Fc-L10-ShK(2-35) peptibodies to examine their stability and half-life in vivo. As a control, PK was also performed on CHO-derived recombinant human Fc (IgG1). All molecules were delivered as a single, intravenous bolus dose. Using an ELISA specific for the human Fc region (Example 8, "protocol 3"), the bivalent Fc-L10-ShK(2-35) peptibody showed a long terminal half-life and slow elimination rate in rats similar to CHO-Fc (Figure 19A). Despite showing a slow elimination rate, the bivalent Fc-L10-ShK(2-35) peptibody exhibited a large distribution phase that was significantly greater than the CHO-Fc molecule. Similar results were obtained from IV, single-dose rat PK studies on bivalent Fc-L10-ShK(1-35) and bivalent Fc-L10-OSK1[K7S], two peptibodies that have been described earlier (Example 1 and Example 41 of WO 2008/088422A2). To more fully understand what might be causing the extensive distribution of the bivalent peptibodies in vivo, we developed a solid-surface affinity capture/SDS-PAGE western blot procedure which allowed us to isolate the peptibody away from serum proteins for efficient SDS-PAGE and western blot analysis of their molecular weight and integrity. Serum samples from the intravenous, single-dose rat PK study were added to microtiter plates coated with

an anti-human Fc antibody to enable affinity capture. Plates were then washed and captured samples were released by addition of SDS/Laemalli sample buffer and run on a polyacrylamide gel. After western blotting to a membrane, samples were visualized by standard immunobloting techniques using an anti-human Fc-specific antibody, secondary-HRP conjugate and luminescent substrate. Figure 19B indicates that during the extensive distribution phase, at 0.25 hours after IV administration of Fc-L10-ShK(2-35), the peptibody is intact. During the slow elimination phase, from 1 to 48 hours after IV injection, two bands are observed, the top or higher molecular weight band being consistent with full-length Fc-L10-ShK[2-35] and the small band being consistent with Fc alone. Since the peptibody is intact during the large and extensive distribution phase, this implies the bivalent peptibody was not proteolyzed but was sequestered within the animal. In support of this, we do not see large serum quantities of Fc alone, which would be expected to occur if the ShK peptide portion of Fc-ShK were quickly cleaved off. Therefore, the SDS-PAGE/western blot data (Figure 19B) imply that during the rapid distribution, the peptibody was intact and not proteolyzed, but during the slow elimination phase it was a monovalent heterodimer containing one Fc-ShK[2-35] chain and one Fc chain. Similar findings were observed from huFc affinity capture, SDS-PAGE/western blot analysis of serum samples from rat PK studies (intravenous) on bivalent Fc-L10-ShK[1-35] and on bivalent Fc-L10-OSK1[K7S] (Figure 19C). Although the exact reason for the rapid and extensive distribution phase of bivalent Kv1.3 peptibodies in rats is unknown, the avidity of bivalent peptibodies containing two toxin units would be much greater than that of a monovalent peptibody. Whether this is Kv1.3 target specific or non-specific is unknown. It should be remembered that toxin peptides, such as ShK and OSK1 are highly basic and could be reacting in a non-specific manner. Irrespective of the cause, because monovalent peptibodies were present in the slow terminal phase, we investigated whether direct IV injection of a monovalent peptibody would show less distribution in rats. To address this, we first tested in rat IV PK studies a purified monovalent Fc-L10-ShK(1-35) molecule isolated as a by-product from conditioned media expressing the bivalent molecule (Example 1,

WO 2008/088422A2). Figure 19D demonstrates by Fc affinity capture/SDS-PAGE/western blot that the monovalent Fc-L10-ShK(1-35) peptibody showed a distribution phase that was much less extensive and had a slow elimination rate. Therefore, despite both bivalent and monovalent peptibodies exhibiting a slow elimination rate, the monovalent heterodimeric peptibody showed significantly less distribution in rats compared to the bivalent homodimeric forms.

[00678] Whereas, the original monovalent Fc-L10-ShK(1-35) molecule was isolated as a small by-product during mammalian expression of the bivalent molecule, Example 11 herein also describes cloning and mammalian expresion of the monovalent Fc-L10-ShK[2-35] heterodimer. Briefly, to produce recombinant monovalent Fc-L10-ShK[2-35], two recombinant polypeptides are co-expressed in the same cell, those being a human Fc (IgG1) chain and a Fc-L10-ShK[2-35] chain (also with human IgG1 Fc region). Under these conditions it is possible to form three distinct dimers, which include an Fc/Fc homodimer, a Fc-L10-ShK(2-35)/Fc-L10-ShK(2-35) homodimer and a Fc/Fc-L10-ShK(2-35) heterodimer. By optimizing expression conditions, the monovalent Fc/Fc-L10-ShK(2-35) heterodimer (also referred to as just monovalent Fc-L10-ShK(2-35)) was produced efficiently and was readily purified to homogeneity (Example 11). The monovalent Fc-L10-ShK(2-35) molecule had an IC50 of 2.1 nM (Table 4H) in blocking IL-2 secretion from human whole blood. The monovalent Fc-ShK/Fc heterodimer had an extended half-life in vivo and exhibited significantly greater exposure than the bivalent homodimers ShK-Fc/ShK-Fc (Figure 23) and Fc-ShK/Fc-ShK. Since the potency of this construct was about 10-fold less than the PEG-ShK conjugate and conjugates of native ShK had poor Kv1.3/Kv1.1 selectivity, we developed additional monovalent peptibodies and formed conjugates of ShK toxin peptide analogs that were identified to have improved Kv1.3 versus Kv1.1 selectivity. The examples that follow provide additional details of monovalent peptibodies with improved selectivity and in vivo pharmacology. The results from these studies indicated that monovalent ShK toxin peptide analog molecules exhibited greater serum levels and exposure in rats

when compared to the bivalent forms of the same molecule, yet retain the slow elimination rate observed in the original bivalent peptibodies.

[00679] Monovalent Fc/Fc-ShK(1-35 Q16K) heterodimer (IgG2). Example 3 herein describes identification of the ShK[Lys16] toxin peptide analog (SEQ ID NO:13) that shows improved Kv1.3 selectivity over neuronal Kv1.1. To increase the stability of this toxin peptide analog in vivo, we generated a monovalent Fc fusion construct that from N- to C-terminus contained: human Fc(IgG2)-L10 linker-[Lys16]ShK molecule, that was co-expressed with the human Fc(IgG2) chain alone to generate a monovalent heterodimer (see, Example 11). A schematic representation of this monovalent construct is provided in Figure 12A. The monovalent Fc/Fc-L10-ShK(1-35 Q16K) heterodimer [also referred to, interchangeably, as monovalent Fc/Fc-ShK(1-35, Q16K) or monovalent Fc-L10-ShK(1-35, Q16K]; (SEQ ID NOS: 337; 348)] potently blocked T cell inflammation in whole blood, suppressing IL-2 secretion with an IC50 of 0.16 nM (Table 4H). The monovalent Fc-L10-ShK(1-35, Q16K) molecule also potently blocked proliferation of the rat myelin-specific T effector memory cell line, PAS, with an IC50 of 0.244 nM as described in Figure 32 and Example 9. Unexpectedly, studies to examine the Kv1.3 versus Kv1.1 selectivity of the molecule, revealed that the monovalent Fc-L10-ShK(1-35 Q16K) conjugate had significantly better Kv1.3 selectivity than the [Lys16]ShK peptide alone. Whereas the [Lys16]ShK (SEQ ID NO:13) peptide alone showed about 18-fold selectivity for Kv1.3 versus Kv1.1 (Table 4H), the monovalent Fc/Fc-L10-ShK(1-35 Q16K) heterodimer was about 1225-fold more active in blocking Kv1.3 versus Kv1.1, as determined by PatchXpress® electrophysiology. Therefore, the [Lys16]ShK peptide when conjugated shows a unique pharmacology of enhanced selectivity. Since the Nα-20kDa-PEG-[Lys16]ShK conjugate (SEQ ID NO:16) also showed enhanced Kv1.3 selectivity (Example 5, Table 4H) relative to the peptide alone, the combined data suggests that the [Lys16]-ShK (SEQ ID NO:13) peptide when fused at its N-terminus with either PEG or Fc-linker exhibits a distinct pharmacology of improved Kv1.3 versus Kv1.1 selectivity.

[00680] To expland our analysis of the Kv1.3 selectivity of the monovalent Fc-L10-ShK(1-35, Q16K) molecule (SEQ ID NOS: 337; 348), its activity was tested against the five remaining members of the Kv1 family. The monovalent Fc-L10-ShK(1-35, Q16K) molecule blocked Kv1.3 1225 times better than Kv1.1, about 1000 times better than Kv1.2, >3663 times better than Kv1.4, >3663 times better than Kv1.5, about 164 times better than Kv1.6, and >3663 times better than Kv1.7, as determined by PatchXpress® electrophysiology (Table 4H(b)). As whole cell patch clamp Kv1.3 electrophysiology and the whole blood assay of T cell inflammation implies that the true activity of the monovalent Fc-L10-ShK(1-35, Q16K) on Kv1.3 may be even greater than determined by Kv1.3 PatchXpress® analysis by a factor of 8 to 10 times (Table 4H(b)), its fold Kv1.3 selectivity over other Kv1 family members may be even greater.

[00681] To assess the pharmacokinetics and stability of the molecule in vivo, single-dose PK studies were performed in rats. After a single 6 mg/kg subcutaneous dose, the monovalent Fc/Fc-L10-ShK(1-35, Q16K) heterodimer exhibited an extended half-life in vivo (Figure 20). Since the sandwich ELISA used to measure serum levels of the molecule (Example 8, "protocol 2") requires binding of two antibodies, one an antibody specific to human Fc region and the other an antibody recognizing [Lys16]ShK (SEQ ID NO:13), the data here indicate that the conjugate had prolonged half-life and remained intact in vivo as a Fc-L10-ShK(1-35 Q16K) fusion protein (Figure 20, open squares; Table 4I below). The monovalent Fc/Fc-L10-ShK(1-35 Q16K) molecule exhibited an extended half-life of about 56 hours, that was about 112 times longer than the ShK (SEQ ID NO:1) peptide alone that was reported to have a half-life of 20-30 min (C. Beeton et al., PNAS 98:13942 (2001)).

[00682] The efficacy of the monovalent Fc/Fc-L10-ShK(1-35,Q16K) molecule (SEQ ID NOS: 337; 348) in an animal model of multiple sclerosis was determined using the adoptive-transfer (AT)-EAE model, as described in Example 9. Monovalent Fc-L10-ShK(1-35,Q16K) delayed disease onset and caused dose-dependent reduction in disease severity (Figure 35 and Figure 36A-D). The molecule was highly potent in this model and the estimated effective dose of

monovalent Fc-L10-ShK (1-35,Q16K) to cause 50% reduction in disease severity (ED50), based on area under the curve of the EAE score, was a dose of <2.5 nmol/kg or <138 μg/kg. This ED50 compares favorable to that activity of PEG-[Lys16]ShK in this model, since the 2.5 nmol/kg dose of the larger monovalent Fc-L10-ShK(1-35, Q16K) molecule corresponds to a 10 μg/kg dose of the smaller PEG-[Lys16]ShK molecule whose μg/kg dose and dosing solution is based on the molecular weight of the peptide portion alone.

[00683] Bivalent Fc-ShK(1-35 Q16K) homodimer (IgG2). The bivalent Fc-ShK(1-35, Q16K) homodimer contains from N- to C-terminus: human Fc (IgG2) – L10 linker – [Lys16]ShK (SEQ ID NO:348). A schematic representation of this bivalent construct is provided in Figure 12B. The molecule (homodimer of SEQ ID NO:348) was cloned, expressed and purified as described in Example 11. The purified molecule was tested for activity in the human whole blood assay of inflammation and found to have an IC50 of 1.850 nM in blocking IL-2 secretion (Table 4H). The activity of this bivalent form was about 12 times less than the monovalent form (above) which had an IC50 of 0.16 nM in this same assay. The reason why the bivalent form was less active than the monovalent is unknown. It is possible that the bivalent molecule containing two positively charged [Lys16]ShK (SEQ ID NO:13) peptides at its end, is less stable and/or interferes with Kv1.3 channel binding to some extent.

[00684] Monovalent and bivalent aKLH HC-ShK(1-35, Q16K) Ab. The monovalent anti-KLH Heavy Chain (HC) fusion antibody (Ab) construct contained from N- to C-terminus: human anti-KLH Ab Heavy Chain-peptidyl linker-[Lys16]ShK molecule (SEQ ID NO:342), that was co-expressed with the human aKLH Heavy Chain alone (SEQ ID NO:339) and the human aKLH light chain (SEQ ID NO:338) to form a monovalent aKLH Ab-[Lys16]ShK molecule (heterotetramer of SEQ ID NO:338; SEQ ID NO:339; SEQ ID NO:338; and SEQ ID NO:342). A schematic representation of this monovalent construct is provided in Figure 12F. The monovalent aKLH HC-ShK(1-35, Q16K) Ab (heterotetramer of SEQ ID NO:338; SEQ ID NO:339; SEQ ID NO:338; and SEQ ID NO:342) potently blocked T cell inflammation in whole blood, suppressing IL-2 secretion

with an IC50 of 0.274 nM (Table 4H). The monovalent aKLH HC-ShK(1-35, Q16K) Ab molecule also potently blocked proliferation of the rat myelin-specific T effector memory cell line, PAS, with an IC50 of 0.839 nM as described in Figure 32 and Example 9. Unexpectedly, studies to examine the Kv1.3 versus Kv1.1 selectivity of the molecule, revealed that the monovalent aKLH HC-ShK(1-35, Q16K) Ab (heterotetramer of SEQ ID NO:338; SEQ ID NO:339; SEQ ID NO:338; and SEQ ID NO:342) had significantly better Kv1.3 selectivity than the [Lys16]ShK (SEQ ID NO:13) peptide alone. This monovalent Ab-ShK conjugate was about 1458-fold more active in blocking Kv1.3 versus Kv1.1 (Table 4H and Figure 6E-F), as determined by PatchXpress® electrophysiology. To expand our analysis of the Kv1.3 selectivity of the monovalent aKLH HC-ShK(1-35, Q16K) Ab molecule, its activity was tested against the five remaining members of the Kv1 family. The monovalent aKLH HC-ShK(1-35, Q16K) Ab blocked Kv1.3 1458 times better than Kv1.1, 2013 times better than Kv1.2, >2525 times better than Kv1.4, >2525 times better than Kv1.5, ~156 times better than Kv1.6, and >2525 times better than Kv1.7, as determined by PatchXpress® electrophysiology (Table 4H(a)). As whole cell patch clamp Kv1.3 electrophysiology and the whole blood assay of T cell inflammation suggests the true activity of the monovalent aKLH HC-ShK(1-35, Q16K) Ab (heterotetramer of SEQ ID NO:338; SEQ ID NO:339; SEQ ID NO:338; and SEQ ID NO:342) on Kv1.3 may be even greater than determined by Kv1.3 PatchXpress analysis by a factor of 11 to 13 times (Table 4H(a)), its fold Kv1.3 selectivity over other Kv1 family members may be even greater.

[00685] The efficacy of the monovalent aKLH HC-ShK(1-35,Q16K) Ab molecule (heterotetramer of SEQ ID NO:338; SEQ ID NO:339; SEQ ID NO:338; and SEQ ID NO:342) in an animal model of multiple sclerosis was determined using the adoptive-transfer (AT)-EAE model, as described in Example 9. The monovalent aKLH HC-ShK(1-35,Q16K) Ab delayed disease onset and caused dose-dependent reduction in disease severity (Figure 33 and Figure 34A-D). The molecule was highly potent in this model and the estimated effective dose of monovalent aKLH HC-ShK(1-35,Q16K) Ab to cause 50% reduction in disease

severity (ED50), based on area under the curve of the EAE score, was a dose of 2.4 nmol/ kg or 360 μg/kg. The efficacy of the monovalent aKLH HC-ShK(1-35,Q16K) Ab in reducing disease severity compared favorably to that of PEG-[Lys16]ShK, since a 2.5 nmol/kg (375 μg/kg) dose of the larger monovalent aKLH HC-ShK(1-35,Q16K) Ab provided similar disease amelioration to 10 μg/kg (2.5 nmol/kg) of the smaller PEG-[Lys16]ShK (Figure 33) molecule, whose μg/kg dose and dosing solution is based on the molecular weight of the peptide portion alone.

[00686] To assess the pharmacokinetics and stability of the molecule in vivo, single-dose PK studies were performed in rats. After a single 6 mg/kg subcutaneous dose, the monovalent KLH HC-ShK(1-35 Q16K) Ab conjugate exhibited an extended half-life in vivo (Figure 20, closed circles). Since the sandwich ELISA used to measure serum levels of the molecule (Example 8, "protocol 2") requires binding of two antibodies, one an antibody specific to human Ig region and the other an antibody recognizing [Lys16]ShK (SEQ ID NO:13), the data here indicates that conjugate has prolonged half-life and remains intact in vivo as a monovalent aKLH HC-ShK(1-35 Q16K) Ab fusion protein (Figure 20, Figure 21, and Table 4J). The bivalent aKLH HC-ShK(1-35, Q16K) Ab molecule (schematically represented by Figure 12G) given at the same 6 mg/kg dose, showed a similarly slow elimination rate (Figure 21), but provided about 37 times less exposure (as measured by AUC0-t, Table 4J) relative to the monovalent molecule (Figure 21). The potent and selective monovalent anti-KLH-Ab-[Lys16]ShK molecule exhibited very slow clearance in rats (CL/F = 10.9 mL h^{-1} kg^{-1}) (Table 4J), a rate that was about 188 times slower than ShK-L5 (SEQ ID NO:17; CL/F = 2052 mL h^{-1} kg^{-1}).

[00687] Monovalent aKLH HC-ShK(2-35 Q16K) Ab. This monovalent aKLH Heavy Chain (HC) fusion antibody (Ab) construct contained from N- to C-terminus: human anti-KLH Ab Heavy Chain – linker – [desArg1, Lys16]ShK molecule (SEQ ID NO:387), that was co-expressed with the human aKLH Heavy Chain (SEQ ID NO:339) and the human aKLH light chain (SEQ ID NO:338) to form a monovalent aKLH Ab-[desArg1, Lys16]ShK molecule. A schematic

representation of this monovalent construct is provided in Figure 12F. The monovalent aKLH HC-ShK(2-35, Q16K) Ab (heterotetramer of SEQ ID NO:338; SEQ ID NO:339; SEQ ID NO:338; and SEQ ID NO:387) potently blocked T cell inflammation in whole blood, suppressing IL-2 secretion with an IC50 of 0.570 nM (Table 4H) and unexpectedly was about 1576 fold more potent in blocking the T-cell potassium channel Kv1.3 than the neuronal channel Kv1.1.

[00688] Monovalent Fc-ShK(1-35 Q16K)/aKLH Ab Heterotrimer. The monovalent Fc-ShK(1-35, Q16K)/aKLH Ab heterotrimer or hemibody contained from N- to C-terminus: human Fc (IgG2) – L10 linker – [Lys16]ShK molecule (SEQ ID NO:348), that was co-expressed with the human aKLH Heavy Chain (IgG2) (SEQ ID NO:339) and the human aKLH light chain (SEQ ID NO:338). A schematic representation of this monovalent construct is provided in Figure 12E. The monovalent Fc-ShK(1-35, Q16K)/aKLH Ab heterotrimer (SEQ ID NO:338; SEQ ID NO:339; SEQ ID NO:348) potently blocked T cell inflammation in whole blood, suppressing IL-2 secretion with an IC50 of 0.245 nM (Table 4H). Surpisingly, studies examining the Kv1.3 versus Kv1.1 selectivity of the molecule revealed that the monovalent Fc-ShK(1-35, Q16K)/aKLH Ab heterotrimer had significantly better Kv1.3 selectivity than the [Lys16]ShK peptide alone (SEQ ID NO:13). This monovalent heterotrimer was about 1935 fold more active in blocking Kv1.3 versus Kv1.1 (Table 4H).

[00689] Although we haven't examined the pharmacokinetics (PK) of the Kv1.3 selective monovalent Fc-ShK(1-35, Q16K)/aKLH Ab heterotrimer or hemibody, we have examined the PK profile of a similar hemibody, that being the Fc-ShK(2-35)/aKLH Ab heterotrimer. A schematic of the structure of this molecule is provided in Figure 12E, and the molecule from N- to C-terminus contains: human Fc (IgG2) – ShK(2-35), which is coexpressed with the human aKLH heavy chain and light chains. After a single 2 mg/kg subcutaneous dose, the monovalent Fc-ShK(2-35)/aKLH Ab heterotrimer (also referred to as monovalent Fc-ShK/aKLH Ab heterotrimer) exhibited an extended half-life in rats (Figure 23). Since the sandwich ELISA used to measure serum levels of the molecule (Example 8, "protocol 2") requires binding of two antibodies, one an

antibody specific to human Ig region and the other an antibody recognizing ShK(2-35), the data here indicates that conjugate has prolonged half-life and remains intact in vivo (Figure 23, Table 4K). The large, about 103 kDa monovalent Fc-ShK(2-35)aKLH Ab heterotrimer or hemibody showed greater exposure and about 2-fold less clearance than the about 56 kDa monovalent Fc/Fc-ShK heterodimer (Figure 23, Table 4K). The very small, about 4 kDa ShK-L5 peptide was cleared much more quickly, having a clearance value in rats (CL/F = 2052 mL $h^{-1}kg^{-1}$, Example 5) that was about 91 times faster than the large monovalent Fc-ShK(2-35)/aKLH Ab heterotrimer (CL/F = 22.6 mL $h^{-1}kg^{-1}$) molecule.

[00690] Monovalent and bivalent anti-KLH AbLoop-[Lys16]ShK fusion proteins. Recombinant monovalent and bivalent anti-KLH AbLoop-[Lys16]ShK fusion proteins were constructed as described in Example 11 and U.S. Patent No.7,442,778 B2 to produce full antibodies with [Lys16]ShK toxin peptide analog inserted into loop regions of the Fc domain in one (monovalent) or both (bivalent) HC monomers. The monovalent aKLH HC-loop-ShK(1-35, Q16K) Ab contained three chains: a human aKLH Ab heavy chain, a human aKLH Ab light chain and a human aKLH Ab heavy chain where the [Lys16]ShK peptide was inserted into a loop within the Fc region of the heavy chain. The [Lys16]ShK peptide within the Fc loop contained a flexible linker sequence attached to its N- and C-terminus to allow for independent folding and extension from the loop. A schematic representation of this molecule is provided in provided in Figure 12N. Linker sequences of differing amino acid composition and length were examined. The monovalent anti-KLH AbLoop-[Lys16]ShK fusion Ab protein was a selective inhibitor of Kv1.3 activity (over Kv1.1; >121-fold more selective for Kv1.3; Table 4H and Figure 6E-F). The monovalent aKLH-AbLoop-[Lys16]ShK molecule exhibited the slowest clearance in rats of all the novel toxin-conjugates that we have examined (Figure 20 and Figure 22 and Table 4L).

[00691] The bivalent aKLH HC-loop-ShK(1-35, Q16K) Ab contained two chains: a human aKLH Ab light chain and a human aKLH Ab heavy chain where the [Lys16]ShK peptide was inserted into a loop within the Fc region of the heavy

chain. A schematic representation of this molecule is provided in provided in Figure 12M. To compare the pharmacokinetics and stability in vivo of this bivalent molecule to the monovalent form, single 6 mg/kg subcutaneous doses of each molecule were delivered to rats. Despite showing a slow elimination rate, the bivalent aKLH HC-loop-ShK(1-35, Q16K) Ab gave profoundly less exposure in rats than the monovalent form of the same molecule (monovalent aKLH HC-loop-ShK(1-35, Q16) Ab) (see Figure 22). Exposure as measured by AUC0-t, was about 161 times less for the bivalent aKLH HC-loop-ShK(1-35, Q16K) Ab molecule compared to the monovalent aKLH HC-loop-ShK(1-35Q16K) Ab molecule (Table 4L). Therefore, our novel monovalent forms show an unexpected and vastly better pharmacokinetic profile in vivo compared to typical bivalent forms of the same molecule.

[00692] Monovalent ShK(1-35, Q16K)-Fc/Fc heterodimer. The monovalent ShK(1-35, Q16K)-Fc/Fc heterodimer contains two chains, one being a human Fc(IgG2) chain and the other being ShK(1-35, Q16K) peptide fused to Fc that contains from N- to C-terminus: [Lys16]ShK – L10 linker – human Fc (IgG2). This peptide-fusion protein contained from N- to C-terminus: the 35 amino acid [Lys16]ShK peptide, a ten amino acid GGGGSGGGGS (SEQ ID NO:292) L10 linker sequence and the human Fc (IgG2) sequence. Therefore, the linker-Fc region was attached to the C-terminus of [Lys16]ShK following Cys35. This molecule is also referred to as monovalent ShK(1-35, Q16K)-Fc heterodimer. A schematic representation of this monovalent construct is provided in Figure 12C. The molecule was cloned, expressed and purified as described Example 11. The purified molecule was highly potent having an IC50 of 0.11 nM in blocking IL-2 secretion in the human whole blood assay of inflammation (Table 4H). Despite its excellent potency, the monovalent ShK(1-35, Q16K)-Fc/Fc heterodimer showed only a modest ~10 fold selectivity for Kv1.3 versus Kv1.1 (Table 4H). Therefore, it would appear that this linker-Fc fusion partner attached the C-terminus of [Lys16]ShK does not result in a further enhancement of Kv1.3 selectivity. This contrasts with N-terminal fusions to [Lys16]ShK, such as the monovalent Fc/Fc-ShK(1-35, Q16K) heterodimer (Table 4H) which showed

~1225 fold selectivity and had the Fc-linker sequence attached to the N-terminal Arg1 residue of [Lys16]ShK. An important and notable exception, however, is the [Lys16]ShK-Ala peptide (SEQID NO: 235, Example 3) which contains a single C-terminal Ala residue adding following Cys35 of [Lys16]ShK. This molecule exhibited an enhanced 262 fold improved selectivity for Kv1.3 versus Kv1.1 (Table 4H). Therefore, we envision that the specific amino acid residue added after Cys35 at the C-terminus of [Lys16]ShK, can alter the selectivity profile of the fusion protein. For example, the monovalent ShK(1-35, Q16K)-L10-Fc molecule described in this example contains the linker Gly residue added after Cys35 of [Lys16]ShK. If an Ala residue was added instead following Cys35, an enhanced Kv1.3 selectivity might be observed. Indeed, we do see 262 fold improved Kv1.3 selectivity by the [Lys16]ShK-Ala peptide. Thus, we anticipate that specific amino acid residue at the fusion junction would alter the selectivity profile. These residue can be readily incorporated into the linker sequence between the [Lys16]ShK peptide and the human Fc to improve the conjugates Kv1.3 selectivity.

[00693] Monovalent ShK(1-35, Q16K)-HC aKLH Ab. The monovalent ShK(1-35, Q16K)-HC aKLH Ab contains three chains, one being the human aKLH Ab light chain, another being the human aKLH Ab heavy chain and the third being a peptide-aKLH Ab heavy chain fusion that contained from N- to C-terminus: [Lys16]ShK – L10 linker – human aKLH heavy chain. Therefore, this fusion contained the linker-heavy chain region attached to the C-terminus of [Lys16]ShK following Cys35. A schematic representation of the monovalent ShK(1-35, Q16K)-HC aKLH Ab molecule is provided in Figure 12I. The purified molecule was highly potent having an IC50 of 0.214 nM in blocking IL-2 secretion in the human whole blood assay of inflammation (Table 4H). Despite being very large in size and fused to a human Ig heavy chain, the monovalent [Lys16]-aKLH Ab molecule retained high potency in blocking T cell responses.

[00694] Monovalent aDNP HC-ShK(1-35, Q16K) Ab. The monovalent aDNP Heavy Chain (HC) fusion antibody (Ab) construct contained from N- to C-terminus: human anti-DNP Ab Heavy Chain – linker – [Lys16]ShK molecule,

that was co-expressed with the human aDNP Heavy Chain and the human aDNP light chain to form a monovalent aDNP Ab-[Lys16]ShK molecule. A schematic representation of this monovalent construct is provided in Figure 12F. The monovalent aDNP HC-ShK(1-35, Q16K) Ab potently blocked T cell inflammation in whole blood, suppressing IL-2 secretion with an IC50 of 0.278 nM (Table 4H). Studies to examine the Kv1.3 versus Kv1.1 selectivity of the molecule, unexpectedly revealed that the monovalent aDNP HC-ShK(1-35, Q16K) Ab conjugate had significantly better Kv1.3 selectivity than the [Lys16]ShK peptide alone. This monovalent Ab-ShK conjugate was >5806 fold more active in blocking Kv1.3 versus Kv1.1 (Table 4H).

Table 4H Data demonstrating conjugates of [Lys16]ShK have improved Kv1.3 selectivity. Toxin peptides and toxin peptide analogs were PEGylated as described in Example 4. Immunoglobulin-containing compounds were recombinantly expressed and purified as described in Example 11. Electrophysiology was by PatchXpress® (PX), except asterisks indicate data from whole cell patch clamp.

SEQ ID NO or citation	Conjugate Type	Designation	Kv1.3 (PX) IC50 (nM)	Kv1.1 (PX) IC50 (nM)	Kv1.1 / Kv1.3 Selectivity Ratio by PX	WB (IL-2) IC50 (nM)	WB (IFNg) IC50 (nM)	Potency Relative to ShK (WB, IL2)
1	none	ShK(1-35)	0.062	0.087	1.40	0.067	0.078	1.00
317	none	ShK-Dap22	0.012*	0.847*	70.58	3.763	3.112	56.16
17	none	ShK-L5	0.221	0.214	0.97	0.031	0.046	0.46
13	none	[Lys16]ShK	0.207	3.677	17.76	0.110	0.158	1.64
235	none	[Lys16]ShK-Ala	0.06	15.726	262.10	0.138	0.266	2.06
8	PEG	20kDa-PEG-ShK	0.299*	1.628*	5.44	0.380	0.840	5.67
16	PEG	20kDa-PEG-[Lys16]ShK	0.94	997	1060.64	0.092	0.160	1.37
316	PEG	20kDa-PEG-[Lys16]ShK-Ala	0.596	2156	3617.45	0.754	1.187	11.25
277	PEG	30kDa-PEG-[Lys16]ShK	1.204	1072	890.37	0.282	0.491	4.21
315	PEG	20kDa-brPEG-[Lys16]ShK	2.095	1574	751.31	0.198	0.399	2.96
Example 1, WO2008/088422A2	IgG1	Bivalent Fc-L10-ShK[1-35] homodimer	0.015*	0.067*	4.47	0.386	0.320	5.76
Example 2, WO2008/088422A2	IgG1	Bivalent Fc-L10-ShK[2-35] homodimer	0.116*	0.411*	3.54	0.585	2.285	8.73
Example 2, WO2008/088422A2	IgG1	Monovalent Fc/Fc-L10-ShK[2-35] heterodimer	ND	ND	ND	2.149	5.199	32.07
337; 348	IgG2	Monovalent Fc/Fc-ShK(1-35 Q16K) heterodimer	2.73	3344	1224.91	0.160	0.499	2.39
348; 348	IgG2	Bivalent Fc-ShK(1-35 Q16K) homodimer	ND	ND	ND	1.850	3.140	27.61
338; 339; 348	IgG2	Monovalent Fc-ShK(1-35 Q16K)/KLH Ab Heterotrimer	0.98	1896	1934.69	0.245	0.665	3.66

Table 4H continued:

SEQ ID NO or citation	Conjugate Type	Designation	Kv1.3 (PX) IC50 (nM)	Kv1.1 (PX) IC50 (nM)	Kv1.1 / Kv1.3 Selectivity Ratio by PX	WB (IL-2) IC50 (nM)	WB (IFNg) IC50 (nM)	Potency Relative to ShK (WB, IL2)
407; 406; 407; 405	IgG2	Monovalent aDNP HC-ShK(1-35 Q16K) Ab	0.574	>3333	>5806.62	0.278	0.660	4.15
338; 339; 338; 342	IgG2	Monovalent aKLH HC-ShK(1-35 Q16K) Ab	3.96	5774	1458.08	0.274	0.657	4.09
338; 342; 338; 342	IgG2	Bivalent aKLH HC-ShK(1-35 Q16K) Ab	ND	ND	ND	1.392	3.568	20.78
338; 339; 338; 387	IgG2	Monovalent aKLH HC-ShK(2-35 Q16K) Ab	1.66	2617	1576.51	0.570	0.820	8.51
402; 278	IgG1	Monovalent ShK(1-35 Q16K)-Fc/Fc heterodimer	1.45	14.77	10.19	0.110	0.120	1.64
338; 339; 338; 403	IgG2	Monovalent ShK(1-35 Q16K)-HC aKLH Ab	ND	ND	ND	0.214	0.332	3.19
338; 344; 338; 343	IgG1	Monovalent aKLH HC-loop-ShK(1-35 Q16K) Ab	8.264	>1000	>121.01	1.604	5.386	23.94
338; 344; 338; 344	IgG1	Bivalent aKLH HC-loop-ShK(1-35 Q16K) Ab	ND	ND	ND	3.910	55.235	58.36

Table 4H(a). Potency and selectivity of monovalent aKLH HC-ShK(1-35,Q16K) (heterotetramer of SEQ ID NO:338; SEQ ID NO:339; SEQ ID NO:338; and SEQ ID NO:342). * = Whole cell patch clamp (WCPC).

Assay	**IC50 nM (n)**
Human whole blood (TG induced IL-2)	0.292 (2)
Kv1.1	5774 (6)
Kv1.2	7973 (4)
Kv1.3	3.96 (4) *0.354(4)
Kv1.4	>10000 (3)
Kv1.5	>10000 (3)
Kv1.6	>616 (6)
Kv1.7	>10000 (3)

Table 4H(b). Selectivity of monovalent Fc-L10-ShK(1-35,Q16K) (SEQ ID NO: 337; 348). * = Whole cell patch clamp (WCPC).

Assay	**IC50 nM (n)**
Human whole blood (TG induced IL-2)	0.262 (24)
Kv1.1	3344 (5)
Kv1.2	2725 (4)
Kv1.3	2.73 (7) *0.336(5)
Kv1.4	>10000 (3)
Kv1.5	>10000 (3)
Kv1.6	448 (11)
Kv1.7	>10000 (4)

Table 4I. Pharmacokinetics of monovalent Fc/Fc-[Lys16]ShK (SEQ ID NO: 337; 348) in Sprague-Dawley rats (n = 3).

CMPD	Tmax (h)	Cmax (ng/ml)	AUC0-t ($ng \cdot hr \cdot mL^{-1}$)	AUC0-inf ($ng \cdot hr \cdot mL^{-1}$)	CL/F ($mL \cdot hr^{-1} \cdot kg^{-1}$)	HL (h)
Monovalent Fc/Fc-[Lys16]ShK	4±3.46	1530±1230	39600±13900	43900±14600	146±47.1	56.3±19.3

Table 4J. Pharmacokinetic data for recombinant monovalent and bivalent anti-KLH Ab-[Lys16]ShK fusion proteins administered by subcutaneous injection (dose = 6 mg/kg) to Sprague-Dawley rats (n = 3).

CMPD	Tmax (h)	Cmax (ng/ml)	AUC0-t ($ng \cdot hr \cdot mL^{-1}$)	AUC0-inf ($ng \cdot hr \cdot mL^{-1}$)	CL/F ($mL \cdot hr^{-1} \cdot kg^{-1}$)	HL (h)
Monovalent	32±13.9	5890±1770	481000±157000	594000±182000	10.9±3.47	32±13.9
Bivalent	60±50.5	126±83.4	12900±9750	17800±17100	655±551	60±50.5

Table 4K. Pharmacokinetic data for recombinant monovalent Fc/Fc-ShK heterodimer, monovalent Fc-ShK/aKLH Ab heterotrimer and bivalent ShK-Fc/ShK-Fc homodimer fusion proteins administered by subcutaneous injection (dose = 2 mg/kg) to Sprague-Dawley rats (n = 3).

CMPD	Tmax (h)	Cmax (ng/ml)	AUC0-t ($ng \cdot hr \cdot mL^{-1}$)	AUC0-inf ($ng \cdot hr \cdot mL^{-1}$)	CL/F ($mL \cdot hr^{-1} \cdot kg^{-1}$)	MRT (h)
Monovalent Fc/Fc-ShK (heterodimer)	18.7 ± 9.2	728 ± 64.6	42469 ± 6566	44012 ± 7484	46.4 ± 8.6	46.8 ± 6.6
Monovalent Fc-ShK/KLH Ab (heterotrimer)	32.0 ± 13.9	1107 ± 26.2	83355.2 ± 5673	89158.6 ± 7915	22.6 ± 1.9	63.0 ± 8.7
Bivalent ShK-Fc/ShK-Fc (homodimer)	18.7 ± 9.2	27.0 ± 4.7	1418.8 ± 232	1460.7 ± 238	1395.0 ± 239	43.6 ± 2.3

Table 4L. Pharmacokinetic data for recombinant monovalent and bivalent anti-KLH AbLoop-[Lys16]ShK fusion proteins administered by subcutaneous injection (dose = 6 mg/kg) to Sprague-Dawley rats (n = 3).

CMPD	Tmax (h)	Cmax (ng/ml)	AUC0-t ($ng \cdot hr \cdot mL^{-1}$)	AUC0-inf ($ng \cdot hr \cdot mL^{-1}$)	CL/F ($mL \cdot hr^{-1} \cdot kg^{-1}$)	HL (h)
monovalent	40.7±35.6	7870±605	878000±259000	2730000±2060000	3.11±2.28	245±151
bivalent	3.33±1.15	102±41.3	5460±3930	6070±4510	1440±985	49.5±13.1

Example 13

Single-Chain (sc) Fc-Toxin Peptide Analog Fusion Proteins

[00695] The fusion of immunoglobulin Fc domains with therapeutic proteins has proven to be a powerful drug delivery platform with broad utility. Traditionally, this platform allows recombinant fusion of therapeutic peptides and proteins to either the N-terminus or C-terminus of an immunoglobulin Fc domain. An extension of this technology involves fusing select therapeutic polypeptides as internal sequences within the Fc sequence (U.S. Patent No.7,442,778 B2). These insertions are termed FcLoop constructs because they target elements of secondary structure predicted to be loop domains. In vivo, these Fc fusion proteins typically manifest prolonged pharmacokinetic lifetimes due to Fc recycling pathways which result in very efficacious therapeutic drugs. However, Fc fusion proteins, whether they're N- or C-terminal or FcLoop fusions are typically bivalent due to the dimeric nature of Fc (see, Example 12).

[00696] As described in Example 12, in some instances, it may be useful for an Fc fusion to be monovalent with respect to the therapeutic warhead, i.e., a toxin peptide analog moiety. In the case of Shk toxin peptide analog fusions it was observed that the bivalent forms of Fc-Shk or antibody FcLoop-Shk were cleared in vivo much more rapidly than their monovalent counterparts (see, Table 4H, Table 4I, Table 4J, Table 4K, and Table 4L). This example demonstrates the design, purification and efficacy of a single-chain Fc-Shk molecule using either an FcLoop insertion or C-terminal fusion to attach a single copy of the Shk toxin peptide analog to an Fc domain, which can be expressed recombinantly with a polypeptide linker of the correct composition and length to connect the C-terminus of one Fc subunit to the N-terminus of the other Fc subunit, thus creating a single-chain Fc (scFc). Initial linker design occurred in silico by examining crystal structures of huFc domains and determining the approximate distance between N-and C-termini. Next, FcLoop-Shk fusions with different length linkers using either 20 or 25 amino acids and FcLoop-Shk insertions in either the first sequential monomeric subunit of Fc or the second were constructed as illustrated schematically in Figure 24. Also shown is the C-terminal fusion of scFc with a 20 amino acid linker between the Fc monomer portions of the molecule and another 10 amino acid linker before the ShK portion. Figure 25A-C gives the polypeptide sequences of these three constructs. These constructs were expressed in E. coli and were refolded and purified (Figure 26A-B). Finally, the purified molecules were tested for activity in vitro and found to be efficacious (Table 4M, below).

[00697] Preparation of scFc-Shk.

[00698] Cloning and Expression of scFc-Shk: Two distinct nucleotide sequences (79% identical) encoding human Fc were used to create a scFc. The first sequence had minor modifications from wild-type Fc sequence to remove rare codons and minimize secondary structure near the 5'-end of the mRNA. The second was fully optimized for expression in bacteria by Verdezyne (Laguna Hills, CA). The two Fc sequences were first separated by 45 nucleotides encoding three repeats of a Gly-Gly-Gly-Gly-Ser (G_4S; SEQ ID NO:291) linker. Later constructs would add one or two additional five-residue repeats to the linker.

[00699] Formation of the expression construct was multi-fold. The expression vector (pET30, Novagen/EMD Biosciences, San Diego, CA) and a first Fc domain coding sequence, along with 20 nucleotides of linker were amplified from a pre-existing expression construct, using primers
5'-TAATGAATTCGAGCTCCGTCGACAAGCT-3' (SEQ ID NO:420) and
5'-CCACCGGATCCACCACCACCTTTACCCGGAGACAGGGAGAGGCT-3' (SEQ ID NO:421). This PCR and all other amplifications in this section used PfuUltra II DNA polymerase (Stratagene, La Jolla CA) according to manufacturer's instructions. A Touchdown PCR profile was used throughout with the annealing temperature dropping from 60°C to 51°C over ten cycles, followed by 20 cycles at 55°C. A second reaction amplified: 15 nucleotides of linker homologous to the 3'-end of the first reaction; the remaining 25 nucleotides of the linker; the second Fc; and another 16 nucleotides homologous to the 5'-end of the first reaction which also included the stop codon, using primers
5'-TGGTGGATCCGGTGGTGGTGGCTCCGGT-3' (SEQ ID NO:422) and
5'-GAGCTCGAATTCATTATTTACCCGGAGACAGAGACAGGGA-3'(SEQ ID NO:423). The two PCR products were joined by Polymerase Chain Reacion "Splicing by Overlap Extension" (SOE) reaction at the 15-nucleotide homologous linker region, using primers (5'-TGGTGGATCCGGTGGTGGTGGCTCCGGT-3' (SEQ ID NO:422) and
5'- CCACCGGATCCACCACCACCTTTACCCGGAGACAGGGAGAGGCT-3' (SEQ ID NO:421). This final PCR product was then treated with DpnI (New England Biolabs, NEB, Ipswich MA) and transformed into competent cells. The stop codon containing 16-nucleotide homologous region served as a site for in vivo recircularization of the vector.

[00700] Linker length was extended from 15 residues to 20, by amplifying the entire construct with two primers designed to insert 15 nucleotides encoding another G_4S in front of the previous three repeats: 5'-GGTGGCGGTGGCTCTGGTGGTGGTGGATCCGGTGGT-3' (SEQ ID NO:424) and

5'- AGAGCCACCGCCACCTTTACCCGGAGACAGGGAGAGGCT-3' (SEQ ID NO:425). This PCR product was treated with DpnI(NEB) and transformed into competent cells. During the screening process, one clone was sequenced to reveal a repeat of this newly inserted 15-nucleotide region and yielded the 25-residue linker sequence.

[00701] ShK (SEQ ID NO:1), flanked by Gly-Gly dipeptide linkers, was cloned into the first Fc domain of the Fc-L25-Fc chain by a method similar to that used to create the original scFc. One PCR amplified the entire vector, adding 6 nucleotides at the 5'- and 3'-ends encoding the flanking Gly-Gly dipeptide linkers, using primers
5'- GGCGGTACCAAGAACCAGGTCAGCCTGA-3' (SEQ ID NO:426) and
5'- ACCGCCCAGCTCATCACGGGATGGGGGCA-3' (SEQ ID NO:427). Another reaction amplified Shk (SEQ ID NO:1), with flanking Gly-Gly dipeptide linkers and a total of 18 nucleotides at it's 5'-end and 20 nucleotides at it's 3-end that were homologous to the corresponding termini of the first reaction, using primers
5'- CGTGATGAGCTGGGCGGTCGTTCTTGCATCGATACAATCCCT-3' (SEQ ID NO:428) and
5'- ACCTGGTTCTTGGTACCGCCACAGGTGCCACAGGTTTTACGA-3' (SEQ ID NO:429). A PCR SOE reaction using primers
5'- GGCGGTACCAAGAACCAGGTCAGCCTGA-3' (SEQ ID NO:426) and
5'- ACCTGGTTCTTGGTACCGCCACAGGTGCCACAGGTTTTACGA-3' (SEQ ID NO:429) gave a product that was transformed into competent cells after treatment with DpnI (NEB). Unfortunately, the only clone was found to have a mutation that needed to be removed by a subsequent round of mutagenesis, using primers
5'- CACTCAATGAAATACCGTCTCAGTTTCTGTCGT-3'(SEQ ID NO:430) and
5'- GTATTTCATTGAGTGTTTGCATTGAAAGGCA-3' (SEQ ID NO:431).

[00702] The same DNA sequence encoding Gly-Gly-ShK-Gly-Gly (SEQ ID NO:360) was inserted into the second Fc domain of the DNA encoding the Fc-L25-Fc polypeptide chain of the following amino acid sequence:
MDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY
KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSGG
GGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK

EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK// SEQ ID NO:413,
by two rounds of PCR. Three overlapping plus-strand primers and three overlapping minus strand primers covered the region. The first pair of primers:
5'- AAACCTGTGGCACCTGTGGCGGTACCAAAAACCAGGTGTCCCTGA -3' (SEQ ID NO:414); and
5'- TATCGATGCAAGAACGACCGCCCAGTTCGTCACGAGACGGCGGCA -3' (SEQ ID NO:415), was added to reaction 1.1; this first pair and a second pair of primers:
5'- AAATACCGTCTCAGTTTCTGTCGTAAAACCTGTGGCACCTGTGGC -3' (SEQ ID NO:416); and
5'- CAGTACAGCGGGACTTAGGGATTGTATCGATGCAAGAACGACCGC -3' (SEQ ID NO:417), were both added to reaction 1.2; and
primer pair 1 (SEQ ID NO:414 and SEQ ID NO:415) and primer pair 2 (SEQ ID NO:416 and SEQ ID NO:417) and the third pair of primers:
5'- CAATGCAAACACTCAATGAAATACCGTCTCAGTTTCTGTCGTAAA-3' (SEQ ID NO:418); and
5'- TGAGTGTTTGCATTGAAAGGCAGTACAGCGGGACTTAGGGATT-3' (SEQ ID NO:419), were all added to reaction 1.3. A second round of PCRs used the first's rounds product as template. Reaction 2.1 added the second primer pair (SEQ ID NO:416 and SEQ ID NO:417) to the product from reaction 1.1. The second and third primer pairs (SEQ ID NO:416 and SEQ ID NO:417; SEQ ID NO:418 and SEQ ID NO:419, respectively) were also added to reaction 1.1's product in reaction 2.2. Reactions 2.3 and 2.4 had the third primer pair (SEQ ID NO:418 and SEQ ID NO:419) being added to the reaction 1.2 and 1.3 products. All four of these second round reaction products were then mixed and used to transform competent cells. A single clone was found with the correct insert, but the 25-residue linker was found to have lost one G_4S repeat.

[00703] The Fc-L20-Fc DNA construct encoding the following:
(a) the Fc-L20-Fc amino acid sequence:
MDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY
KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSDK

THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF
YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPGK// SEQ ID NO:408,

and

(b) the CH2.L10.[Lys16]Shk-Ala DNA construct encoding an amino acid sequence containing L10 linker (SEQ ID NO:292; underlined) and [Lys16]ShK-Ala (SEQID NO:235; in boldface type):

MKPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
ISGGGGSGGGGS**RSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCGTC**A// SEQ ID NO:409,

were used as templates for creating Fc-L20-Fc-L10-[Lys16]Shk-Ala (amino acid sequence shown in Figure 25C; SEQ ID NO:410). One PCR amplified the Fc-L20-Fc vector, using primers:

5'- TAATGAATTCGAGCTCCGTCGACAAGCT-3' 9SEQ ID NO:432) and

5'- CCCGGAGACAGAGACAGGGA-3' (SEQ ID NO:433),

while another amplified 15 nucleotides homologous to the 3'-end of the first amplicon, the L10-[Lys16]ShK-Ala, and 15 nucleotides homologous to the 5'-end of the first amplicon, using primers:

5'- GTCTCTGTCTCCGGGTAAAGGCGGCGGCGGCA-3' (SEQ ID NO:434) and

5'- AGCTCGAATTCATTAAGCACAGGTGCCACAGGTT-3' (SEQ ID NO:435).

Clontech's (a Takara company, Shiga, Japan) In-Fusion™ technology was used to fuse the homologous regions.

[00704] All expression constructs were transformed into chemically-competent BL21Star (Invitrogen, Carlsbad, California). Autoinduction (AI) medium was inoculated and grown overnight at 37°C. Harvest was generally 16-24 h after inoculation. The AI medium was prepared by adding 20 mL of 50x Master Mix (10g Glucose; 500 mL Glycerol; 100 g Lactose; 5 g Aspartate; 100 mM $MgSO_4$; dH_2O to 1L) to 1L TB (Teknova).

[00705] Purification of scFc-Shk: Cell pastes containing expressed scFc-Shk constructs were resuspended in water at 0.1 g/mL and lysed with 3 passes through a microfluidizer. The lysate was centrifuged to pellet the inclusion bodies, which were then washed once with 1% deoxycholate, followed by a second water wash. The washed inclusion

bodies were then solubilized in 7.2 M guanidine hydrochloride, 1 0 mM dithiothreitol (DTT), pH8.5 at 0.1 g/mL. The solubilized inclusion bodies were then refolded by by 1:25 dilution at 4°C into a refolding buffer containing: 2 M urea, 150 mM arginine, 50 mM Tris, 3 mM cysteine and 1 mM cystamine at pH 8.5. The diluted refold reaction was gently stirred about 48hrs at 4°C, then clarified by centrifugation followed by filtration. Refolded scFc-Shk fusions were purified by affinity chromatography using Protein A Sepharose resin (GE Healthcare) in PBS and eluted with 0.1 N acetic acid, pH 3.5. The eluted peak was neutralized and dialyzed into 10 mM sodium acetate, 50 mM NaCl, pH5. The Protein A-purified scFc-Shk constructs were further purified by ion exchange chromatography using SP Sepharose HP resin (GE Healthcare) in 10mM NaOAc, 50mM NaCl, pH5 using a linear 50 mM-1M NaCl gradient. The eluted peaks were evaluated by SDS-PAGE and pooled based on size. The scFc-Shk constructs underwent a final purification step using hydrophobic interaction chromatography on Phenyl Sepharose HP (GE Healthcare) in 10 mM NaOAc, 50 mM NaCl, pH 5 and eluted with a linear 1 M $(NH_4)_2SO_4$ with eluted peaks further evaluated by SDS-PAGE. Final pools were concentrated, dialyzed into 10 mM sodium acetate, 9% sucrose, pH 5 and sterile filtered. The finished product was characterized by SDS-PAGE (Figure 26A), RP-HPLC (Figure 26B) and in vitro whole blood activity assay was run as described in Example 2 (Table 4M).

Table 4M. In vitro whole blood assay (inhibition of IL-2) average results demonstrating equipotence with conventional FcLoop-Shk bivalent peptibody.

Construct	***In Vitro* IC50 (nM)**
FcLoopShk/FcLoopShk (bivalent Fc)	**2.0**
Fc.L20FcLoopShk (scFc)	**2.01**
FcLoopShk.L25.Fc (scFc)	**1.91**

Table 5. Reference Peptides

SEQ ID NO:	Amino Acid Sequence	Designation	Kv1.3 (IWQ) IC50 (nM, Avg)	Kv1.1 (IWQ) IC50 (nM, Avg)	Kv1.1 / Kv1.3 Selectivity Ratio	WB (IL-2) IC50 (nM, Avg)	WB (IFNg) IC50 (nM, Avg)	WB (IL-2) Potency Relative to ShK	WB (IFNg) Potency Relative to ShK
1	RSCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC	ShK(1-35) (bench mark compound)	0.146	0.018	0.123	0.043	0.052	1.00	1.00
8	RSCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC	20kDa PEG-ShK	1.208	0.110	0.091	0.387	0.855	9.00	16.44
9	GVIINVKCKISRQCLEPCKKAGMRFGKCMNGKCACTPK	[Ala34]Osk-1	0.100	1.071	10.710	0.052	0.074	1.21	1.42
2	RTCKDLIPVSECTDIRCRTSMKYRLNLCRKTCGSC	HmK	0.900	0.015	0.017	0.85	1.195	19.77	22.98
10	RTCKDLIPVSECTDIKCRTS[Nva]KYRLNLCRKTCGSC	[Lys16,Nva21]HmK-amide	23.030	0.580	0.025	6.387	9.826	148.53	188.96
11	RSCIDTIPKSRCTAF[1-Nal]CKHSMKYRLSFCRKTCGTC	[1-Nal16]ShK	0.699	169.177	**242.027**	28.86	9.552	671.16	183.69
12	RSCIDTIPKSRCTAFQCKHSMKYRL[1-Nal]FCRKTCGTC	[1-Nal26]ShK	0.352	1.869	5.310	0.357	0.573	8.30	11.02
13	RSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCGTC	[Lys16]ShK	0.220	2.085	9.477	0.113	0.194	2.63	3.73
14	RSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCGTC	[Lys16]ShK-amide	0.174	0.600	3.448	0.223	0.278	5.19	5.35
15	RSCIDTIPKSRCTAFKCKHS[Nle]KYRLSFCRKTCGTC	[Lys16,Nle21]ShK-amide	0.153	13.220	**86.405**	0.823	1.099	19.14	21.13
16	RSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCGTC	20kDa PEG-[Lys16]ShK	0.665	1082.214	**1627.389**	0.104	0.215	2.42	4.13
17	[pY][Aeea]RSCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC	ShK(L5) (bench mark compound)	0.085	0.015	0.176	0.031	0.046	0.72	0.88
18	[pY][Aeea]RSCIDTIPKSRCTAFQCKHS[Nle]KYRLSFCRKTCGTC	[Nle21]ShK(L5)-amide (bench mark compound)	0.170	0.030	0.176	0.16	0.492	3.72	9.46

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Table 6. Arginine Scan

SEQ ID NO:	Amino Acid Sequence	Designation	Kv1.3 (IWQ) IC50 (nM, Avg)	Kv1.1 (IWQ) IC50 (nM, Avg)	Kv1.1 / Kv1.3 Selectivity Ratio	WB (IL-2) IC50 (nM, Avg)	WB (IFNg) IC50 (nM, Avg)	WB (IL-2) Potency Relative to ShK	WB (IFNg) Potency Relative to ShK
19	RRCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC	[Arg2]ShK	0.074	0.004	0.054	0.097	0.087	2.26	1.67
20	RSCRDTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC	[Arg4]ShK	0.110	0.006	0.055	0.051	0.081	1.19	1.56
21	RSCIRTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC	[Arg5]ShK	3.300	2.050	0.621	47.255	91.733	1098.95	1764.10
22	RSCIDRIPKSRCTAFQCKHSMKYRLSFCRKTCGTC	[Arg6]ShK	0.178	0.051	0.287	0.246	0.4	5.72	7.69
23	RSCIDTRPKSRCTAFQCKHSMKYRLSFCRKTCGTC	[Arg7]ShK	0.301	0.021	0.070	6.391	11.111	148.63	213.67
24	RSCIDTIRKSRCTAFQCKHSMKYRLSFCRKTCGTC	[Arg8]ShK	0.070	0.004	0.057	0.187	1.422	4.35	27.35
25	RSCIDTIPRSRCTAFQCKHSMKYRLSFCRKTCGTC	[Arg9]ShK	0.096	0.004	0.042	0.108	0.116	2.51	2.23
26	RSCIDTIPKRRCTAFQCKHSMKYRLSFCRKTCGTC	[Arg10]ShK	0.070	0.004	0.057	0.029	0.068	0.67	1.31
27	RSCIDTIPKSRCRAFQCKHSMKYRLSFCRKTCGTC	[Arg13]ShK	0.149	0.024	0.161	0.503	0.629	11.70	12.10
28	RSCIDTIPKSRCTRFQCKHSMKYRLSFCRKTCGTC	[Arg14]ShK	0.077	0.004	0.052	0.266	0.307	6.19	5.90
29	RSCIDTIPKSRCTARQCKHSMKYRLSFCRKTCGTC	[Arg15]ShK	0.088	0.022	0.250	0.162	0.204	3.77	3.92
30	RSCIDTIPKSRCTAFRCKHSMKYRLSFCRKTCGTC	[Arg16]ShK	0.164	0.066	0.402	0.652	0.818	15.16	15.73
31	RSCIDTIPKSRCTAFQCRHSMKYRLSFCRKTCGTC	[Arg18]ShK	0.110	0.005	0.045	0.012	0.023	0.28	0.44
32	RSCIDTIPKSRCTAFQCKRSMKYRLSFCRKTCGTC	[Arg19]ShK	0.100	0.004	0.040	0.039	0.116	0.91	2.23
33	RSCIDTIPKSRCTAFQCKHRMKYRLSFCRKTCGTC	**[Arg20]ShK**	3.842	100.000	26.028	36.906	31.291	858.28	601.75
34	RSCIDTIPKSRCTAFQCKHSRKYRLSFCRKTCGTC	[Arg21]ShK	0.283	0.045	0.159	1.76	4.655	40.93	89.52
35	RSCIDTIPKSRCTAFQCKHSMRYRLSFCRKTCGTC	[Arg22]ShK	18.737	33.330	1.779	100	100	2325.58	1923.08
36	RSCIDTIPKSRCTAFQCKHSMKRRLSFCRKTCGTC	**[Arg23]ShK**	0.154	3.300	21.429	100	100	2325.58	1923.08
37	RSCIDTIPKSRCTAFQCKHSMKYRRSFCRKTCGTC	[Arg25]ShK	0.107	0.001	0.009	0.095	0.2	2.21	3.85
38	RSCIDTIPKSRCTAFQCKHSMKYRLRFCRKTCGTC	[Arg26]ShK	0.279	0.089	0.319	0.208	0.572	4.84	11.00
39	RSCIDTIPKSRCTAFQCKHSMKYRLSRCRKTCGTC	[Arg27]ShK	100.000	5.037	0.050	12.958	59.73	301.35	1148.65
40	RSCIDTIPKSRCTAFQCKHSMKYRLSFCRRTCGTC	[Arg30]ShK	0.040	0.006	0.150	0.014	0.027	0.33	0.52
41	RSCIDTIPKSRCTAFQCKHSMKYRLSFCRKRCGTC	[Arg31]ShK	3.300	0.597	0.181	11.978	54.791	278.56	1053.67
42	RSCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCRTC	[Arg33]ShK	0.102	0.004	0.039	0.068	0.232	1.58	4.46
43	RSCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCGRC	[Arg34]ShK	0.182	0.008	0.044	0.418	0.486	9.72	9.35

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Table 7. Glutamate Scan

SEQ ID NO:	Amino Acid Sequence	Designation	Kv1.3 (IWQ) IC50 (nM, Avg)	Kv1.1 (IWQ) IC50 (nM, Avg)	Kv1.1 / Kv1.3 Selectivity Ratio	WB (IL-2) IC50 (nM, Avg)	WB (IFNg) IC50 (nM, Avg)	WB (IL-2) Potency Relative to ShK	WB (IFNg) Potency Relative to ShK
44	ESCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC	[Glu1]ShK	0.144	0.010	0.069	0.213	0.249	4.95	4.79
45	RECIDTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC	[Glu2]ShK	0.111	0.002	0.018	0.029	0.039	0.67	0.75
46	RSCEDTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC	[Glu4]ShK	0.070	0.006	0.086	0.031	0.049	0.72	0.94
47	RSCIETIPKSRCTAFQCKHSMKYRLSFCRKTCGTC	[Glu5]ShK	3.300	1.186	0.359	16.91	24.557	393.26	472.25
48	RSCIDEIPKSRCTAFQCKHSMKYRLSFCRKTCGTC	[Glu6]ShK	0.170	0.026	0.153	0.073	0.109	1.70	2.10
49	RSCIDTEPKSRCTAFQCKHSMKYRLSFCRKTCGTC	[Glu7]ShK	1.253	0.034	0.027	4.999	19.512	116.26	375.23
50	RSCIDTIEKSRCTAFQCKHSMKYRLSFCRKTCGTC	[Glu8]ShK	0.097	0.007	0.072	0.072	0.238	1.67	4.58
51	RSCIDTIPESRCTAFQCKHSMKYRLSFCRKTCGTC	[Glu9]ShK	0.128	0.006	0.047	0.036	0.056	0.84	1.08
52	RSCIDTIPKERCTAFQCKHSMKYRLSFCRKTCGTC	[Glu10]ShK	0.134	0.007	0.052	0.023	0.055	0.53	1.06
53	RSCIDTIPKSECTAFQCKHSMKYRLSFCRKTCGTC	[Glu11]ShK	0.969	0.031	0.032	1.322	4.542	30.74	87.35
54	RSCIDTIPKSRCEAFQCKHSMKYRLSFCRKTCGTC	[Glu13]ShK	0.157	0.002	0.013	0.785	1.099	18.26	21.13
55	RSCIDTIPKSRCTEFQCKHSMKYRLSFCRKTCGTC	[Glu14]ShK	0.110	0.008	0.073	0.035	0.132	0.81	2.54
56	RSCIDTIPKSRCTAEQCKHSMKYRLSFCRKTCGTC	[Glu15]ShK	0.266	0.112	0.421	0.312	0.575	7.26	11.06
57	RSCIDTIPKSRCTAFECKHSMKYRLSFCRKTCGTC	[Glu16]ShK	0.206	0.058	0.282	0.351	0.373	8.16	7.17
58	RSCIDTIPKSRCTAFQCEHSMKYRLSFCRKTCGTC	[Glu18]ShK	0.117	0.004	0.034	0.047	0.083	1.09	1.60
59	RSCIDTIPKSRCTAFQCKESMKYRLSFCRKTCGTC	[Glu19]ShK	0.256	0.018	0.070	0.202	0.215	4.70	4.13
60	RSCIDTIPKSRCTAFQCKHEMKYRLSFCRKTCGTC	[Glu20]ShK	100.000	13.426	0.134	92	92	2139.53	1769.23
61	RSCIDTIPKSRCTAFQCKHSEKYRLSFCRKTCGTC	[Glu21]ShK	3.333	0.071	0.021	18.468	28.089	429.49	540.17
62	RSCIDTIPKSRCTAFQCKHSMEYRLSFCRKTCGTC	[Glu22]ShK	33.300	33.300	1.000	100	100	2325.58	1923.08
63	RSCIDTIPKSRCTAFQCKHSMKERLSFCRKTCGTC	[Glu23]ShK	33.300	33.300	1.000	100	100	2325.58	1923.08
64	RSCIDTIPKSRCTAFQCKHSMKYELSFCRKTCGTC	[Glu24]ShK	0.260	0.014	0.054	1.11	4.073	25.81	78.33
65	RSCIDTIPKSRCTAFQCKHSMKYRESFCRKTCGTC	[Glu25]ShK	0.240	0.031	0.129	0.494	0.678	11.49	13.04
66	RSCIDTIPKSRCTAFQCKHSMKYRLEFCRKTCGTC	[Glu26]ShK	0.317	0.039	0.123	0.202	0.4	4.70	7.69
67	RSCIDTIPKSRCTAFQCKHSMKYRLSECRKTCGTC	[Glu27]ShK	0.000	0.000		8.155	34.03	189.65	654.42
68	RSCIDTIPKSRCTAFQCKHSMKYRLSFCEKTCGTC	[Glu29]ShK	0.142	0.009	0.063	0.73	1.955	16.98	37.60
69	RSCIDTIPKSRCTAFQCKHSMKYRLSFCRETCGTC	[Glu30]ShK	0.272	0.002	0.007	0.019	0.043	0.44	0.83
70	RSCIDTIPKSRCTAFQCKHSMKYRLSFCRKECGTC	[Glu31]ShK	0.220	0.006	0.027	0.242	0.309	5.63	5.94
71	RSCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCETC	[Glu33]ShK	0.060	0.005	0.083	0.054	0.176	1.26	3.38
72	RSCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCGEC	[Glu34]ShK	0.067	0.008	0.119	0.073	0.123	1.70	2.37

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Table 8. 1-Nal Scan

SEQ ID NO:	Amino Acid Sequence	Designation	Kv1.3 (IWQ) IC50 (nM, Avg)	Kv1.1 (IWQ) IC50 (nM, Avg)	Kv1.1 / Kv1.3 Selectivity Ratio	WB (IL-2) IC50 (nM, Avg)	WB (IFNg) IC50 (nM, Avg)	WB (IL-2) Potency Relative to ShK	WB (IFNg) Potency Relative to ShK
73	[1-Nal]SCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC	[1-Nal1]ShK	0.225	0.005	0.022	0.222	0.247	5.16	4.75
74	R[1-Nal]CIDTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC	[1-Nal2]ShK	0.250	0.002	0.008	0.223	0.258	5.19	4.96
75	RSC[1-Nal]DTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC	[1-Nal4]ShK	0.150	0.002	0.013	0.274	0.317	6.37	6.10
76	RSCI[1-Nal]TIPKSRCTAFQCKHSMKYRLSFCRKTCGTC	[1-Nal5]ShK	3.300	3.300	1.000	70.4	31.937	1637.21	614.17
77	RSCID[1-Nal]IPKSRCTAFQCKHSMKYRLSFCRKTCGTC	[1-Nal6]ShK	0.150	0.034	0.227	0.86	0.962	20.00	18.50
78	RSCIDT[1-Nal]PKSRCTAFQCKHSMKYRLSFCRKTCGTC	[1-Nal7]ShK	0.699	0.036	0.052	11.623	18.007	270.30	346.29
79	RSCIDTI[1-Nal]KSRCTAFQCKHSMKYRLSFCRKTCGTC	[1-Nal8]ShK	0.000	0.000		0	0		
80	RSCIDTIP[1-Nal]SRCTAFQCKHSMKYRLSFCRKTCGTC	[1-Nal9]ShK	0.473	0.011	0.023	0.367	0.666	8.53	12.81
81	RSCIDTIPK[1-Nal]RCTAFQCKHSMKYRLSFCRKTCGTC	[1-Nal10]ShK	0.206	0.004	0.019	0.205	0.255	4.77	4.90
82	RSCIDTIPKS[1-Nal]CTAFQCKHSMKYRLSFCRKTCGTC	[1-Nal11]ShK	0.922	0.027	0.029	1.137	1.645	26.44	31.63
83	RSCIDTIPKSRC[1-Nal]AFQCKHSMKYRLSFCRKTCGTC	[1-Nal13]ShK	0.235	0.016	0.068	3.563	4.856	82.86	93.38
84	RSCIDTIPKSRCT[1-Nal]FQCKHSMKYRLSFCRKTCGTC	[1-Nal14]ShK	0.000	0.000		0.089	0.11	2.07	2.12
85	RSCIDTIPKSRCTA[1-Nal]QCKHSMKYRLSFCRKTCGTC	[1-Nal15]ShK	0.072	0.009	0.125	0.184	0.291	4.28	5.60
11	RSCIDTIPKSRCTAF[1-Nal]CKHSMKYRLSFCRKTCGTC	**[1-Nal16]ShK**	0.699	169.177	**242.027**	28.86	9.552	671.16	183.69
87	RSCIDTIPKSRCTAFQC[1-Nal]HSMKYRLSFCRKTCGTC	[1-Nal18]ShK	0.471	0.004	0.008	0.419	0.375	9.74	7.21
88	RSCIDTIPKSRCTAFQCK[1-Nal]SMKYRLSFCRKTCGTC	[1-Nal19]ShK	0.277	0.001	0.004	0.153	0.255	3.56	4.90
89	RSCIDTIPKSRCTAFQCKH[1-Nal]MKYRLSFCRKTCGTC	[1-Nal20]ShK	2.110	3.300	1.564	100	100	2325.58	1923.08
90	RSCIDTIPKSRCTAFQCKHS[1-Nal]KYRLSFCRKTCGTC	[1-Nal21]ShK	0.669	0.038	0.057	6.586	8.131	153.16	156.37
91	RSCIDTIPKSRCTAFQCKHSM[1-Nal]YRLSFCRKTCGTC	[1-Nal22]ShK	33.300	33.330	1.001	100	100	2325.58	1923.08
92	RSCIDTIPKSRCTAFQCKHSMK[1-Nal]RLSFCRKTCGTC	[1-Nal23]ShK	3.300	3.300	1.000	100	100	2325.58	1923.08
93	RSCIDTIPKSRCTAFQCKHSMKY[1-Nal]LSFCRKTCGTC	[1-Nal24]ShK	0.000	0.000		0	0		
94	RSCIDTIPKSRCTAFQCKHSMKYR[1-Nal]SFCRKTCGTC	[1-Nal25]ShK	0.106	0.012	0.113	0.061	0.084	1.42	1.62
12	RSCIDTIPKSRCTAFQCKHSMKYRL[1-Nal]FCRKTCGTC	**[1-Nal26]ShK**	0.352	1.869	5.310	0.357	0.573	8.30	11.02
95	RSCIDTIPKSRCTAFQCKHSMKYRLS[1-Nal]CRKTCGTC	**[1-Nal27]ShK**	0.144	2.027	14.076	1.072	2.84	24.93	54.62
96	RSCIDTIPKSRCTAFQCKHSMKYRLSFC[1-Nal]KTCGTC	[1-Nal29]ShK	3.300	3.300	1.000	4.717	8.082	109.70	155.42
97	RSCIDTIPKSRCTAFQCKHSMKYRLSFCR[1-Nal]TCGTC	[1-Nal30]ShK	0.160	0.005	0.031	0.082	0.148	1.91	2.85
98	RSCIDTIPKSRCTAFQCKHSMKYRLSFCRK[1-Nal]CGTC	[1-Nal31]ShK	0.120	0.004	0.033	0.032	0.129	0.74	2.48
99	RSCIDTIPKSRCTAFQCKHSMKYRLSFCRKTC[1-Nal]TC	[1-Nal33]ShK	0.182	0.006	0.033	0.439	1.429	10.21	27.48
100	RSCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCG[1-Nal]C	[1-Nal34]ShK	0.077	0.004	0.052	0.018	0.063	0.42	1.21

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Table 9. Alanine Scan

SEQ ID NO:	Amino Acid Sequence	Designation	Kv1.3 (IWQ) IC50 (nM, Avg)	Kv1.1 (IWQ) IC50 (nM, Avg)	Kv1.1 / Kv1.3 Selectivity Ratio	WB (IL-2) IC50 (nM, Avg)	WB (IFNg) IC50 (nM, Avg)	WB (IL-2) Potency Relative to ShK	WB (IFNg) Potency Relative to ShK
101	ASCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC	[Ala1]ShK	0.168	0.007	0.042	0.073	0.102	1.70	1.96
102	RACIDTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC	[Ala2]ShK	0.156	0.003	0.019	0.054	0.087	1.26	1.67
103	RSCADTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC	[Ala4]ShK	0.176	0.005	0.028	0.012	0.008	0.28	0.15
104	RSCIATIPKSRCTAFQCKHSMKYRLSFCRKTCGTC	[Ala5]ShK	0.000	0.000		0	0		
105	RSCIDAIPKSRCTAFQCKHSMKYRLSFCRKTCGTC	[Ala6]ShK	0.208	0.012	0.058	0.065	0.032	1.51	0.62
106	RSCIDTAPKSRCTAFQCKHSMKYRLSFCRKTCGTC	[Ala7]ShK	0.188	0.005	0.027	0.886	2.223	20.60	42.75
107	RSCIDTIAKSRCTAFQCKHSMKYRLSFCRKTCGTC	[Ala8]ShK	0.078	0.004	0.051	0.039	0.061	0.91	1.17
108	RSCIDTIPASRCTAFQCKHSMKYRLSFCRKTCGTC	[Ala9]ShK	0.116	0.010	0.086	0.053	0.103	1.23	1.98
109	RSCIDTIPKARCTAFQCKHSMKYRLSFCRKTCGTC	[Ala10]ShK	0.193	0.440	2.280	1.211	1.779	28.16	34.21
110	RSCIDTIPKSACTAFQCKHSMKYRLSFCRKTCGTC	[Ala11]ShK	0.431	0.001	0.002	1.237	1.899	28.77	36.52
111	RSCIDTIPKSRCAAFQCKHSMKYRLSFCRKTCGTC	[Ala13]ShK	0.156	0.006	0.038	0.582	0.954	13.53	18.35
112	RSCIDTIPKSRCTAAQCKHSMKYRLSFCRKTCGTC	[Ala15]ShK	0.084	0.002	0.024	0.032	0.048	0.74	0.92
113	RSCIDTIPKSRCTAFACKHSMKYRLSFCRKTCGTC	[Ala16]ShK	0.196	0.001	0.005	0.058	0.078	1.35	1.50
114	RSCIDTIPKSRCTAFQCAHSMKYRLSFCRKTCGTC	[Ala18]ShK	0.160	0.001	0.006	0.025	0.025	0.58	0.48
115	RSCIDTIPKSRCTAFQCKASMKYRLSFCRKTCGTC	[Ala19]ShK	0.237	0.006	0.025	0.379	0.472	8.81	9.08
116	RSCIDTIPKSRCTAFQCKHAMKYRLSFCRKTCGTC	[Ala20]ShK	0.195	0.017	0.087	0.3	0.825	6.98	15.87
117	RSCIDTIPKSRCTAFQCKHSAKYRLSFCRKTCGTC	[Ala21]ShK	0.227	0.001	0.004	0.275	0.641	6.40	12.33
118	RSCIDTIPKSRCTAFQCKHSMAYRLSFCRKTCGTC	[Ala22]ShK	0.454	0.573	1.262	2.326	5.487	54.09	105.52
119	RSCIDTIPKSRCTAFQCKHSMKARLSFCRKTCGTC	[Ala23]ShK	0.621	2.095	3.374	3.752	5.44	87.26	104.62
120	RSCIDTIPKSRCTAFQCKHSMKYALSFCRKTCGTC	[Ala24]ShK	0.062	0.005	0.081	0.043	0.216	1.00	4.15
121	RSCIDTIPKSRCTAFQCKHSMKYRASFCRKTCGTC	[Ala25]ShK	0.116	0.006	0.052	0.195	0.408	4.53	7.85
122	RSCIDTIPKSRCTAFQCKHSMKYRLAFCRKTCGTC	[Ala26]ShK	0.137	0.001	0.007	0.068	0.114	1.58	2.19
123	RSCIDTIPKSRCTAFQCKHSMKYRLSACRKTCGTC	[Ala27]ShK	0.207	0.008	0.039	1.569	8.777	36.49	168.79
124	RSCIDTIPKSRCTAFQCKHSMKYRLSFCAKTCGTC	[Ala29]ShK	4.771	0.490	0.103	4.28	7.815	99.53	150.29
125	RSCIDTIPKSRCTAFQCKHSMKYRLSFCRATCGTC	[Ala30]ShK	0.178	0.005	0.028	0.258	0.394	6.00	7.58
126	RSCIDTIPKSRCTAFQCKHSMKYRLSFCRKACGTC	[Ala31]ShK	0.039	0.004	0.103	0.043	0.284	1.00	5.46
127	RSCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCATC	[Ala33]ShK	0.075	0.005	0.067	0.049	0.1	1.14	1.92
128	RSCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCGAC	[Ala34]ShK	0.123	0.004	0.033	0.063	0.075	1.47	1.44

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Table 10. Lysine Scan

SEQ ID NO:	Amino Acid Sequence	Designation	Kv1.3 (IWQ) IC50 (nM, Avg)	Kv1.1 (IWQ) IC50 (nM, Avg)	Kv1.1 / Kv1.3 Selectivity Ratio	WB (IL-2) IC50 (nM, Avg)	WB (IFNg) IC50 (nM, Avg)	WB (IL-2) Potency Relative to ShK	WB (IFNg) Potency Relative to ShK
129	KSCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC	[Lys1]ShK	0.158	0.006	0.038	0.035	0.073	0.81	1.40
130	RKCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC	[Lys2]ShK	0.149	0.006	0.040	0.099	0.093	2.30	1.79
131	RSCKDTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC	[Lys4]ShK	0.153	0.004	0.026	0.023	0.054	0.53	1.04
132	RSCIKTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC	[Lys5]ShK	3.300	1.579	0.478	100	100	2325.58	1923.08
133	RSCIDKIPKSRCTAFQCKHSMKYRLSFCRKTCGTC	[Lys6]ShK	0.085	0.002	0.024	0.117	0.21	2.72	4.04
134	RSCIDTKPKSRCTAFQCKHSMKYRLSFCRKTCGTC	**[Lys7]ShK**	0.792	8.689	10.971	4.671	13.468	108.63	259.00
135	RSCIDTIKKSRCTAFQCKHSMKYRLSFCRKTCGTC	[Lys8]ShK	0.083	0.005	0.060	0.154	0.974	3.58	18.73
136	RSCIDTIPKKRCTAFQCKHSMKYRLSFCRKTCGTC	[Lys10]ShK	0.174	0.004	0.023	0.052	0.082	1.21	1.58
137	RSCIDTIPKSKCTAFQCKHSMKYRLSFCRKTCGTC	[Lys11]ShK	0.137	0.002	0.015	0.095	0.252	2.21	4.85
138	RSCIDTIPKSRCKAFQCKHSMKYRLSFCRKTCGTC	[Lys13]ShK	0.157	0.005	0.032	0.257	0.363	5.98	6.98
139	RSCIDTIPKSRCTKFQCKHSMKYRLSFCRKTCGTC	[Lys14]ShK	0.073	0.005	0.068	0.075	0.202	1.74	3.88
140	RSCIDTIPKSRCTAKQCKHSMKYRLSFCRKTCGTC	[Lys15]ShK	0.295	0.046	0.156	0.465	0.991	10.81	19.06
13	RSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCGTC	**[Lys16]ShK**	0.220	2.085	9.477	0.113	0.194	2.63	3.73
141	RSCIDTIPKSRCTAFQCKKSMKYRLSFCRKTCGTC	[Lys19]ShK	0.189	0.004	0.021	0.041	0.089	0.95	1.71
142	RSCIDTIPKSRCTAFQCKHKMKYRLSFCRKTCGTC	**[Lys20]ShK**	1.332	15.408	11.568	0.108	0.192	2.51	3.69
143	RSCIDTIPKSRCTAFQCKHSKKYRLSFCRKTCGTC	[Lys21]ShK	0.822	0.048	0.058	3.589	4.822	83.47	92.73
144	RSCIDTIPKSRCTAFQCKHSMKKRLSFCRKTCGTC	[Lys23]ShK	0.000	0.000		0	0		
145	RSCIDTIPKSRCTAFQCKHSMKYKLSFCRKTCGTC	[Lys24]ShK	0.067	0.004	0.060	0.044	0.108	1.02	2.08
146	RSCIDTIPKSRCTAFQCKHSMKYRKSFCRKTCGTC	[Lys25]ShK	0.101	0.030	0.297	0.226	0.366	5.26	7.04
147	RSCIDTIPKSRCTAFQCKHSMKYRLKFCRKTCGTC	[Lys26]ShK	0.115	0.037	0.322	0.273	0.495	6.35	9.52
148	RSCIDTIPKSRCTAFQCKHSMKYRLSKCRKTCGTC	[Lys27]ShK	0.928	0.136	0.147	3.485	11.751	81.05	225.98
149	RSCIDTIPKSRCTAFQCKHSMKYRLSFCKKTCGTC	[Lys29]ShK	0.158	0.181	1.146	0.04	0.071	0.93	1.37
150	RSCIDTIPKSRCTAFQCKHSMKYRLSFCRKKCGTC	[Lys31]ShK	1.417	0.174	0.123	5.984	27.906	139.16	536.65
151	RSCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCKTC	[Lys33]ShK	0.122	0.005	0.041	0.218	0.317	5.07	6.10
152	RSCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCGKC	[Lys34]ShK	0.150	0.006	0.040	0.067	0.136	1.56	2.62

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Table 11. Position 16 Analogs

SEQ ID NO:	Amino Acid Sequence	Designation	Kv1.3 (IWQ) IC50 (nM, Avg)	Kv1.1 (IWQ) IC50 (nM, Avg)	Kv1.1 / Kv1.3 Selectivity Ratio	WB (IL-2) IC50 (nM, Avg)	WB (IFNg) IC50 (nM, Avg)	WB (IL-2) Potency Relative to ShK	WB (IFNg) Potency Relative to ShK
153	RSCIDTIPKSRCTAFOCKHSMKYRLSFCRKTCGTC	[Orn16]ShK	0.140	0.740	5.286	0.138	0.16	3.21	3.08
154	RSCIDTIPKSRCTAF[Dab]CKHSMKYRLSFCRKTCGTC	[Dab16]ShK	0.082	0.011	0.134	0.086	0.223	2.00	4.29
155	RSCIDTIPKSRCTAF[Ahp]CKHS[Nle]KYRLSFCRKTCGTC	[Ahp16,Nle21]ShK-amide	1.100	3.300	3.000	7.202	10.352	167.49	199.08
156	RSCIDTIPKSRCTAFNCKHS[Nle]KYRLSFCRKTCGTC	[Asn16,Nle21]ShK-amide	0.171	0.009	0.053	0.689	1.075	16.02	20.67
157	RSCIDTIPKSRCTAFHCKHS[Nle]KYRLSFCRKTCGTC	[His16,Nle21]ShK-amide	0.139	0.016	0.115	0.24	0.148	5.58	2.85

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Table 12. PEGylated Toxin Peptide Analogs

SEQ ID NO:	Amino Acid Sequence	Designation	Kv1.3 (IWQ) IC50 (nM, Avg)	Kv1.1 (IWQ) IC50 (nM, Avg)	Kv1.1 / Kv1.3 Selectivity Ratio	WB (IL-2) IC50 (nM, Avg)	WB (IFNg) IC50 (nM, Avg)	WB (IL-2) Potency Relative to ShK	WB (IFNg) Potency Relative to ShK
277	RSCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC	30kDaPEG-ShK	1.931	0.182	0.094	0.372	2.425	8.65	46.63
16	RSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCGTC	20kDaPEG-[Lys16]ShK	0.665	1082.214	**1627.389**	0.104	0.215	2.42	4.13
158	RSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCGTC	30kPEG-[Lys16]ShK	0.454	33.300	**73.348**	0.282	0.491	6.56	9.44
159	RSCIDTIPKSRCTAF[1-Nal]CKHSMKYRLSFCRKTCGTC	20kDaPEG-[1-Nal16]ShK	39.080	333.000	8.521	100	100	2325.58	1923.08
160	RSCIDTIPKSRCTAFQCKHSMKYRLS[1-Nal]CRKTCGTC	20kDaPEG-[1-Nal27]ShK	8.301	33.300	4.012	6.416	11.037	149.21	212.25
161	RSCIDTIPKSRCTAFKCKHSAKYRLSFCRKTCGTC	20kDaPEG-[Lys16,Ala21]ShK	4.573	33.300	7.282	4.802	7.922	111.67	152.35
162	RSCIDTIPKSRCTAFKCKHSMKYRLS[1-Nal]CRKTCGTC	20kDaPEG-[Lys16,1-Nal27]ShK	3.410	33.300	9.765	1.659	1.127	38.58	21.67
163	RSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCGTC	20kDaPEG-[Lys16]ShK-amide	0.605	219.863	**363.410**	0.089	0.164	2.07	3.15
164	RSCIDTIPKSRCTAFKCKHS[Nle]KYRLSFCRKTCGTC	20kDaPEG-[Lys16,Nle21]ShK-amide	1.429	33.300	23.303	0.707	1.754	16.44	33.73
165	RSCIDTIPKSRCTAFKCKHS[Nva]KYRLSFCRKTCGTC	20kDaPEG-[Lys16,Nva21]ShK-amide	1.610	1000.000	**621.118**	100	100	2325.58	1923.08
166	RSCIDTIPKSRCTAFKCKHSQKYRLSFCRKTCGTC	20kDaPEG-[Lys16,Gln21]ShK-amide	1.740	536.944	**308.589**	0.398	0.942	9.26	18.12
167	RSCIDTIPKSRCTAAKCKHS[Nle]KYRLSFCRKTCGTC	20kDaPEG-[Ala15,Lys16,Nle21]ShK-amide	2.691	3.300	1.226	0.631	1.618	14.67	31.12
168	RSCIDTIPKSRCTAFKCEHS[Nle]KYRLSFCRKTCGTC	20kDaPEG-[Lys16,Glu18,Nle21]ShK-amide	9.022	33.300	3.691	2.888	8.846	67.16	170.12
169	RSCEDTIPKSRCTAFKCKHS[Nle]KYRLSFCRKTCGTC	20kDaPEG-[Glu4,Lys16,Nle21]ShK-amide	2.372	3.300	1.391	1.731	3.782	40.26	72.73
170	RSCIDTIPKSRCTAFKCKHS[Nle]KYRLSFCRKTCGTCA	20kDaPEG-[Lys16,Nle21]ShK-Ala	2.435	1000.000	**410.678**	0.505	0.78	11.74	15.00
171	RSCIDTIPKSRCTAFKCKHS[Nva]KYRLSFCRKTCGTCA	20kDaPEG-[Lys16,Nva21]ShK-Ala	5.970	1000.000	**167.504**	0.655	0.693	15.23	13.33
172	RSCEDTIPKSRCTAFKCKHS[Nva]KYRLSFCRKTCGTCA	20kDaPEG-[Glu4,Lys16,Nva21]ShK-Ala	5.830	33.300	5.712	3.241	9.463	75.37	181.98
173	RSCEDTIPKSRCTAFKCKHSQKYRLSFCRKTCGTCA	20kDaPEG-[Glu4,Lys16,Gln21]ShK-Ala	7.780	33.300	4.280	2.781	14.023	64.67	269.67
174	RSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCGTC	[Lys16(20KPeg)]ShK	6.900	33.300	4.826	1.126	1.485	26.19	28.56

Table 13. Combination Toxin Peptide Analogs {Free Acid}

SEQ ID NO:	Amino Acid Sequence	Designation	Kv1.3 (IWQ) IC50 (nM, Avg)	Kv1.1 (IWQ) IC50 (nM, Avg)	Kv1.1 / Kv1.3 Selectivity Ratio	WB (IL-2) IC50 (nM, Avg)	WB (IFNg) IC50 (nM, Avg)	WB (IL-2) Potency Relative to ShK	WB (IFNg) Potency Relative to ShK
175	RSCIDTIPKSRCTAFQCKHS[Nle]KYRLSFCRKTCGTC	[Nle21]ShK	0.040	0.015	0.375	0.153	0.303	3.56	5.83
176	RSCIDTIPKSRCTAAKCKHSMKYRLSFCRKTCGTC	[Ala15,Lys16]ShK	0.960	9.081	9.459	0.057	0.052	1.33	1.00
177	RSCIDTIPKSRCTAFKCRHSMKYRLSFCRKTCGTC	[Lys16,Arg18]ShK	0.425	1.090	2.565	0.077	0.097	1.79	1.87
178	RSCIDTIPKSRCTAFKCKHSAKYRLSFCRKTCGTC	[Lys16,Ala21]ShK	0.076	4.690	61.711	0.872	1.334	20.28	25.65
179	RSCIDTIPKSRCTAFKCKHS[Nle]KYRLSFCRKTCGTC	[Lys16,Nle21]ShK	0.130	29.258	225.062	0.249	0.848	5.79	16.31
180	RSCIDTIPKSRCTAFKCKHSMKYRLS[1-Nal]CRKTCGTC	[Lys16,1-Nal27]ShK	0.113	2.806	24.832	0.924	5.678	21.49	109.19
181	RSCIDTIPKSRCTAFKCKHSMKYRLSFCKKTCGTC	[Lys16,Lys29]ShK	0.071	1.172	16.507	0.112	0.143	2.60	2.75
182	RSCIDTIPKSRCTAFKCKHSMKYRLSFCRRTCGTC	[Lys16,Arg30]ShK	0.092	0.837	9.098	0.169	0.189	3.93	3.63
183	RSCIDTIPKSRCTAFKCKHSMKYRLSFCRETCGTC	[Lys16,Glu30]ShK	0.073	3.300	45.205	0.032	0.081	0.74	1.56
184	RSCIDTIPKSRCTAFKCKHSMKYRLS[1-Nal]CKRTCGTC	[Lys16,29;1-Nal27;Arg30]ShK	0.110	3.383	30.755	3.372	3.831	78.42	73.67
185	RSCEDTIPKRRCTAAKCKHSMKYRLSFCRRTCGTC	[Glu4,Arg10,30,Ala15,Lys16]ShK	0.140	8.857	63.264	0.209	0.305	4.86	5.87

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Table 14. Combination Toxin Peptide Analogs {C-Terminal Amide}

SEQ ID NO:	Amino Acid Sequence	Designation	Kv1.3 (IWQ) IC50 (nM, Avg)	Kv1.1 (IWQ) IC50 (nM, Avg)	Kv1.1 / Kv1.3 Selectivity Ratio	WB (IL-2) IC50 (nM, Avg)	WB (IFNg) IC50 (nM, Avg)	WB (IL-2) Potency Relative to ShK	WB (IFNg) Potency Relative to ShK
14	RSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCGTC	[Lys16]ShK-amide	0.174	0.600	3.448	0.223	0.278	5.19	5.35
186	RSCIDTIPKSRCTAAKCKHSMKYRLSFCRKTCGTC	[Ala15,Lys16]ShK-amide	0.091	1.662	18.264	0.167	0.193	3.88	3.71
187	RSCIDTIPKSRCTAFKCKHSMKYRLS[1-Nal]CRKTCGTC	[Lys16,1-Nal27]ShK-amide	0.299	2.247	7.515	3.948	2.943	91.81	56.60
188	RSCEDTIPKSRCTAFKCKHSMKYRLSFCRKTCGTC	[Glu4,Lys16]ShK-amide	0.127	4.523	35.614	0.071	0.068	1.65	1.31
189	RSCIDTIPKSRCTAFKCKHSMKYRLSFCRRTCGTC	[Lys16,Arg30]ShK-amide	0.129	0.689	5.341	0.151	0.236	3.51	4.54
190	RSCIDTIPKSRCTAFKCKHSMKYRLSFCRETCGTC	[Lys16,Glu30]ShK-amide	0.028	1.412	**50.429**	0.105	0.144	2.44	2.77
191	RSCEDTIPKSRCTAFKCKHSMKYRL[1-Nal]FCKRTCGTC	[Glu4;Lys16,29;1-Nal26;Arg30]ShK-amide	0.384	33.300	**86.719**	0.712	0.598	16.56	11.50
192	RSCIDTIPKSRCTAFKCKHSMKYRLYFCKKTCGTC	[Lys16,29;Tyr26]ShK-amide	0.084	33.300	**396.429**	1.04	0.754	24.19	14.50
193	RSCIDTIPKSRCTAFKCKHSMKYRL[2Nal]FCKKTCGTC	[Lys16,29;2-Nal26]ShK-amide	0.762	33.300	43.701	0.222	0.148	5.16	2.85
194	RSCIDTIPKSRCTAFKCKHS[Nle]KYRLSFCRETCGTC	[Lys16,Nle21,Glu30]ShK-amide	1.882	33.300	17.694	3.183	5.027	74.02	96.67
195	RSCIDTIPKSRCTAFKCEHSMKYRLSFCRKTCGTC	[Lys16,Glu18]ShK-amide	0.065	3.436	**52.862**	0.119	0.234	2.77	4.50
196	RSCIDTIPKSRCTAFKCKHS[Nle]KYRLSFCRRTCGTC	[Lys16,Nle21,Arg30]ShK-amide	0.423	12.665	29.941	0.953	1.904	22.16	36.62
15	RSCIDTIPKSRCTAFKCKHS[Nle]KYRLSFCRKTCGTC	[Lys16,Nle21]ShK-amide	0.153	13.220	**86.405**	0.823	1.099	19.14	21.13
197	RSCEDTIPKSRCTAFKCKHS[Nle]KYRL[1-Nal]FCKRTCGTC	[Glu4;Lys16,29;1-Nal26;Arg30;Nle21]ShK-amide	1.655	3.300	1.994	10.683	17.391	248.44	334.44
198	RSCEDTIPESRCTAFKCRHSMKYRLSFCKRTCGTC	[Glu4,9;Lys16,29;Arg18,30]ShK-amide	0.062	3.300	**53.226**	0.151	0.345	3.51	6.63
199	RSCEDTIPKERCTAFKCRHSMKYRLSFCKRTCGTC	[Glu4,10;Lys16,29;Arg18,30]ShK-amide	0.064	3.300	**51.563**	0.114	0.217	2.65	4.17
200	RSCEDTIPEERCTAFKCRHSMKYRLSFCKRTCGTC	[Glu4,9,10;Lys16,29;Arg18,30]ShK-amide	0.059	3.300	**55.932**	0.204	0.513	4.74	9.87
201	RSCEDTIPKSRCTAFKCKHSMKYRLSFCKRTCGTC	[Glu4;Lys16,29;Arg30]ShK-amide	0.104	3.300	31.731	0.067	0.28	1.56	5.38
202	RSCIDTIPKSRCTAFKCKHSMKYRLSWCKKTCGTC	[Lys16,29;Trp27]ShK-amide	0.121	3.300	27.273	0.264	0.606	6.14	11.65
203	RSCIDTIPKSRCTAFKCKHS[Nle]KYRLSFCKRTCGTC	[Lys16,29;Nle21,Arg30]ShK-amide	0.484	33.300	**68.802**	1.721	2.838	40.02	54.58
204	RSCIDTIPKSRCTAFKCEHS[Nle]KYRLSFCRKTCGTC	[Lys16,Glu18,Nle21]ShK-amide	0.280	33.300	**118.929**	0.477	0.929	11.09	17.87
205	RSCEDTIPKSRCTAFKCKHS[Nle]KYRLSFCRKTCGTC	[Glu4,Lys16,Nle21]ShK-amide	0.149	33.300	**223.490**	0.57	0.965	13.26	18.56
206	RSCIDTIPKSRCTAAKCKHS[Nle]KYRLSFCRKTCGTC	[Ala15,Lys16,Nle21]ShK-amide	0.127	13.944	**109.795**	0.952	1.866	22.14	35.88
207	RSCIDTIPKSRCTAFKCKHSMKYRLS[2Nal]CKKTCGTC	[Lys16,29;2-Nal27]ShK-amide	10.108	33.300	3.294	13.112	24.747	304.93	475.90

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Table 14 continued. Combination Toxin Peptide Analogs {C-Terminal Amide}

SEQ ID NO:	Amino Acid Sequence	Designation	Kv1.3 (IWQ) IC50 (nM, Avg)	Kv1.1 (IWQ) IC50 (nM, Avg)	Kv1.1 / Kv1.3 Selectivity Ratio	WB (IL-2) IC50 (nM, Avg)	WB (IFNg) IC50 (nM, Avg)	WB (IL-2) Potency Relative to ShK	WB (IFNg) Potency Relative to ShK
208	RSCIDTIPKSRCTAFKCRHSMKYRLSFCKRTCGTC	[Lys16,29;Arg18,30]ShK-amide	0.401	1.896	4.728	0.26	0.242	6.05	4.65
209	RSCIDTIPKSRCTAFKCKHSMKYRLFFCKKTCGTC	[Lys16,29;Phe26]ShK-amide	0.542	3.300	6.089	1.417	1.363	32.95	26.21
210	RSCIDTIPKSRCTAFKCKHSMKYRLSFCKATCGTC	[Lys16,29;Ala30]ShK-amide	0.197	3.300	16.751	0.186	0.289	4.33	5.56
211	RSCEDTIPKSRCTAFKCRHS[Nle]KYRLSFCKRTCGTC	[Glu4;Lys16,29;Arg18,30;Nle21]ShK-amide	0.269	3.300	12.268	1.961	1.331	45.60	25.60
212	RSCEDTIPKSRCTAFKCRHSMKYRLSFCKRTCGTC	[Glu4;Lys16,29;Arg18,30]ShK-amide	0.192	3.300	17.188	0.203	0.353	4.72	6.79
213	RSCIDTIPKSRCTAFKCKHSMKYRLSFCKRTCGTC	[Lys16,29;Arg30]ShK-amide	0.131	2.469	18.847	0.339	0.281	7.88	5.40
214	RSCEDTIPKSRCTAAKCEHS[Nle]KYRLSFCRKTCGTC	[Glu4,18,Ala15,Lys16,Nle21]ShK-amide	0.346	3.300	9.538	1.215	1.597	28.26	30.71
215	RSCEDTIPKSRCTAFKCEHS[Nle]KYRLSFCRKTCGTC	[Glu4,18,Lys16,Nle21]ShK-amide	0.239	3.300	13.908	0.603	0.934	14.02	17.96
216	RSCEDTIPKSRCTAAKCKHS[Nle]KYRLSFCRKTCGTC	[Glu4,Ala15,Lys16,Nle21]ShK-amide	0.168	3.300	19.643	0.856	0.901	19.91	17.33
217	RSCIDTIPKSRCTAAKCEHS[Nle]KYRLSFCRKTCGTC	[Glu18,Ala15,Lys16,Nle21]ShK-amide	0.202	3.300	16.337	1.355	1.291	31.51	24.83
218	RSCEDTIPKSRCTAFKCKHS[Nle]KYRLSFCRRTCGTC	[Glu4,Lys16,Nle21,Arg30]ShK-amide	0.132	33.300	**252.273**	1.181	1.341	27.47	25.79
219	RSCIDTIPKSRCTAFKCEHS[Nle]KYRLSFCRRTCGTC	[Glu18,Lys16,Nle21,Arg30]ShK-amide	0.226	33.300	**147.345**	1.22	0.969	28.37	18.63
220	RSCEDTIPKSRCTAFKCEHS[Nle]KYRLSFCRRTCGTC	[Glu4,18,Lys16,Nle21,Arg30]ShK-amide	0.260	3.300	12.692	0.97	1.985	22.56	38.17
221	RSCIDTIPKSRCTAAKCKHS[Nle]KYRLSFCRRTCGTC	[Ala15,Lys16,Nle21,Arg30]ShK-amide	0.329	33.300	**101.216**	1.348	1.714	31.35	32.96
222	RSCIDTIPESRCTAFKCKHS[Nle]KYRLSFCRKTCGTC	[Glu9,Lys16,Nle21]ShK-amide	0.263	33.300	**126.616**	0.257	0.342	5.98	6.58
223	RSCIDTIPKSRCTAAKCEHS[Nle]KYRLSFCRRTCGTC	[Glu18,Ala15,Lys16,Nle21,Arg30]ShK-amide	0.398	33.300	**83.668**	1.321	2.028	30.72	39.00
224	RSCEDTIPKSRCTAAKCKHS[Nle]KYRLSFCRRTCGTC	[Glu4,Ala15,Lys16,Nle21,Arg30]ShK-amide	0.118	3.300	27.966	0.647	0.816	15.05	15.69
225	RSCIDTIPKERCTAFKCKHS[Nle]KYRLSFCRKTCGTC	[Glu10,Lys16,Nle21]ShK-amide	0.127	3.300	25.984	0.776	1.257	18.05	24.17
226	RSCIDTIPKSRCTAFKCKHSMKYRL[1-Nal]FCKKTCGTC	[Lys16,29;1-Nal26]ShK-amide	0.341	3.300	9.677	0.808	1.975	18.79	37.98

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Table 14 continued. Combination Toxin Peptide Analogs {C-Terminal Amide}

SEQ ID NO:	Amino Acid Sequence	Designation	Kv1.3 (IWQ) IC50 (nM, Avg)	Kv1.1 (IWQ) IC50 (nM, Avg)	Kv1.1 / Kv1.3 Selectivity Ratio	WB (IL-2) IC50 (nM, Avg)	WB (IFNg) IC50 (nM, Avg)	WB (IL-2) Potency Relative to ShK	WB (IFNg) Potency Relative to ShK
227	RSCIDTIPKSRCTAFKCKHS[Nle]KYRL[1-Nal]FCKRTCGTC	[Lys16,29;1-Nal26;Arg30;Nle21]ShK-amide	2.334	3.300	1.414	9.442	8.222	219.58	158.12
228	RSCIDTIPKSRCTAFKCKHSMKYRLSYCKKTCGTC	[Lys16,29;Tyr27]ShK-amide	0.042	3.300	78.571	0.738	1.172	17.16	22.54
229	RSCEDTIPKSRCTAFKCKHSTKYRLSFCRKTCGTC	[Glu4,Lys16,Thr21]ShK-amide	0.085	3.300	38.824	1.384	3.942	32.19	75.81
230	RSCEDTIPKSRCTAFKCKHSSKYRLSFCRKTCGTC	[Glu4,Lys16,Ser21]ShK-amide	0.098	3.300	33.673	0.86	1.994	20.00	38.35
231	RSCIDTIPKSRCTAFKCKHS[Nle]KYRLSFCKATCGTC	[Lys16,29;Ala30,Nle21]ShK-amide	0.057	24.070	422.281	0.791	1.7	18.40	32.69
232	RSCEDTIPKSRCTAFKCKHS[Nva]KYRLSFCRKTCGTC	[Glu4,Lys16,Nva21]ShK-amide	0.086	3.300	38.372	0.405	0.427	9.42	8.21
233	RSCEDTIPKSRCTAFKCKHS[Abu]KYRLSFCRKTCGTC	[Glu4,Lys16,Abu21]ShK-amide	0.330	33.000	100.000	1.115	1.765	25.93	33.94
234	RSCEDTIPKSRCTAFKCKHSQKYRLSFCRKTCGTC	[Glu4,Lys16,Gln21]ShK-amide	0.000	0.000		0.23	0.423	5.35	8.13

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Table 15. Toxin Peptide Analogs with C-Terminal Extension

SEQ ID NO:	Amino Acid Sequence	Designation	Kv1.3 (IWQ) IC50 (nM, Avg)	Kv1.1 (IWQ) IC50 (nM, Avg)	Kv1.1/ Kv1.3 Selectivity Ratio	WB (IL-2) IC50 (nM, Avg)	WB (IFNg) IC50 (nM, Avg)	WB (IL-2) Potency Relative to ShK	WB (IFNg) Potency Relative to ShK
235	RSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCGTCA	[Lys16]ShK-Ala	0.060	9.500	**158.333**	0.138	0.266	3.21	5.12
236	RSCIDTIPKSRCTAFKCKHS[Nle]KYRLSFCRKTCGTCA	[Lys16,Nle21]ShK-Ala	0.071	33.300	**469.014**	0.305	0.515	7.09	9.90
237	RSCIDTIPKSRCTAFKCKHSNKYRLSFCRKTCGTCA	[Lys16,Asn21]ShK-Ala	0.360	33.000	**91.667**	0.891	0.693	20.72	13.33
238	RSCIDTIPKSRCTAFKCKHS[Nva]KYRLSFCRKTCGTCA	[Lys16,Nva21]ShK-Ala	0.540	13.490	24.981	0.164	0.307	3.81	5.90
239	RSCIDTIPKSRCTAFKCKHS[Nva]KYRLSFCRKTCGTCA	[Lys16,Nva21]ShK-Ala-amide	0.080	7.606	**95.075**	0.431	1.455	10.02	27.98
240	RSCIDTIPKSRCTAFKCKHSQKYRLSFCRKTCGTCA	[Lys16,Gln21]ShK-Ala	0.350	4.010	11.457	0.168	0.206	3.91	3.96
241	RSCEDTIPKSRCTAFKCKHSQKYRLSFCRKTCGTCA	[Glu4,Lys16,Gln21]ShK-Ala	0.145	26.350	**181.724**	0.137	0.265	3.19	5.10
242	RSCEDTIPKSRCTAFKCKHS[Nva]KYRLSFCRKTCGTCA	[Glu4,Lys16,Nva21]ShK-Ala	0.133	33.300	**250.376**	0.208	0.368	4.84	7.08
243	RSCEDTIPKSRCTAFKCKHS[Nle]KYRLSFCRKTCGTCA	[Glu4,Lys16,Nle21]ShK-Ala	0.060	33.300	**555.000**	0.327	0.987	7.60	18.98
244	RSCIDTIPKSRCTAFKCKHS[Nle]KYRLSFCRKTCGTCE	[Lys16,Nle21]ShK-Glu	0.090	22.490	**249.889**	0.386	0.621	8.98	11.94
245	RSCIDTIPKSRCTAFKCKHS[Nle]KYRLSFCRKTCGTCY	[Lys16,Nle21]ShK-Tyr	0.215	109.305	**508.395**	0.418	0.485	9.72	9.33
246	RSCIDTIPKSRCTAFKCKHS[Nle]KYRLSFCRKTCGTCV	[Lys16,Nle21]ShK-Val	0.000	0.000		2.046	5.647	47.58	108.60
247	RSCIDTIPKSRCTAFKCKHS[Nva]KYRLSFCRKTCGTC[bAla]	[Lys16,Nva21]ShK-betaAla	0.260	12.580	48.385	0.304	0.231	7.07	4.44

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Table 16. Toxin Peptide Analogs with C-Terminal Extension {and C-Terminal Amide}

SEQ ID NO:	Amino Acid Sequence	Designation	Kv1.3 (IWQ) IC50 (nM, Avg)	Kv1.1 (IWQ) IC50 (nM, Avg)	Kv1.1 / Kv1.3 Selectivity Ratio	WB (IL-2) IC50 (nM, Avg)	WB (IFNg) IC50 (nM, Avg)	WB (IL-2) Potency Relative to ShK	WB (IFNg) Potency Relative to ShK
248	RSCIDTIPKSRCTAFKCKHS[Nva]KYRLSFCRKTCGTCD	[Lys16,Nva21]ShK-Asp-amide	0.280	12.670	45.250	0.255	0.349	5.93	6.71
249	RSCIDTIPKSRCTAFKCKHS[Nva]KYRLSFCRKTCGTCE	[Lys16,Nva21]ShK-Glu-amide	0.210	7.970	37.952	0.218	0.353	5.07	6.79
250	RSCIDTIPKSRCTAFKCKHS[Nle]KYRLSFCRKTCGTCD	[Lys16,Nle21]ShK-Asp-amide	0.390	16.600	42.564	0.295	0.403	6.86	7.75
251	RSCIDTIPKSRCTAFKCKHS[Nva]KYRLSFCRKTCGTCS	[Lys16,Nva21]ShK-Ser-amide	0.090	10.730	**119.222**	0.467	0.662	10.86	12.73
252	RSCIDTIPKSRCTAFKCKHS[Nva]KYRLSFCRKTCGTCT	[Lys16,Nva21]ShK-Thr-amide	0.030	4.430	**147.667**	0.385	0.702	8.95	13.50
253	RSCIDTIPKSRCTAFKCKHS[Nva]KYRLSFCRKTCGTC[Aib]	[Lys16,Nva21]ShK-Aib-amide	0.030	5.730	**191.000**	0.483	0.722	11.23	13.88
254	RSCIDTIPKSRCTAFKCKHS[Nva]KYRLSFCRKTCGTCA	[Lys16,Nva21]ShK-Ala-amide	0.080	7.606	**95.075**	0.431	1.455	10.02	27.98

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Table 17. Position 21 Toxin Peptide Analogs

SEQ ID NO:	Amino Acid Sequence	Designation	Kv1.3 (IWQ) IC50 (nM, Avg)	Kv1.1 (IWQ) IC50 (nM, Avg)	Kv1.1 / Kv1.3 Selectivity Ratio	WB (IL-2) IC50 (nM, Avg)	WB (IFNg) IC50 (nM, Avg)	WB (IL-2) Potency Relative to ShK	WB (IFNg) Potency Relative to ShK
255	RSCIDTIPKSRCTAFQCKHS[Nle]KYRLSFCRKTCGTC	[Nle21]ShK-amide	0.040	0.015	0.375	0.325	1.133	7.56	21.79
15	RSCIDTIPKSRCTAFKCKHS[Nle]KYRLSFCRKTCGTC	[Lys16,Nle21]ShK-amide	0.153	13.220	**86.405**	0.823	1.099	19.14	21.13
257	RSCIDTIPKSRCTAFKCKHS[Met(O)]KYRLSFCRKTCGTC	[Lys16,Met(O)21]ShK-amide	0.106	2.106	19.868	0.24	0.289	5.58	5.56
258	RSCIDTIPKSRCTAFKCKHSSKYRLSFCRKTCGTC	[Lys16,Ser21]ShK-amide	0.068	3.300	48.529	1.448	1.705	33.67	32.79
259	RSCIDTIPKSRCTAFKCKHSTKYRLSFCRKTCGTC	[Lys16,Thr21]ShK-amide	0.058	3.300	**56.897**	1.554	2.978	36.14	57.27
260	RSCIDTIPKSRCTAFKCKHS[Nva]KYRLSFCRKTCGTC	[Lys16,Nva21]ShK-amide	0.470	7.374	15.689	0.486	0.932	11.30	17.92
261	RSCIDTIPKSRCTAFKCKHSQKYRLSFCRKTCGTC	[Lys16,Gln21]ShK-amide	0.161	3.151	19.571	0.42	0.812	9.77	15.62
262	RSCIDTIPKSRCTAFKCKHSHKYRLSFCRKTCGTC	[Lys16,His21]ShK-amide	0.219	3.300	15.068	5.498	9.248	127.86	177.85
263	RSCIDTIPKSRCTAFKCKHSYKYRLSFCRKTCGTC	[Lys16,Tyr21]ShK-amide	0.246	3.300	13.415	2.835	3.19	65.93	61.35
264	RSCIDTIPKSRCTAFKCKHSNKYRLSFCRKTCGTC	[Lys16,Asn21]ShK-amide	0.167	3.300	19.760	3.236	2.727	75.26	52.44
265	RSCIDTIPKSRCTAFKCKHSVKYRLSFCRKTCGTC	[Lys16,Val21]ShK-amide	0.344	33.300	**96.802**	4.801	6.654	111.65	127.96
266	RSCIDTIPKSRCTAFKCKHSLKYRLSFCRKTCGTC	[Lys16,Leu21]ShK-amide	0.213	23.808	**111.775**	0.889	0.636	20.67	12.23
267	RSCIDTIPKSRCTAFKCKHSWKYRLSFCRKTCGTC	[Lys16,Trp21]ShK-amide	0.782	3.300	4.220	3.864	3.195	89.86	61.44
268	RSCIDTIPKSRCTAFKCKHS[Abu]KYRLSFCRKTCGTC	[Lys16,Abu21]ShK-amide	0.246	3.300	13.415	1.202	1.526	27.95	29.35
269	RSCIDTIPKSRCTAFKCKHS[Cha]KYRLSFCRKTCGTC	[Lys16,Cha21]ShK-amide	0.499	3.300	6.613	1.739	1.325	40.44	25.48
270	RSCIDTIPKSRCTAFKCKHS[Chg]KYRLSFCRKTCGTC	[Lys16,Chg21]ShK-amide	0.205	3.300	16.098	0.875	0.727	20.35	13.98
271	RSCIDTIPKSRCTAFKCKHS[Hyp]KYRLSFCRKTCGTC	[Lys16,Hyp21]ShK-amide	3.300	3.300	1.000	100	100	2325.58	1923.08
272	RSCIDTIPKSRCTAFKCKHSFKYRLSFCRKTCGTC	[Lys16,Phe21]ShK-amide	0.246	3.300	13.415	1.474	2.041	34.28	39.25
273	RSCIDTIPKSRCTAFKCKHSIKYRLSFCRKTCGTC	[Lys16,Ile21]ShK-amide	0.140	33.300	**237.857**	2.494	5.772	58.00	111.00
274	RSCIDTIPKSRCTAFKCKHSDKYRLSFCRKTCGTC	[Lys16,Asp21]ShK-amide	0.350	33.300	**95.143**	2.305	3.634	53.60	69.88

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Abbreviations

Abbreviations used throughout this specification are as defined below, unless otherwise defined in specific circumstances.

Ac	acetyl (used to refer to acetylated residues)
AcBpa	acetylated p-benzoyl-L-phenylalanine
ACN	acetonitrile
AcOH	acetic acid
ADCC	antibody-dependent cellular cytotoxicity
Aib	aminoisobutyric acid
bA	beta-alanine
Bpa	p-benzoyl-L-phenylalanine
BrAc	bromoacetyl ($BrCH_2C(O)$
BSA	Bovine serum albumin
Bzl	Benzyl
Cap	Caproic acid
CBC	complete blood count
COPD	Chronic obstructive pulmonary disease
CTL	Cytotoxic T lymphocytes
DCC	Dicylcohexylcarbodiimide
Dde	1-(4,4-dimethyl-2,6-dioxo-cyclohexylidene)ethyl
DNP	2,4-dinitrophenol
DOPC	1,2-Dioleoyl-sn-Glycero-3-phosphocholine
DOPE	1,2-Dioleoyl-sn-Glycero-3-phosphoethanolamine
DPPC	1,2-Dipalmitoyl-sn-Glycero-3-phosphocholine
DSPC	1,2-Distearoyl-sn-Glycero-3-phosphocholine
DTT	Dithiothreitol
EAE	experimental autoimmune encephalomyelitis
ECL	enhanced chemiluminescence
ESI-MS	Electron spray ionization mass spectrometry
FACS	fluorescence-activated cell sorting
Fmoc	fluorenylmethoxycarbonyl
HOBt	1-Hydroxybenzotriazole
HPLC	high performance liquid chromatography
HSL	homoserine lactone
IB	inclusion bodies
KCa	calcium-activated potassium channel (including IKCa, BKCa, SKCa)

KLH	Keyhole Limpet Hemocyanin
Kv	voltage-gated potassium channel
Lau	Lauric acid
LPS	lipopolysaccharide
LYMPH	lymphocytes
MALDI-MS	Matrix-assisted laser desorption ionization mass spectrometry
Me	methyl
MeO	methoxy
MeOH	methanol
MHC	major histocompatibility complex
MMP	matrix metalloproteinase
MW	Molecular Weight
MWCO	Molecular Weight Cut Off
1-Nap	1-napthylalanine
NEUT	neutrophils
Nle	norleucine
NMP	N-methyl-2-pyrrolidinone
OAc	acetate
PAGE	polyacrylamide gel electrophoresis
PBMC	peripheral blood mononuclear cell
PBS	Phosphate-buffered saline
Pbf	2,2,4,6,7-pendamethyldihydrobenzofuran-5-sulfonyl
PCR	polymerase chain reaction
PD	pharmacodynamic
Pec	pipecolic acid
PEG	Poly(ethylene glycol)
pGlu	pyroglutamic acid
Pic	picolinic acid
PK	pharmacokinetic
pY	phosphotyrosine
RBS	ribosome binding site
RT	room temperature (25 °C)
Sar	sarcosine
SDS	sodium dodecyl sulfate
STK	serine-threonine kinases
t-Boc	tert-Butoxycarbonyl
tBu	tert-Butyl

TCR	T cell receptor
TFA	trifluoroacetic acid
THF	thymic humoral factor
Trt	trityl

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CLAIMS

1. A composition of matter comprising an amino acid sequence of the formula:

$X_{aa}^{1}X_{aa}^{2}Cys^{3}X_{aa}^{4}Asp^{5}X_{aa}^{6}X_{aa}^{7}X_{aa}^{8}X_{aa}^{9}X_{aa}^{10}X_{aa}^{11}Cys^{12}X_{aa}^{13}X_{aa}^{14}X_{aa}^{15}X_{aa}^{16}Cys^{17}$
$X_{aa}^{18}X_{aa}^{19}X_{aa}^{20}X_{aa}^{21}X_{aa}^{22}X_{aa}^{23}X_{aa}^{24}X_{aa}^{25}X_{aa}^{26}X_{aa}^{27}Cys^{28}X_{aa}^{29}X_{aa}^{30}$
$X_{aa}^{31}Cys^{32}X_{aa}^{33}X_{aa}^{34}Cys^{35}X_{aa}^{36}X_{aa}^{37}X_{aa}^{38}$//SEQ ID NO:4

or a pharmaceutically acceptable salt thereof,

wherein:

$X_{aa}^{1}X_{aa}^{2}$ is absent; or X_{aa}^{1} is absent and X_{aa}^{2} is Glu, Ser, Ala, or Thr; or X_{aa}^{1} is Arg or Ala and X_{aa}^{2} is Glu, Ser, Ala, or Thr;

X_{aa}^{4} is an alkyl, basic, or acidic amino acid residue;

X_{aa}^{6} is Thr, Tyr, Ala, or Leu;

X_{aa}^{7} is Leu, Ile, Ala, or Lys;

X_{aa}^{8} is Pro, Ala, Arg, Lys, 1-Nal, or Glu;

X_{aa}^{9} is Lys, Ala, Val or an acidic amino acid residue selected from Glu, Asp, and α-aminoadipic acid;

X_{aa}^{10} is Ser, Glu, Arg, or Ala;

X_{aa}^{11} is Arg, Glu; or Ala;

X_{aa}^{13} is Thr, Ala, Arg, Lys, 1-Nal, or Glu;

X_{aa}^{14} is Gln, Ala or an acidic amino acid residue selected from Glu, Asp, and α-aminoadipic acid;

X_{aa}^{15} is an alkyl or aromatic amino acid residue;

X_{aa}^{16} is a basic, alkyl, or aromatic amino acid residue, other than Ala, Gln, Glu or Arg;

X_{aa}^{18} is Ala or an acidic or basic amino acid residue;

X_{aa}^{19} is Thr, Ala or a basic amino acid residue selected from Lys, Arg, His, Orn, D-Orn, Dab, Dap, 1Pip, 2Pal, 3Pal, N-Me-Lys, Nα Methyl-Arg; homoarginine, Cit, Nα-Methyl-Cit, Homocitrulline, Guf, and 4-Amino-Phe, and N-Me-Orn;

X_{aa}^{20} is Ser, Ala, or a basic amino acid residue selected from Lys, Arg, His, Orn, D-Orn, Dab, Dap, 1Pip, 2Pal, 3Pal, N-Me-Lys, Nα Methyl-Arg; homoarginine, Cit, Nα-Methyl-Cit, Homocitrulline, Guf, and 4-Amino-Phe, and N-Me-Orn;

X_{aa}^{21} is an alkyl or aromatic amino acid residue, other than Ala or Met;

X_{aa}^{22} is Lys or Ala;

X_{aa}^{23} is Tyr or Ala;

X_{aa}^{24} is Arg, Lys, or Ala;

X_{aa}^{25} is Tyr, Leu, or Ala;

X_{aa}^{26} is Ser, Thr, Asn, Ala, or an aromatic amino acid residue selected from 1-Nal, 2-Nal, Phe, Trp, and Tyr;

X_{aa}^{27} is Leu, Ala, Asn, or an aromatic amino acid residue selected from 1-Nal, 2-Nal, Phe, Trp, and Tyr;

X_{aa}^{29} is 1-Nal, 2-Nal, Ala, or a basic amino acid residue selected from Lys, Arg, His, Orn, D-Orn, Dab, Dap, 1Pip, 2Pal, 3Pal, N-Me-Lys, Nα Methyl-Arg; homoarginine, Cit, Nα-Methyl-Cit, Homocitrulline, Guf, and 4-Amino-Phe, and N-Me-Orn;

X_{aa}^{30} is Ala or an acidic or basic amino acid residue;

X_{aa}^{31} is Thr, Ala, or an aromatic amino acid residue selected from 1-Nal, 2-Nal, Phe, Trp, and Tyr;

X_{aa}^{33} is Gly, Ala, Arg, Lys, 1-Nal, or Glu;

X_{aa}^{34} is Thr, Ser, Ala, Lys, or an aromatic amino acid residue selected from 1-Nal, 2-Nal, Phe, Trp, and Tyr;

each of X_{aa}^{36}, X_{aa}^{37}, and X_{aa}^{38} is independently absent or is independently a neutral, basic, acidic, or N-alkylated amino acid residue;

and wherein:

there is a disulfide bond between residue Cys^{3} and residue Cys^{35},

there is a disulfide bond between residue Cys^{12} and residue Cys^{28};

there is a disulfide bond between residue Cys^{17} and residue Cys^{32}; and

the carboxy-terminal residue is optionally amidated.

2. The composition of matter of Claim 1 wherein X_{aa}^{4} is selected from Ser, Thr, Ala, Gly, Leu, Ile, Val, Met, Cit, Homocitrulline, Oic, Pro, Hyp, Tic, D-Tic, D-Pro, Guf, and 4-Amino-Phe,Thz, Aib, Sar, Pip, Bip, Phe, Tyr, Lys, His, Trp, Arg, N^{α} Methyl-Arg; homoarginine, 1-Nal, 2-Nal, Orn, D-Orn, Asn, Gln, Glu, Asp, α-aminoadipic acid, and *para*-carboxyl-phenylalanine.

3. The composition of matter of Claim 1, wherein X_{aa}^{4} is selected from Ala, Ile, Lys, Orn, Glu and Asp.

4. The composition of matter of Claim 1, wherein the alkyl or aromatic amino acid residue of X_{aa}^{15} is selected from Ala, 1-Nal, 2-Nal, Phe, Tyr, Val, Ile, and Leu.

5. The composition of matter of Claim 1, wherein the alkyl or aromatic amino acid residue of X_{aa}^{15} is selected from Phe, Ala, and Ile.

6. The composition of matter of Claim 1, wherein X_{aa}^{16} is selected from Lys, Orn, Dab, Dap 1-Nal, 2-Nal, Tyr, Phe, Pip, 2Pal, 3Pal, N-Me-Lys, N-Me-Orn, alpha-methyl-lysine, Lys(N^{ϵ}-Me), Lys(N^{ϵ}-Me)$_2$, Lys(N^{ϵ}-Me)$_3$, para-Methyl-Phe, AMeF, and homoPhe.

7. The composition of matter of Claim 1, wherein the basic or acidic amino acid residue of X_{aa}^{18} and X_{aa}^{30} is each independently selected from Lys, Arg, Orn, Glu, Asp, His, Trp, and Pac.

8. The composition of matter of Claim 1, wherein X_{aa}^{21} is selected from Nle, Nva, Abu, Phe,Tyr, Asn, Gln, Met[O], Val, Ile, Leu, Met[O_2], Cha, Chg, Asn, Trp, para-Methyl-Phe, alpha-methyl-Phe, and homoPhe.

9. The composition of matter of Claim 1, wherein X_{aa}^{36}, X_{aa}^{37}, and X_{aa}^{38}, if present, is each independently selected from Ala, Leu, Lys, Glu, Asp, Phe, Arg, Phe, Asp-amide, Aib-amide, Ser-amide, Tyr, Thr-amide, Glu, Glu-amide, beta-Ala, and N-Me-Ala.

10. The composition of matter of Claim 1, wherein the carboxy-terminal residue is amidated.

11. The composition of matter of Claim 1, wherein X_{aa}^{36} is present.

12. The composition of matter of Claim 1, comprising an amino acid sequence selected from SEQ ID NOS: 15, 155, 164 through 172, 179, 186, 188, 190, 194, 195, 196, 198, 199, 200, 201, 202, 203 through 206, 210, 211, 212, 214 through 225, 231, 232, 233, 236, 238, 239, 242 through 254, 260, 263, and 265 through 273.

13. The composition of matter of Claim 1, wherein:

X_{aa}^{4} is Ala, Ile, Lys, or Glu; and

X_{aa}^{16} is Lys, Orn, Dab, Dap 1-Nal, 2-Nal, Tyr, Phe, Pip, 2Pal, 3Pal, N-Me-Lys, or N-Me-Orn, alpha-methyl-lysine, Lys(N^{ϵ}-Me), Lys(N^{ϵ}-Me)$_2$, Lys(N^{ϵ}-Me)$_3$, para-Methyl-Phe, alpha-methyl-Phe, or homoPhe.

14. The composition of matter of Claim 13, wherein:

X_{aa}^{18} is Lys, Arg, Orn, Glu, Asp, His, Trp, Pac, or Ala.

15. The composition of matter of Claim 14, wherein:

X_{aa}^{20} is Ser, Ala, Lys, Arg, Orn, Dab, Dap, 1Pip, 2Pal, 3Pal, N-Me-Lys, or N-Me-Orn.

16. The composition of matter of Claim 15, wherein:

X_{aa}^{29} is 1-Nal, 2-Nal, Ala, Lys, Arg, Orn, Dab, Dap, 1Pip, 2Pal, 3Pal, N-Me-Lys, N-Me-Orn.

17. The composition of matter of Claim 16, wherein:

X_{aa}^{30} is selected from Lys, Arg, Orn, Glu, Asp, His, Trp, or Pac.

18. The composition of matter of any one of Claims 1 to 17, further comprising an optional linker moiety and a pharmaceutically acceptable, covalently linked half-life extending moiety.

19. The composition of matter of any one of Claims 1 to 17, wherein the composition of matter comprises a toxin peptide analog 33 to about 100 amino acid residues long.

20. The composition of matter of Claim 18, wherein the composition of matter comprises a toxin peptide analog 33 to about 100 amino acid residues long.

21. The composition of matter of Claim 18 or Claim 20, wherein the half-life extending moiety is polyethylene glycol of molecular weight of about 1000 Da to about 100000 Da, an IgG Fc domain, a transthyretin, or a human serum albumin.

22. The composition of matter of Claim 18 or Claim 20, wherein the half-life extending moiety comprises a human immunoglobulin or a human immunoglobulin Fc domain, or both.

23. The composition of matter of Claim 22 having a configuration as set forth in any of Figures 12A-N.

24. The composition of matter of Claim 22, wherein the composition comprises a monovalent heterodimeric Fc-toxin peptide analog fusion.

25. A pharmaceutical composition, comprising the composition of matter of any one of Claims 1 to 24, and a pharmaceutically acceptable carrier.

26. A pharmaceutical composition, comprising the composition of matter of Claim 18 and a pharmaceutically acceptable carrier.

27. A method of preventing or mitigating a relapse of at least one symptom of multiple sclerosis, comprising administering a prophylactically effective amount of the composition of matter of any one of Claims 1 to 24, or the pharmaceutical composition of Claim 25 or Claim 26.

28. A method of treating an autoimmune disorder, comprising administering a therapeutically effective amount of the composition of matter of any one of Claims 1 to 24, or the pharmaceutical composition of Claim 25 or Claim 26.

29. The method of Claim 28, wherein the autoimmune disorder is selected from the group consisting of multiple sclerosis, Type 1 diabetes, psoriasis, inflammatory bowel disease, contact-mediated dermatitis, rheumatoid arthritis, psoriatic arthritis, asthma, allergy, restinosis, systemic sclerosis, fibrosis, scleroderma, glomerulonephritis, Sjogren syndrome, inflammatory bone resorption, transplant rejection, graft-versus-host disease, and lupus.

30. A method of preventing or mitigating a relapse of at least one symptom of multiple sclerosis, comprising administering a prophylactically effective amount of the composition of matter of Claim 18, or the pharmaceutical composition of Claim 26.

31. A method of treating an autoimmune disorder, comprising administering a therapeutically effective amount of the composition of matter of Claim 18, or the pharmaceutical composition of Claim 26.

32. The method of Claim 31, wherein the autoimmune disorder is selected from the group consisting of multiple sclerosis, Type 1 diabetes, psoriasis, inflammatory bowel disease, contact-mediated dermatitis, rheumatoid arthritis, psoriatic arthritis, asthma, allergy, restinosis, systemic sclerosis, fibrosis, scleroderma, glomerulonephritis, Sjogren syndrome, inflammatory bone resorption, transplant rejection, graft-versus-host disease, and lupus.

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33. Use of the composition of matter of any one of Claims 1 to 24 in the preparation of a medicament for:

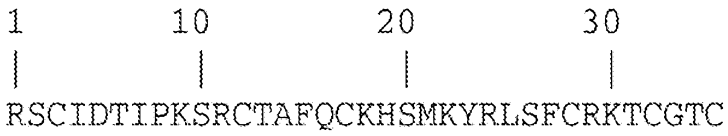
preventing or mitigating a relapse of at least one symptom of multiple sclerosis; or treating an autoimmune disorder.

34. Use of the composition of matter of Claim 18 in the preparation of a medicament for:

preventing or mitigating a relapse of at least one symptom of multiple sclerosis; or treating an autoimmune disorder.

35. A composition of matter of the invention; a pharmaceutical composition of the invention; a method of preventing or mitigating a relapse of at least one symptom of multiple sclerosis; a method of treating an autoimmune disease or use of a composition of matter of the invention, substantially as herein described with reference to any one or more of the examples but excluding comparative examples.

FIG. 1A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20
30
RSCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC

FIG. 1B

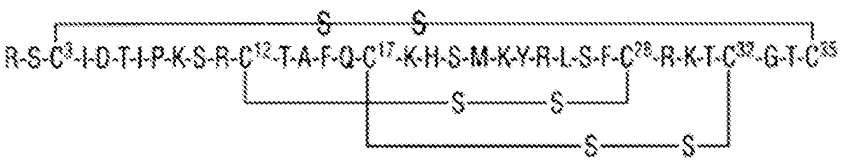

R-S-C-I-D-T-I-P-K-S-R-C-T-A-F-Q-C-K-H-S-M-K-Y-R-L-S-F-C-R-K-T-C-G-T-C
S
S
S
S
S
S

FIG. 1C

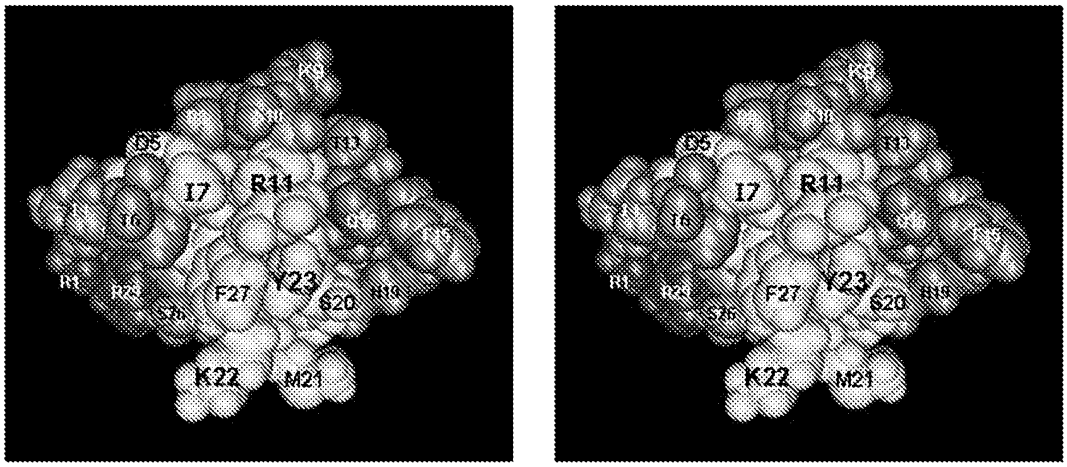

I7
R11
Y23
S20
K22
M21
F27
I7
R11
Y23
S20
K22
M21
F27

FIG. 1D

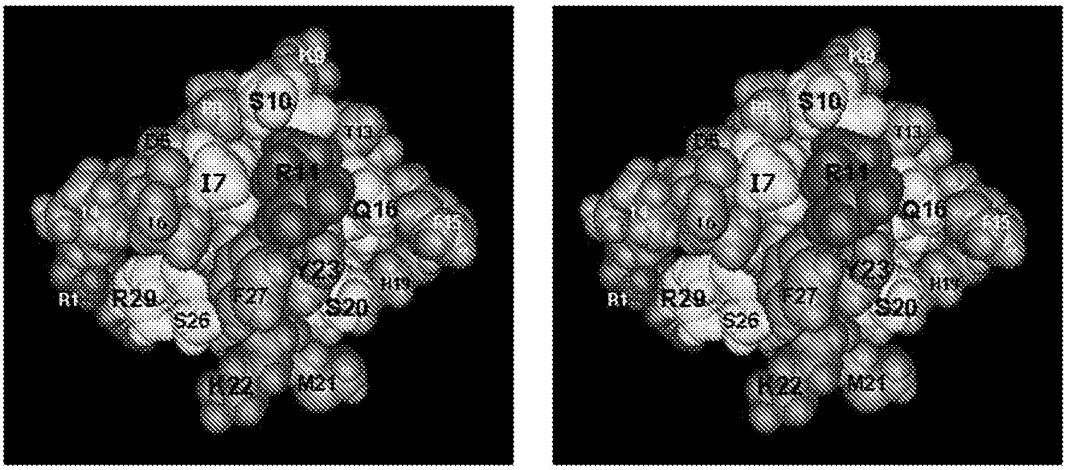

S10
I7
R11
Q16
Y23
R29
S26
F27
S20
K22
M21
S10
I7
R11
Q16
Y23
R29
S26
F27
S20
K22
M21

FIG. 2A

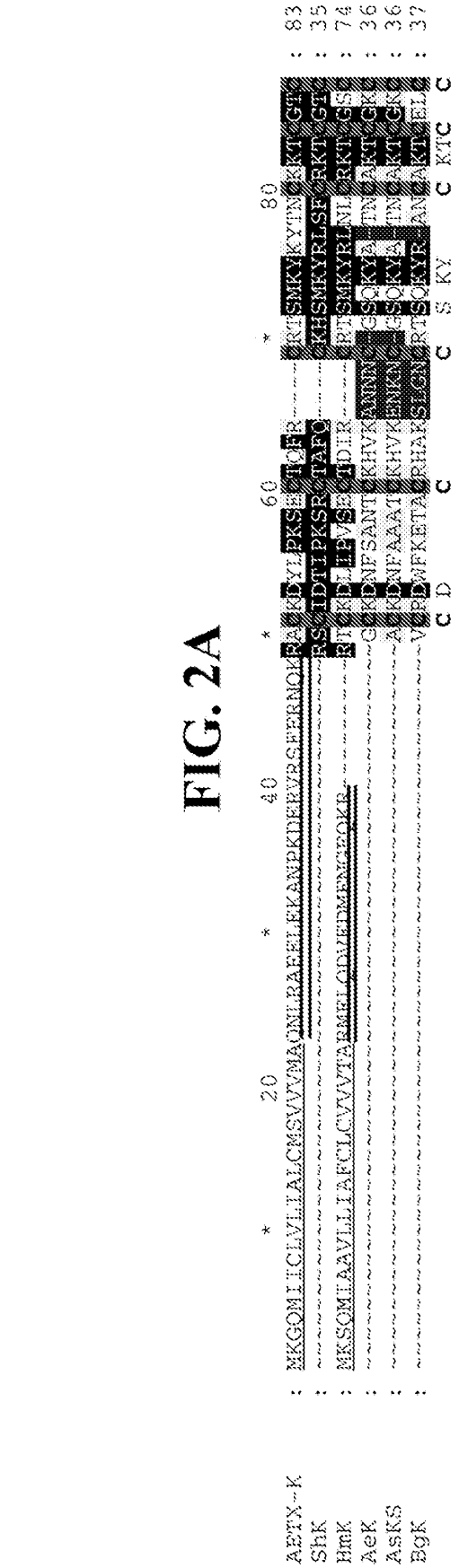


FIG. 2B

Disulfide linkage diagram

Disulfides

3

Disulfide Bonding

C1-C6, C2-C4, C3-C5

FIG. 3

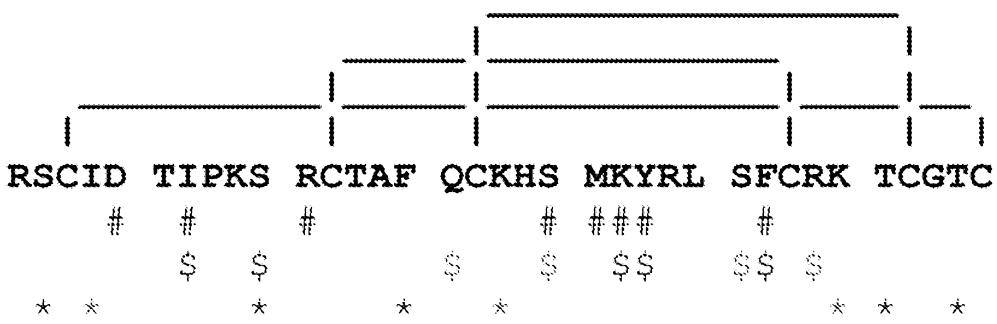


FIG. 4

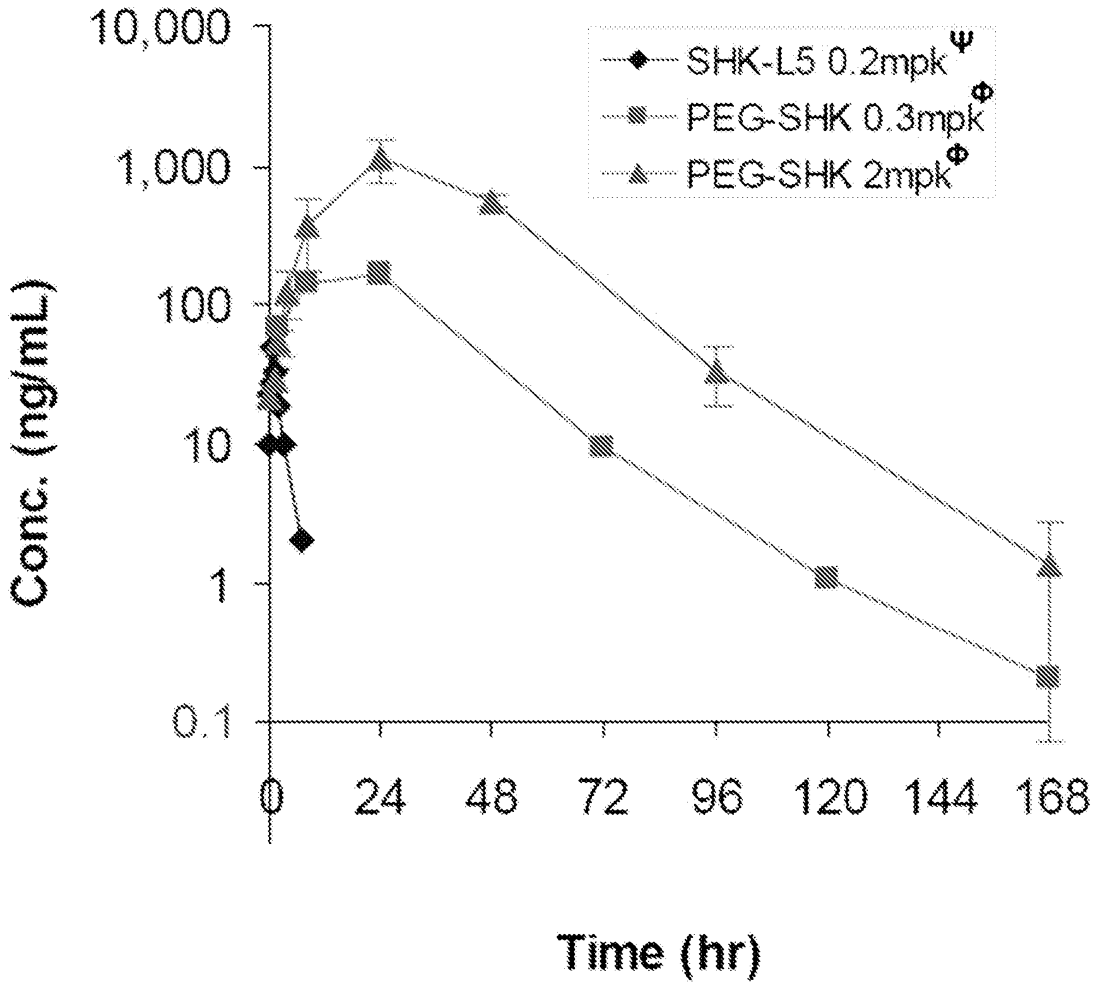

10,000
1,000
100
10
1
0.1
Conc. (ng/mL)
0
24
48
72
96
120
144
168
Time (hr)
SHK-L5 0.2mpk Ψ
PEG-SHK 0.3mpk Φ
PEG-SHK 2mpk Φ

FIG. 5A

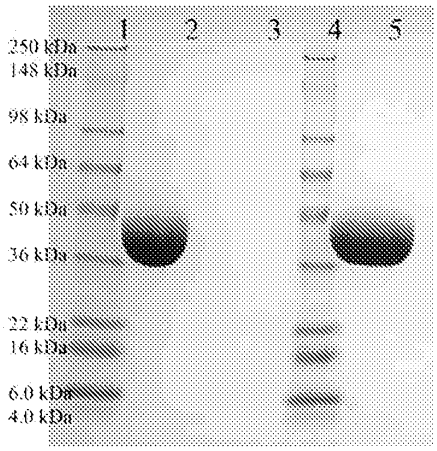

1
2
3
4
5
250 kDa
148 kDa
98 kDa
64 kDa
50 kDa
36 kDa
22 kDa
16 kDa
6.0 kDa
4.0 kDa

FIG. 5B

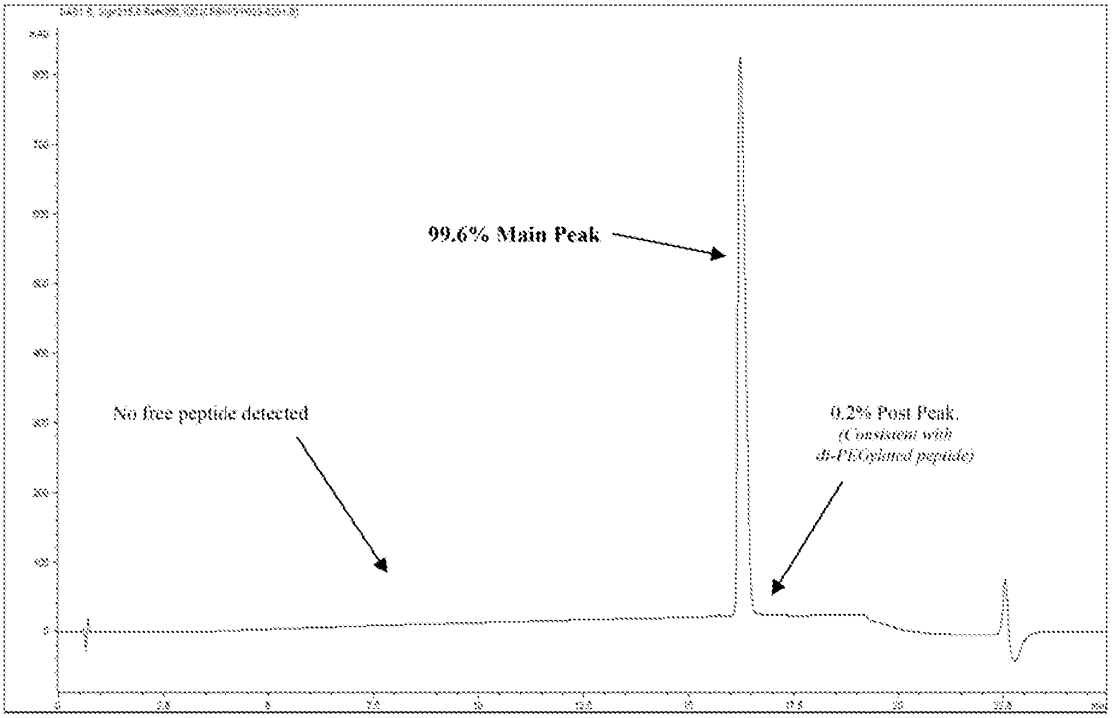

99.6% Main Peak
No free peptide detected
0.2% Post Peak.
(Consistent with
di-PEGylated peptide)

FIG. 5C

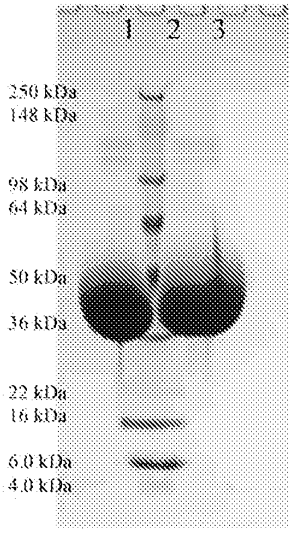

1 2 3
250 kDa
148 kDa
98 kDa
64 kDa
50 kDa
36 kDa
22 kDa
16 kDa
6.0 kDa
4.0 kDa

FIG. 5D

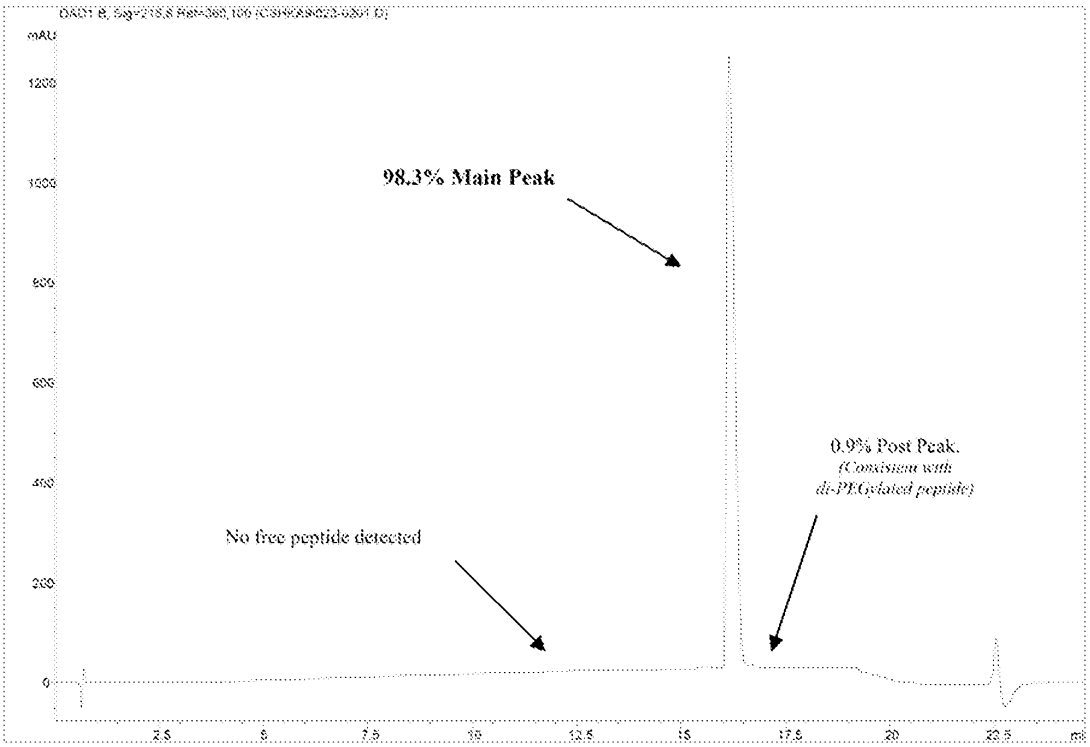
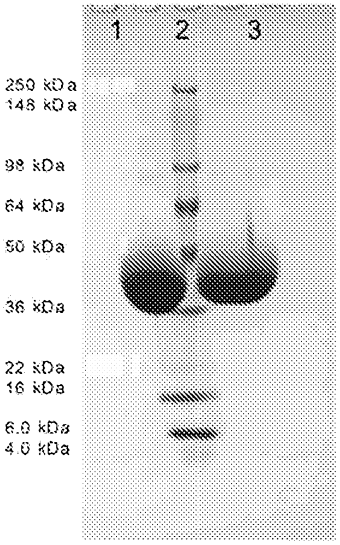

mAU
98.3% Main Peak
0.9% Post Peak.
(Consistent with
di-PEGylated peptide)
No free peptide detected
2.5
5
7.5
10
12.5
15
17.5
20
22.5
min

FIG. 5E

SDS-PAGE of Nα-(20 kDa PEG)-[Lys16, Ala36]ShK:
2.0µg load on a 4 – 20% Tris-Glycine gel



Lane	Sample Loading	
1.	20k PEG [Lys16]ShK-Ala NR	2 µg
2.	SeeBlue +2 M.W.Std	10 µL
3.	20k PEG [Lys16]ShK-Ala Red	2 µg

FIG. 5F

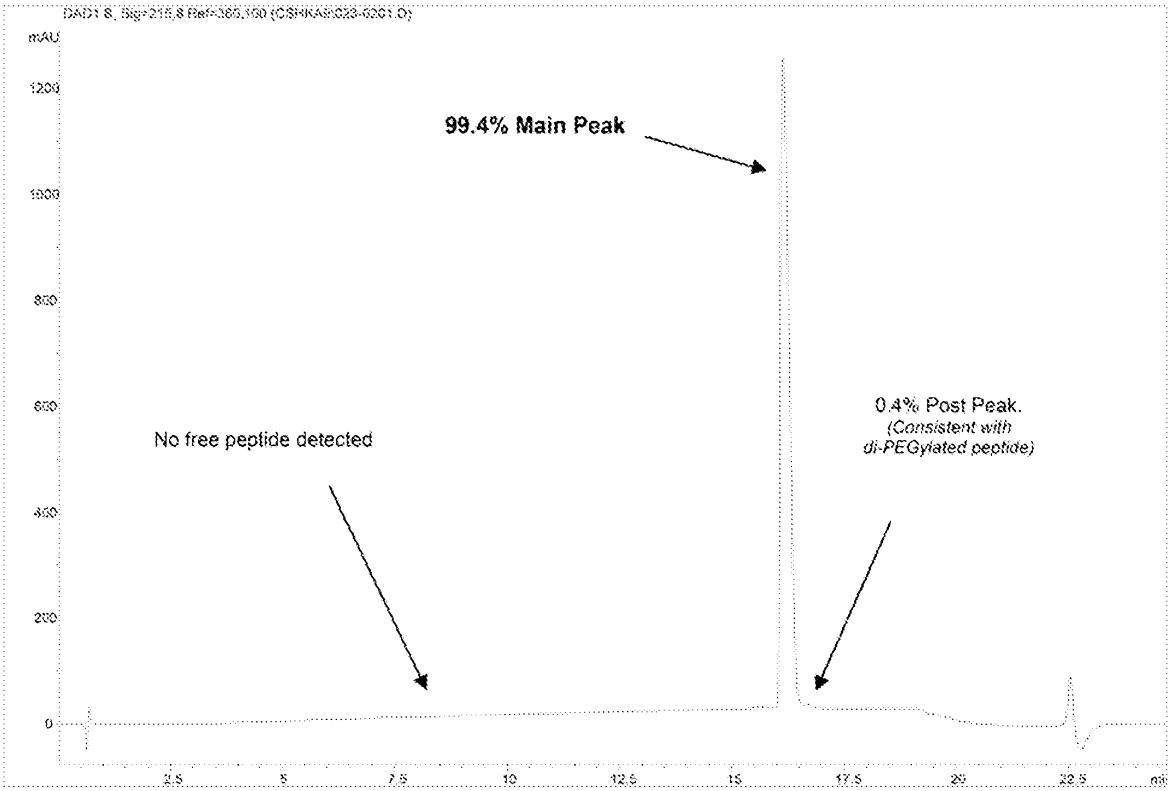

mAU
1200
1000
800
600
400
200
0
99.4% Main Peak
No free peptide detected
0.4% Post Peak.
(Consistent with
di-PEGylated peptide)
2.5
5
7.5
10
12.5
15
17.5
20
22.5

FIG. 6A

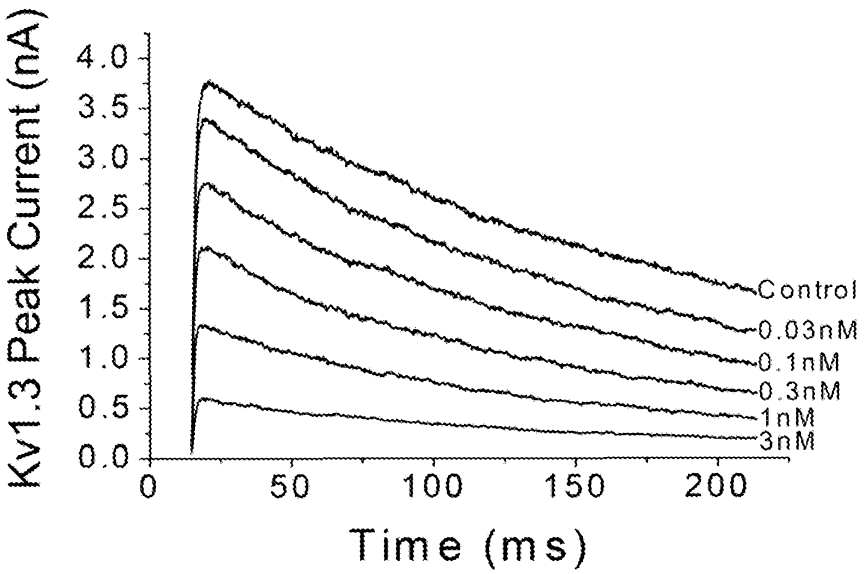

Kv1.3 Peak Current (nA)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
50
100
150
200
Time (ms)
Control
0.03nM
0.1nM
0.3nM
1nM
3nM

FIG. 6B

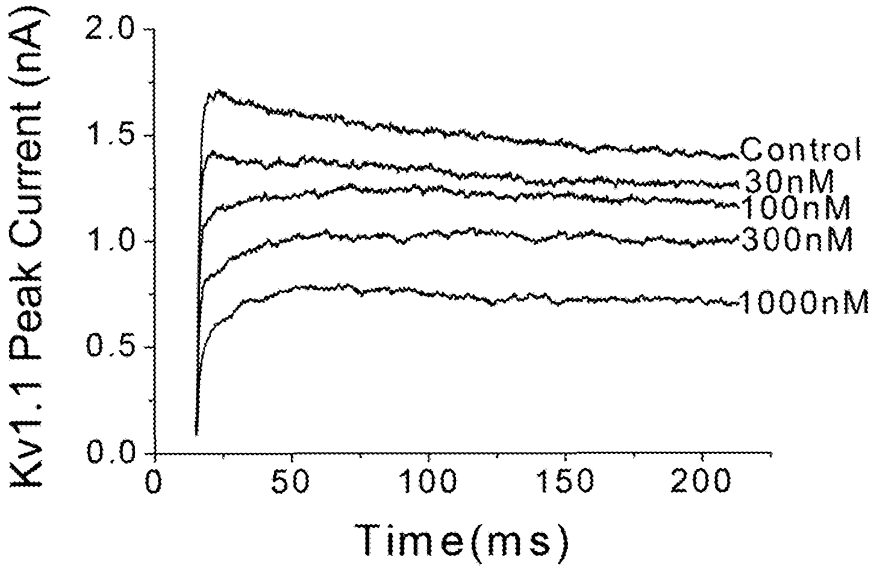

Kv1.1 Peak Current (nA)
2.0
1.5
1.0
0.5
0.0
0
50
100
150
200
Time(ms)
Control
30nM
100nM
300nM
1000nM

FIG. 6C

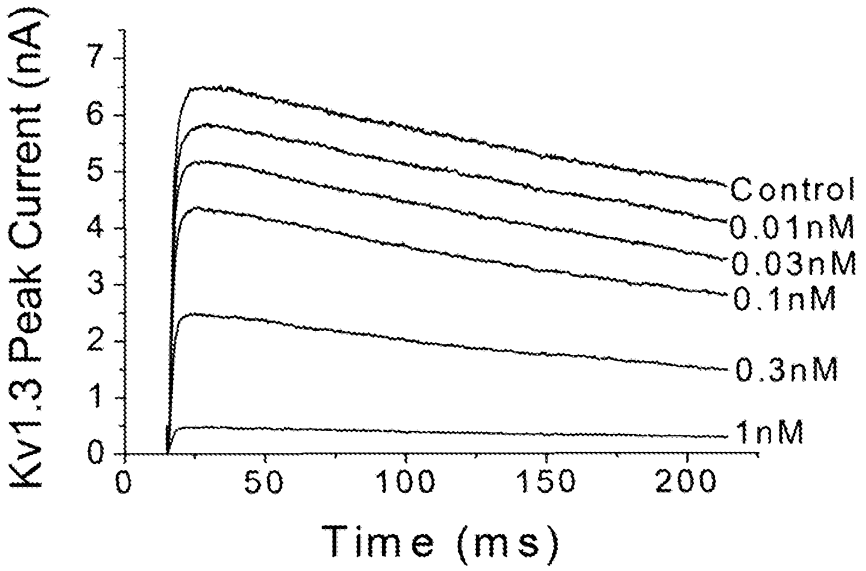

Kv1.3 Peak Current (nA)
0
1
2
3
4
5
6
7
0
50
100
150
200
Time (ms)
Control
0.01nM
0.03nM
0.1nM
0.3nM
1nM

FIG. 6D

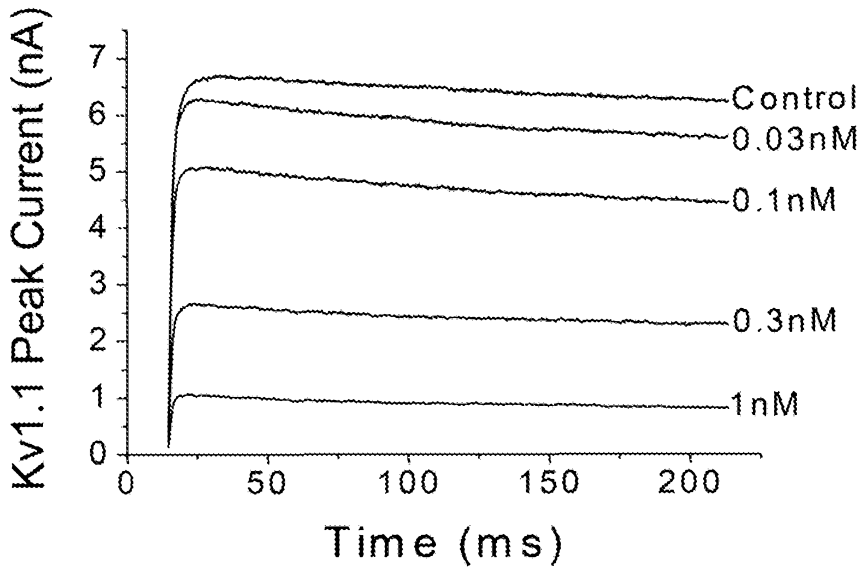

Kv1.1 Peak Current (nA)
0
1
2
3
4
5
6
7
0
50
100
150
200
Time (ms)
Control
0.03nM
0.1nM
0.3nM
1nM

FIG. 6E

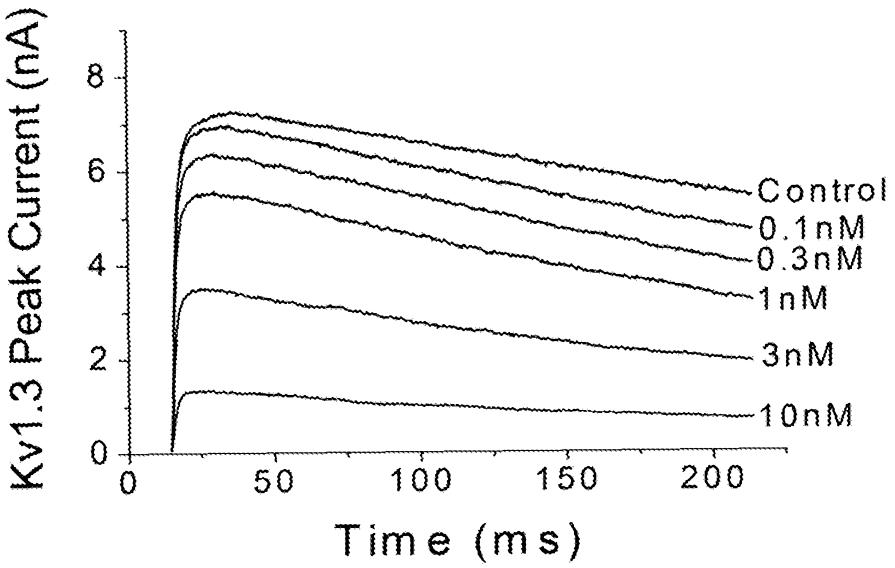

Kv1.3 Peak Current (nA)
8
6
4
2
0
0
50
100
150
200
Time (ms)
Control
0.1nM
0.3nM
1nM
3nM
10nM

FIG. 6F

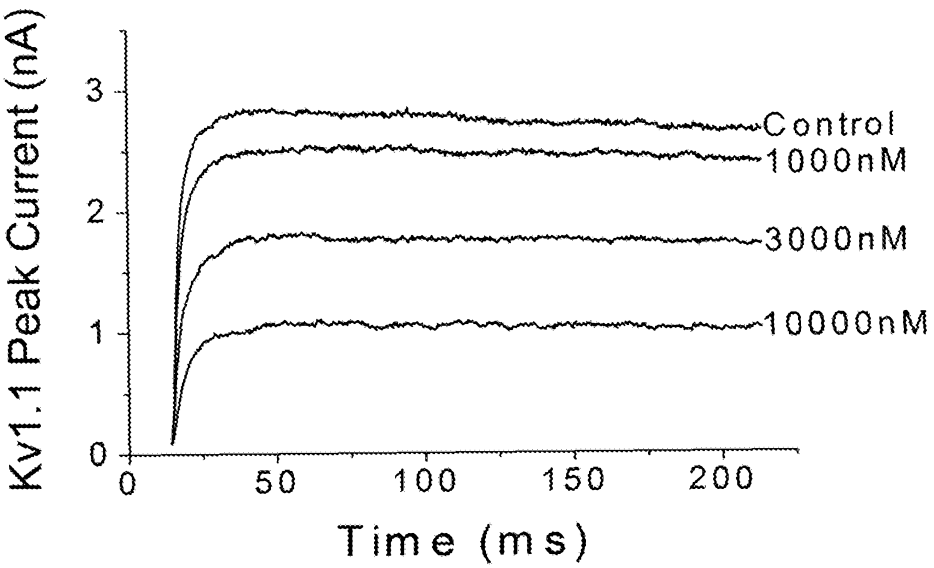

Kv1.1 Peak Current (nA)
3
2
1
0
0
50
100
150
200
Time (ms)
Control
1000nM
3000nM
10000nM

FIG. 7

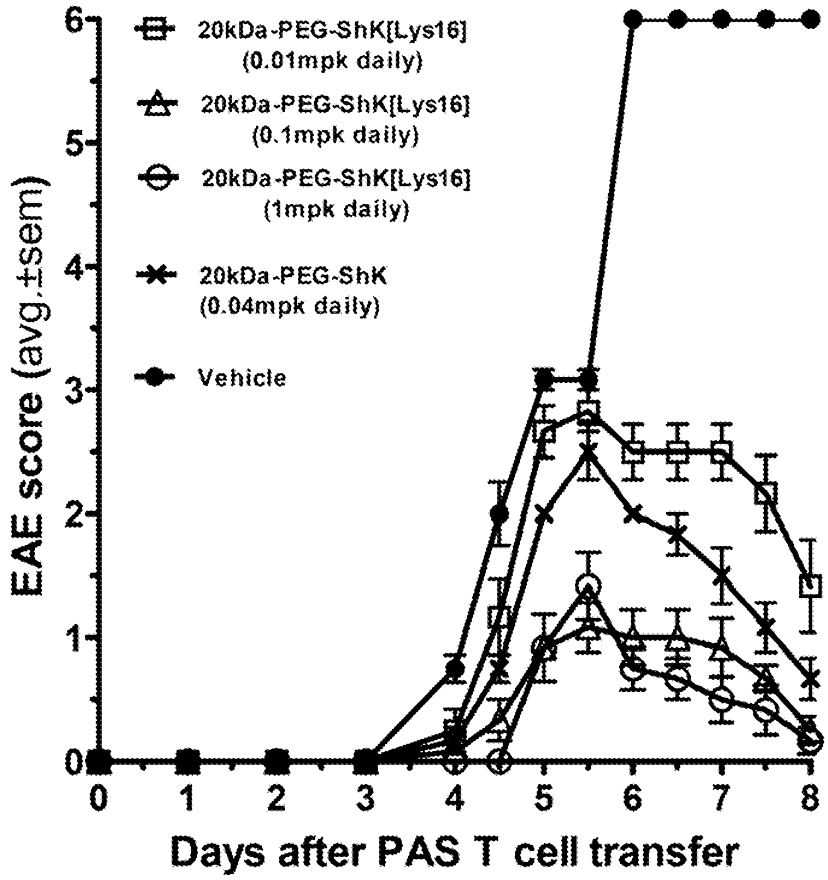

20kDa-PEG-ShK[Lys16]
(0.01mpk daily)
20kDa-PEG-ShK[Lys16]
(0.1mpk daily)
20kDa-PEG-ShK[Lys16]
(1mpk daily)
20kDa-PEG-ShK
(0.04mpk daily)
Vehicle
EAE score (avg.±sem)
Days after PAS T cell transfer
0
1
2
3
4
5
6
7
8

FIG. 8

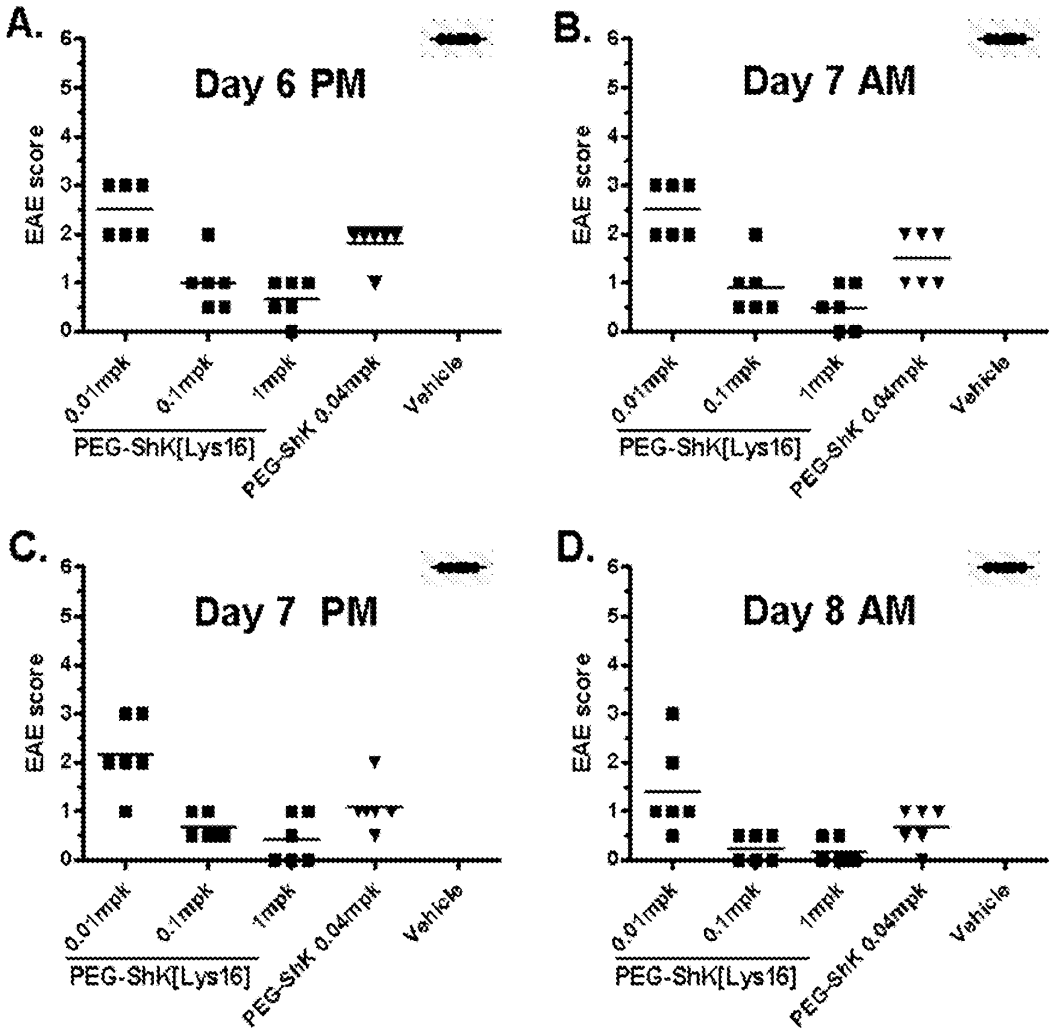

A.
Day 6 PM
B.
Day 7 AM
C.
Day 7 PM
D.
Day 8 AM
EAE score
0
1
2
3
4
5
6
0.01mpk
0.1mpk
1mpk
PEG-ShK 0.04mpk
Vehicle
PEG-ShK[Lys16]

FIG. 9A

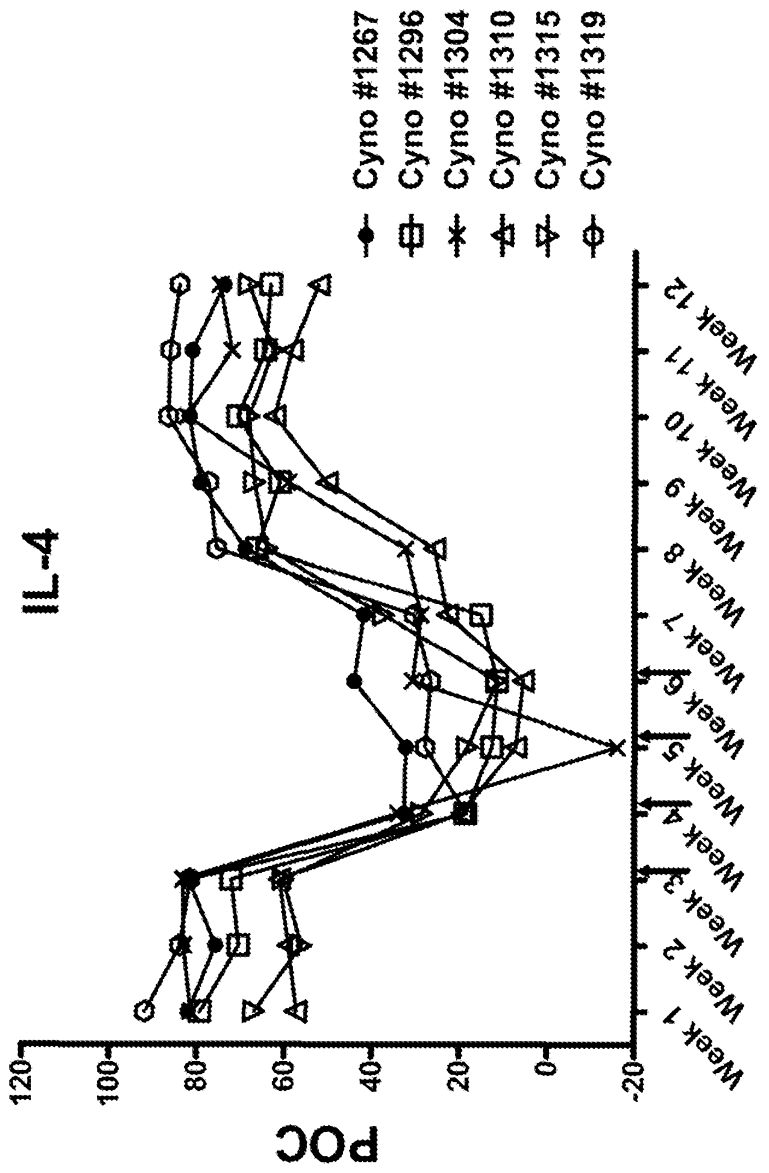

IL-4
pOC
120
100
80
60
40
20
0
-20
Week 1
Week 2
Week 3
Week 4
Week 5
Week 6
Week 7
Week 8
Week 9
Week 10
Week 11
Week 12
Cyno #1267
Cyno #1296
Cyno #1304
Cyno #1310
Cyno #1315
Cyno #1319

FIG. 9B

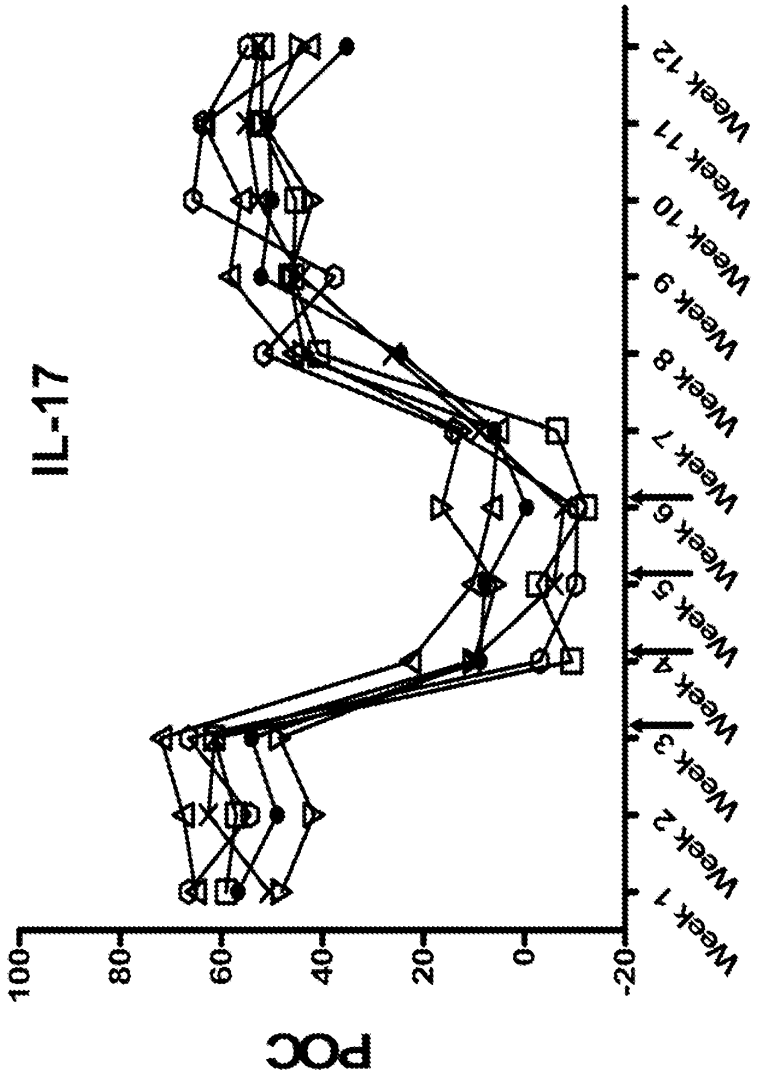

IL-17
PCC
100
80
60
40
20
0
-20
Week 1
Week 2
Week 3
Week 4
Week 5
Week 6
Week 7
Week 8
Week 9
Week 10
Week 11
Week 12

FIG. 9C

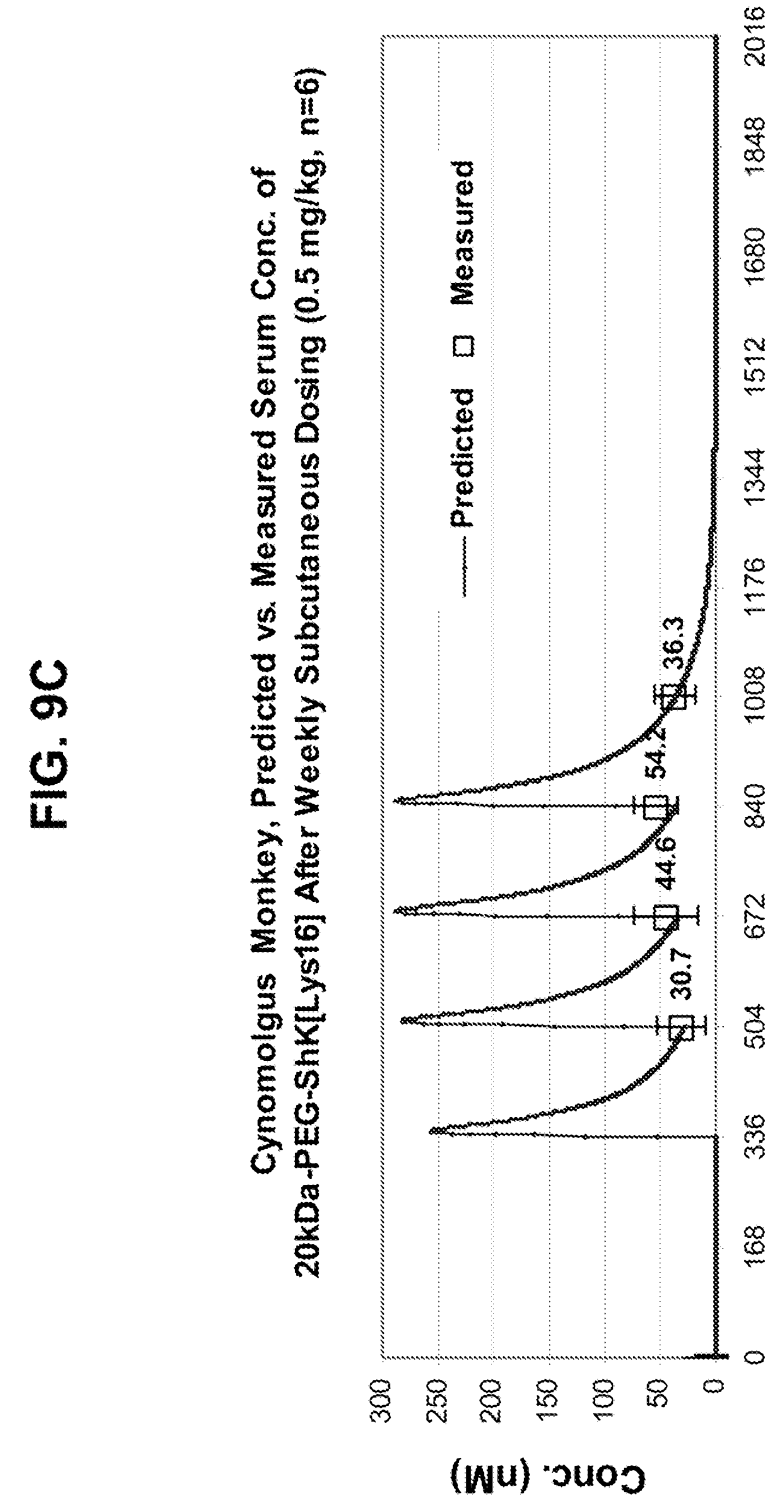

Cynomolgus Monkey, Predicted vs. Measured Serum Conc. of
20kDa-PEG-ShK[Lys16] After Weekly Subcutaneous Dosing (0.5 mg/kg, n=6)
Predicted
Measured
Conc. (nM)
Time (hr)
0
50
100
150
200
250
300
0
168
336
504
672
840
1008
1176
1344
1512
1680
1848
2016
30.7
44.6
54.2
36.3

FIG. 9D

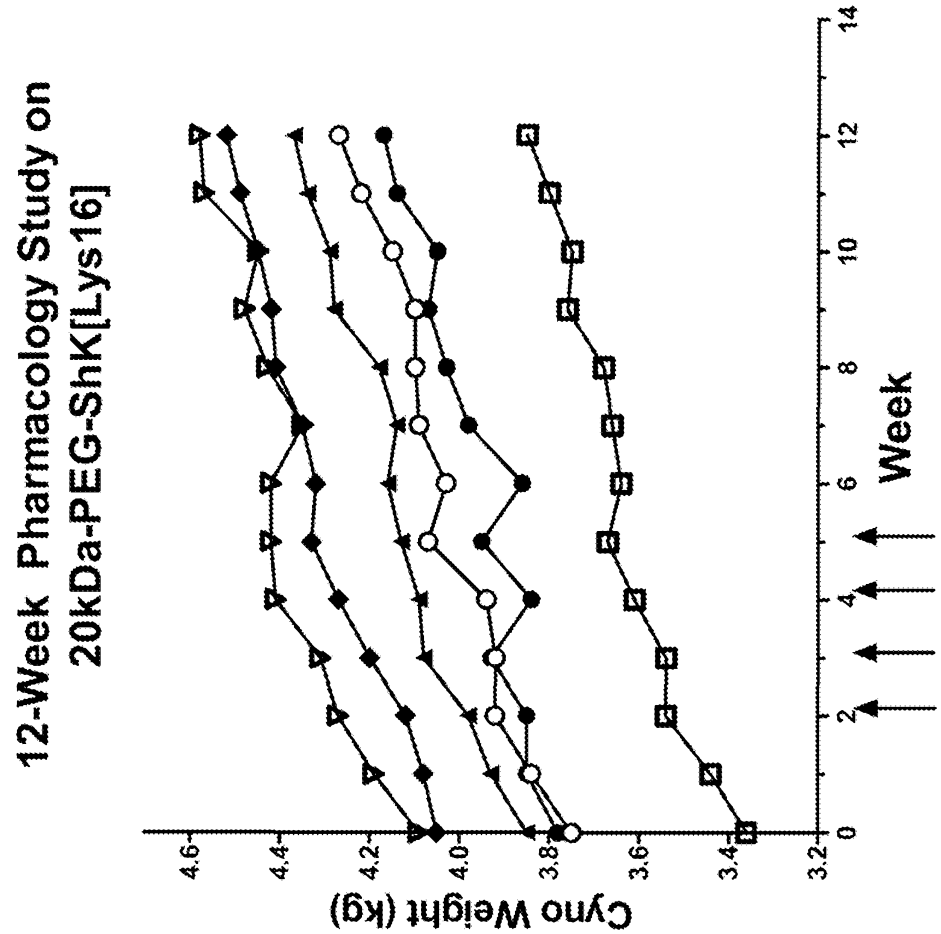

12-Week Pharmacology Study on
20kDa-PEG-ShK[Lys16]
V001304
V001310
V001296
V001315
V001319
V001267
Cyno Weight (kg)
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
0
2
4
6
8
10
12
14
Week

FIG. 10

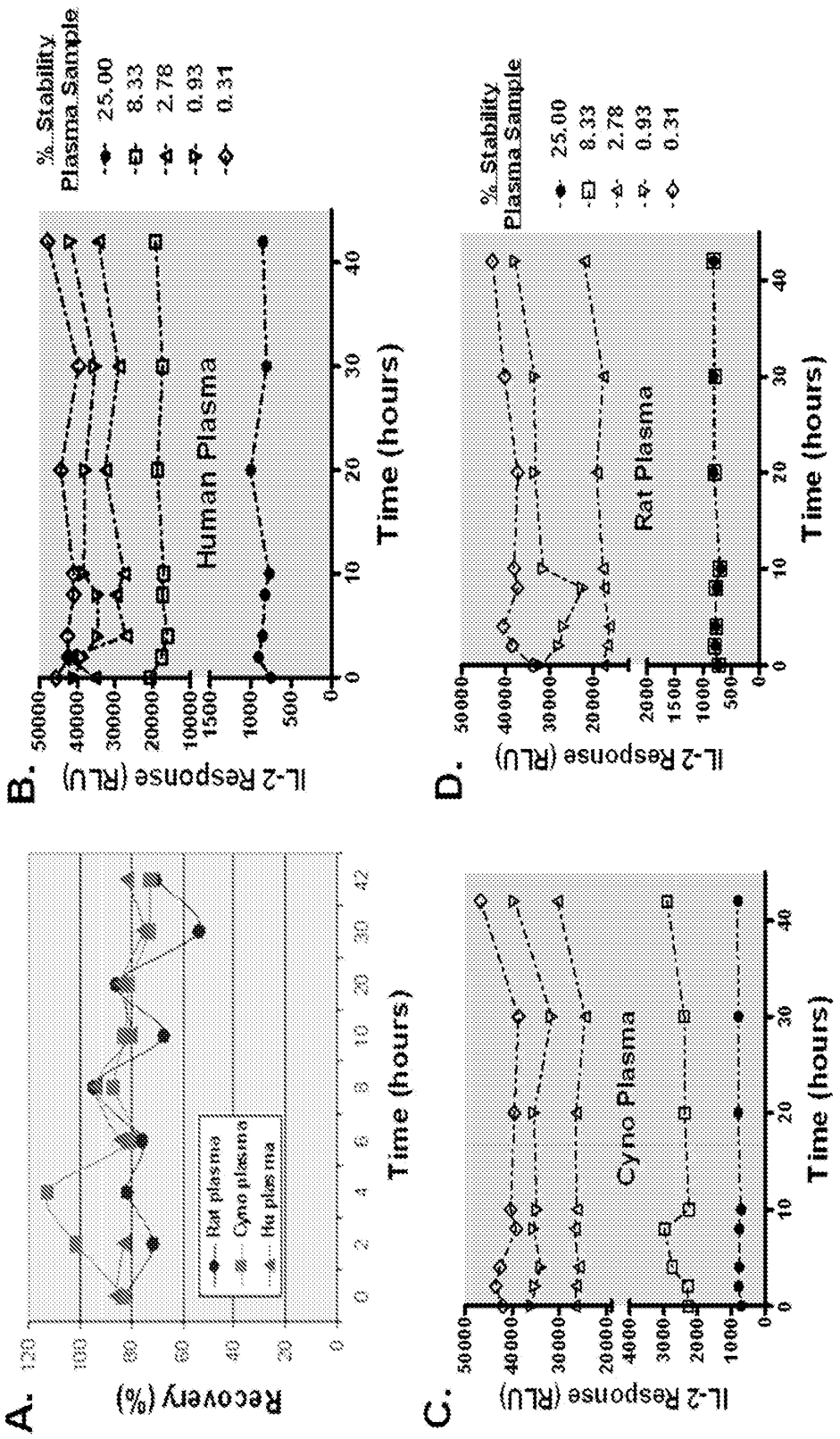

A.
Recovery (%)
Time (hours)
Rat plasma
Cyno plasma
Hu plasma
B.
IL-2 Response (RLU)
Time (hours)
Human Plasma
% Stability
Plasma Sample
25.00
8.33
2.78
0.93
0.31
C.
IL-2 Response (RLU)
Time (hours)
Cyno Plasma
D.
IL-2 Response (RLU)
Time (hours)
Rat Plasma
% Stability
Plasma Sample
25.00
8.33
2.78
0.93
0.31

FIG. 11A

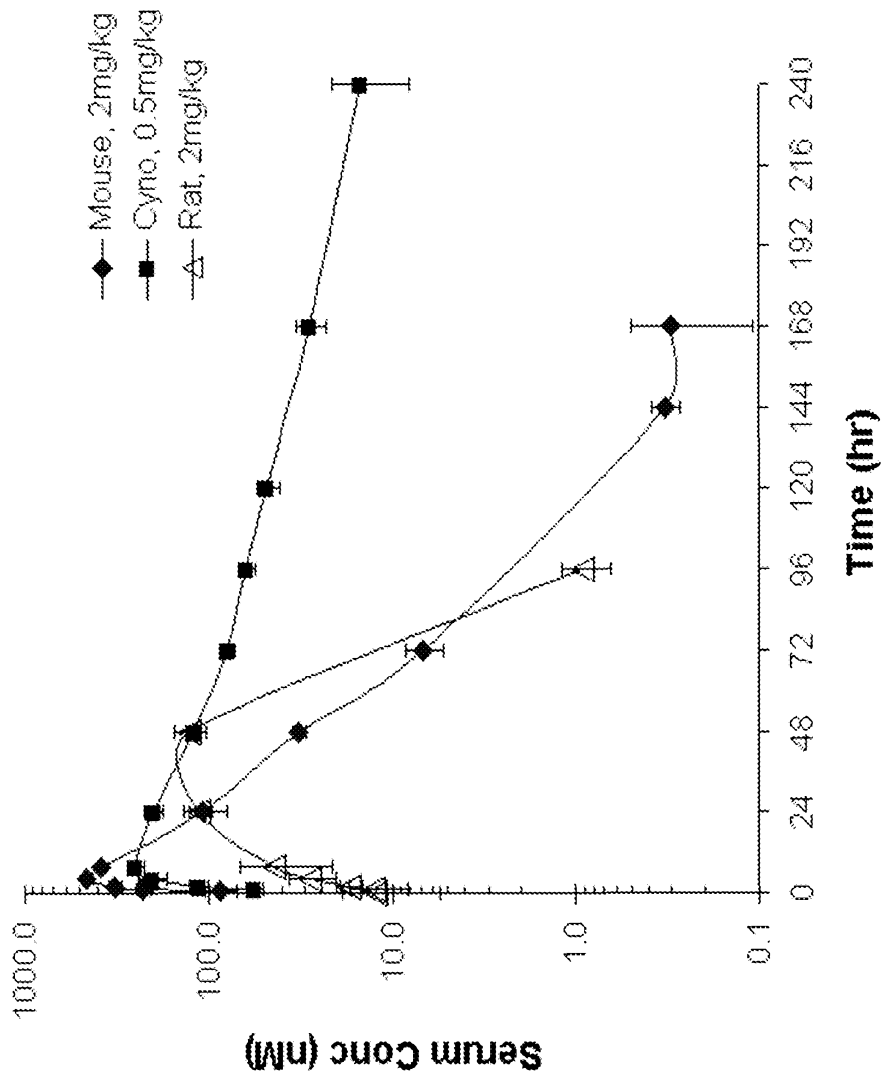

Mouse, 2mg/kg
Cyno, 0.5mg/kg
Rat, 2mg/kg
Serum Conc (nM)
1000.0
100.0
10.0
1.0
0.1
0
24
48
72
96
120
144
168
192
216
240
Time (hr)

FIG. 11B

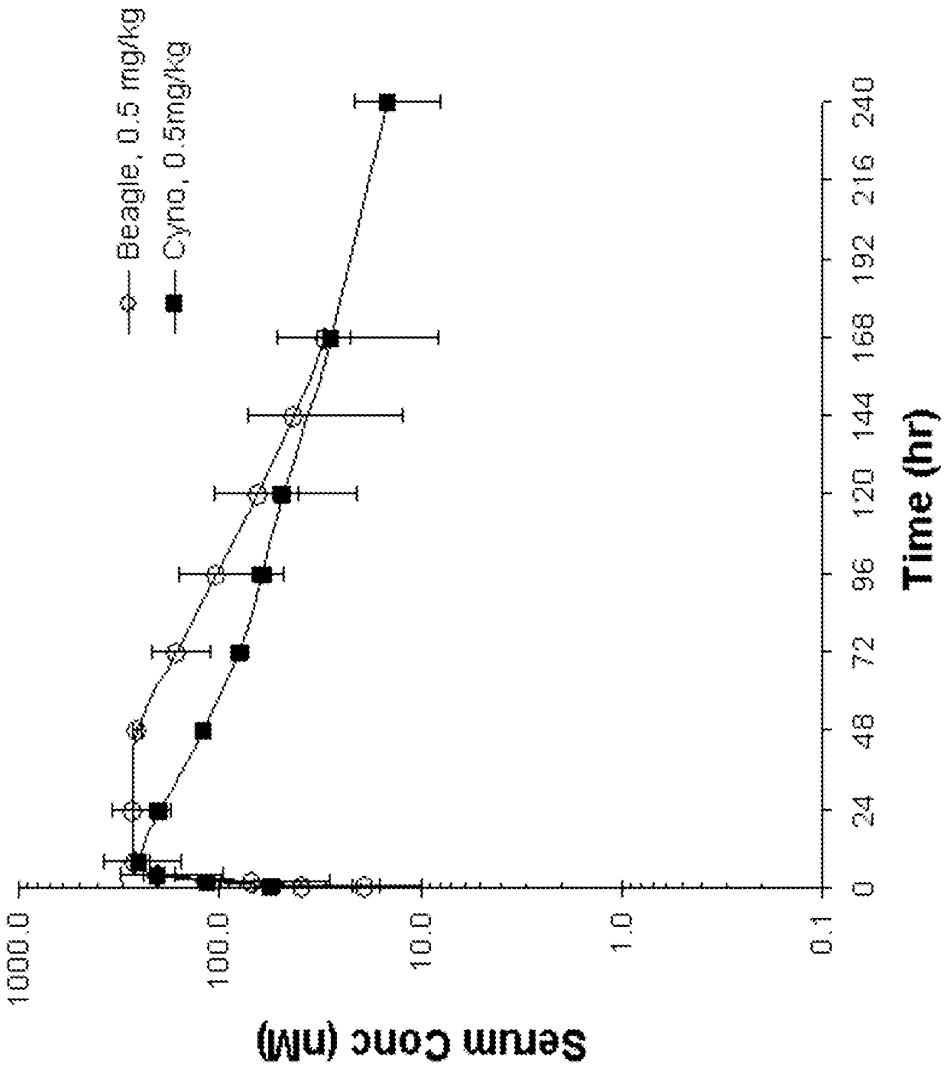

Beagle, 0.5 mg/kg
Cyno, 0.5mg/kg
Serum Conc (nM)
1000.0
100.0
10.0
1.0
0.1
0
24
48
72
96
120
144
168
192
216
240
Time (hr)

FIG. 11C

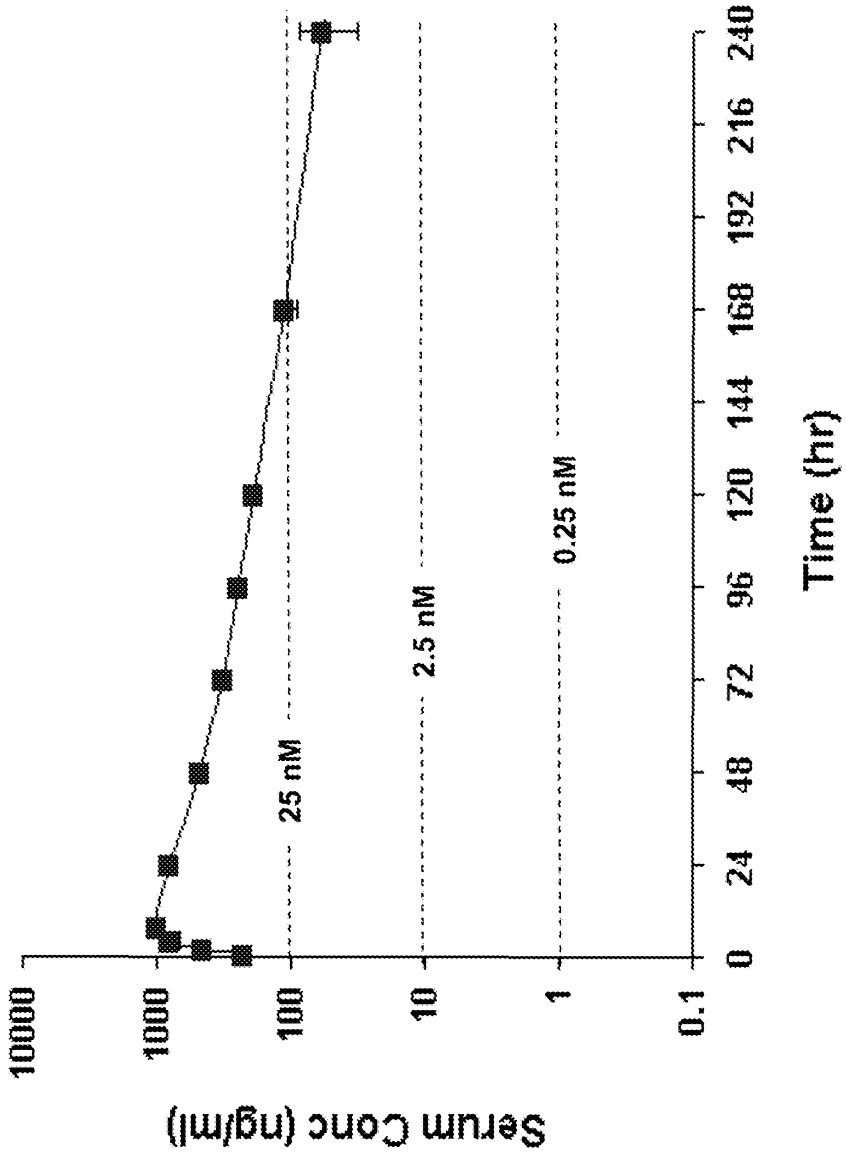

Serum Conc (ng/ml)
10000
1000
100
10
1
0.1
0
24
48
72
96
120
144
168
192
216
240
Time (hr)
25 nM
2.5 nM
0.25 nM

FIG. 12A

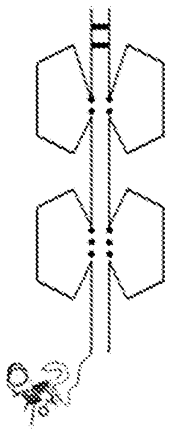


FIG. 12B

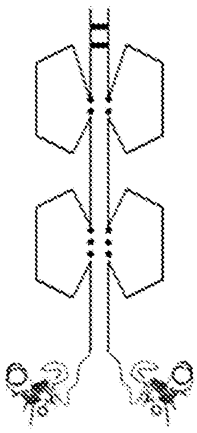


FIG. 12C

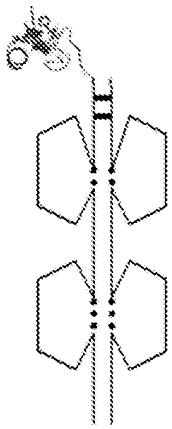


FIG. 12D

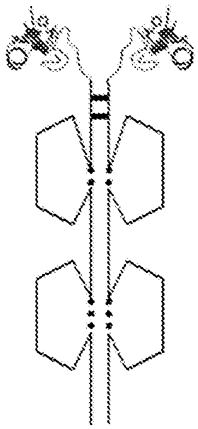


FIG. 12E

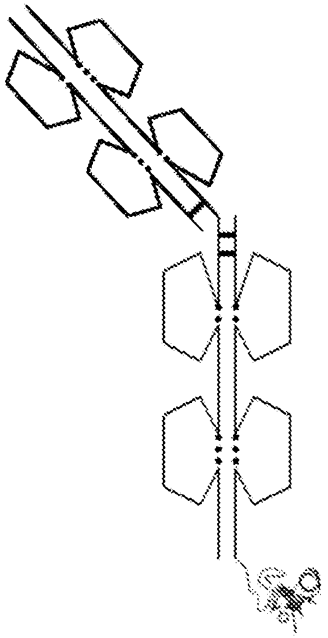


FIG. 12F

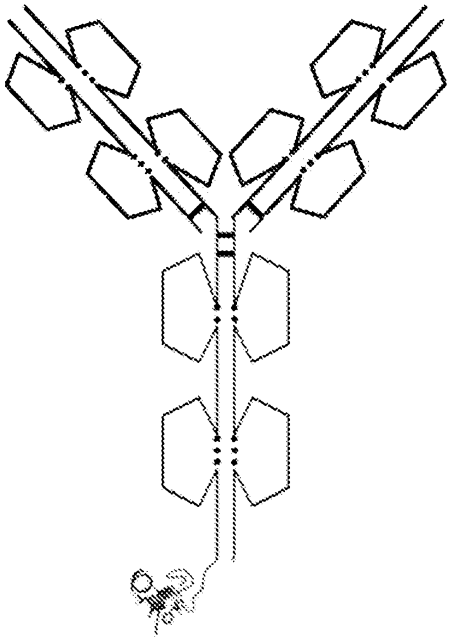


FIG. 12G

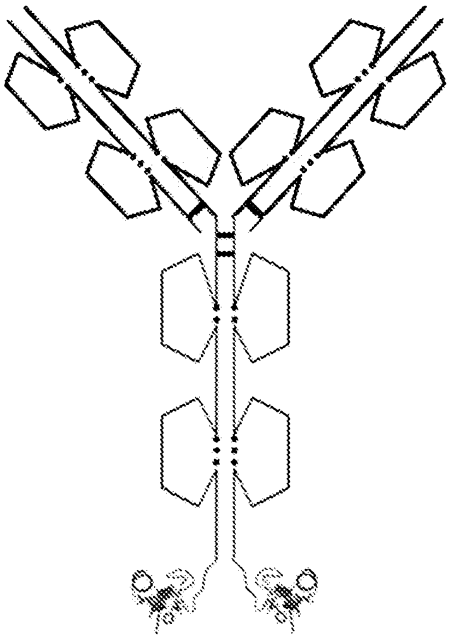


FIG. 12H

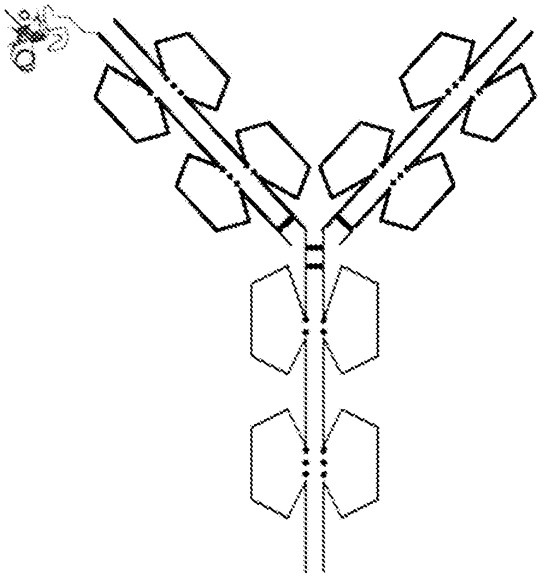


FIG. 12I

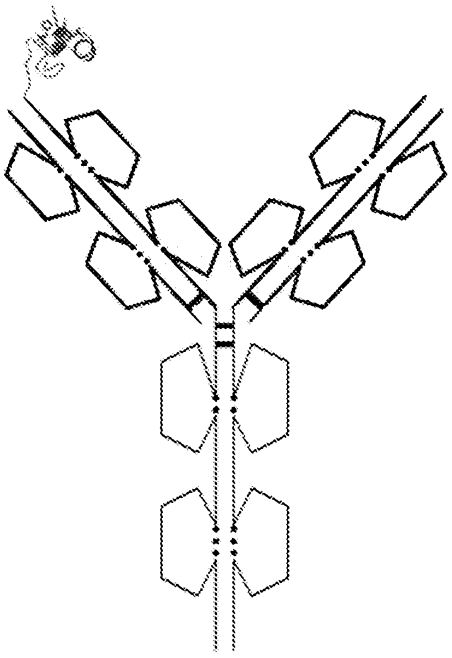


FIG. 12J

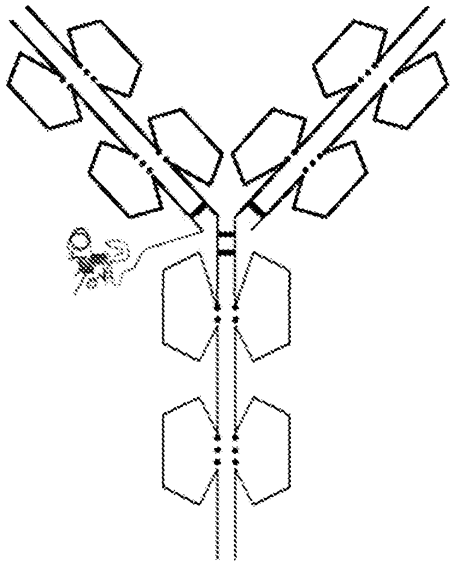


FIG. 12K

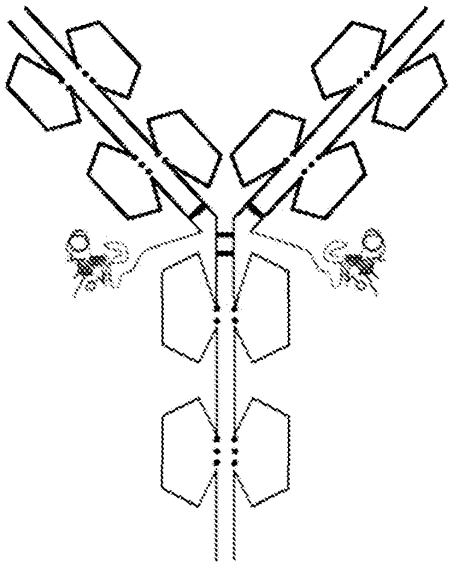


FIG. 12L

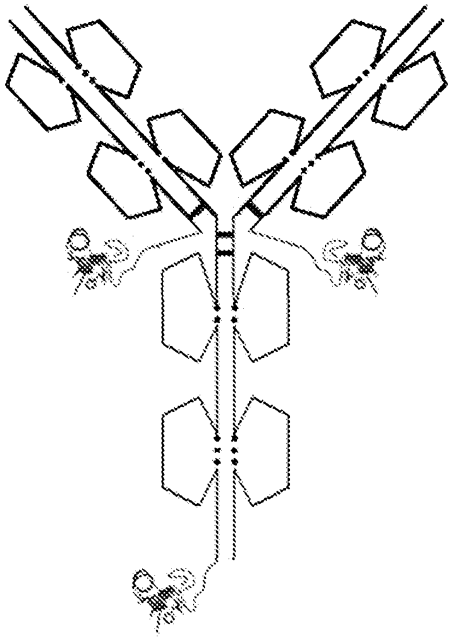


FIG. 12M

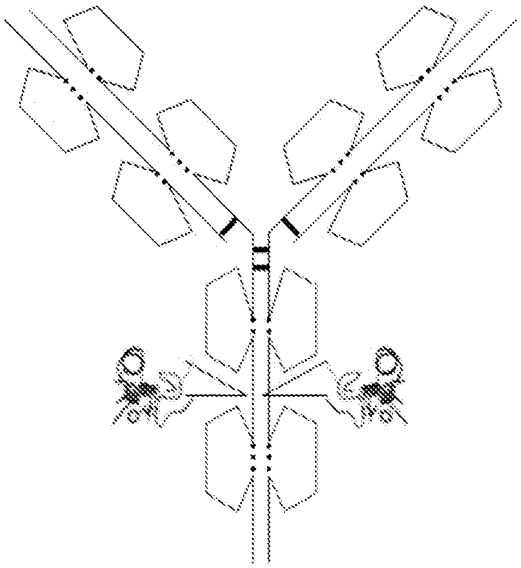


FIG. 12N

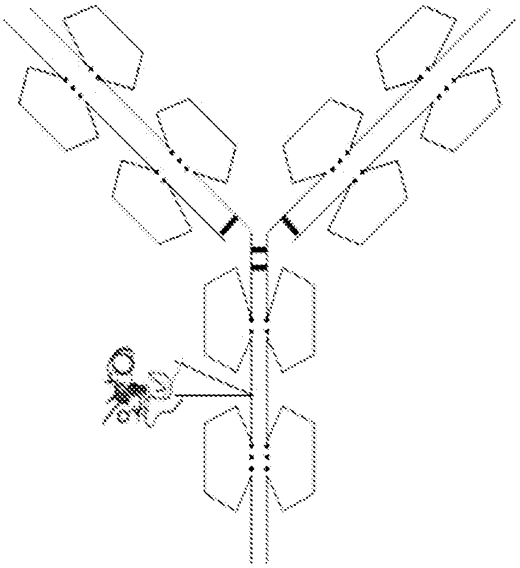


FIG. 13A

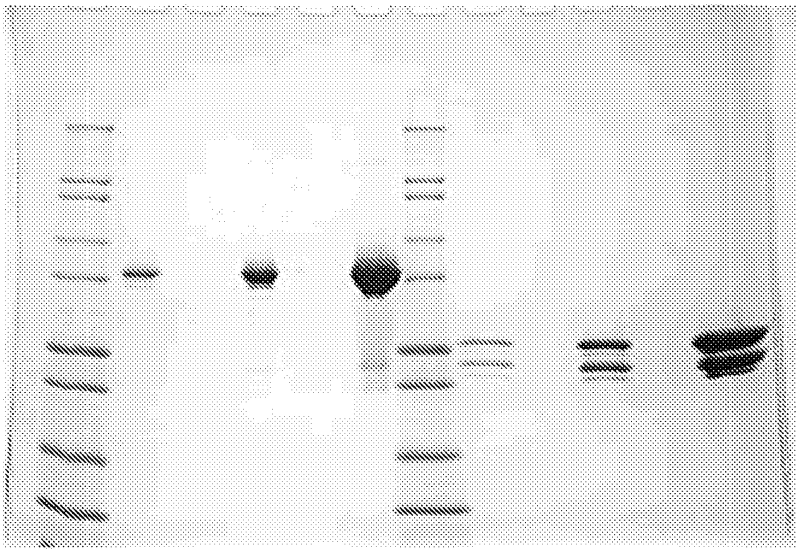


FIG. 13B

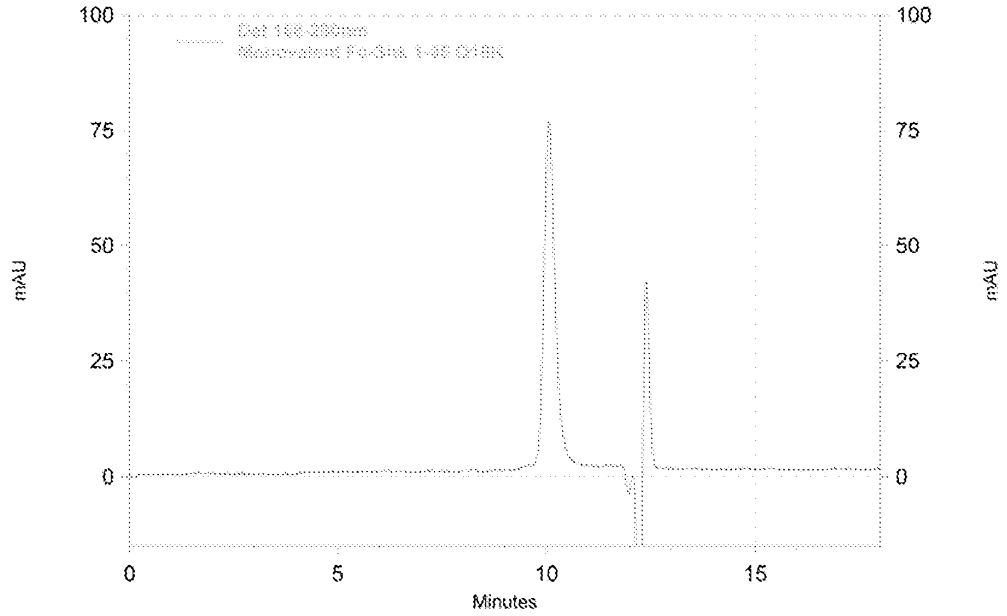


FIG. 13C

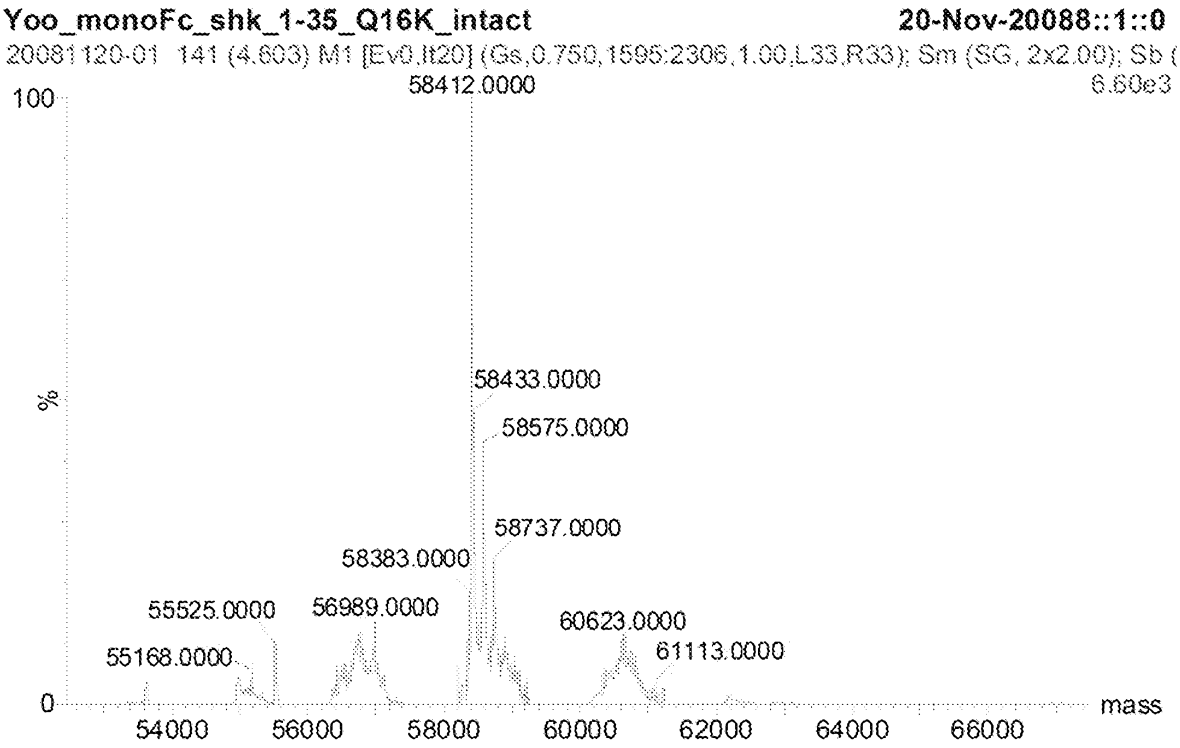

Yoo_monoFc_shk_1-35_Q16K_intact
20-Nov-20088::1::0
20081120-01 141 (4.603) M1 [Ev0,It20] (Gs,0.750,1595:2306,1.00,L33,R33); Sm (SG, 2x2.00); Sb (
6.60e3
100
%
0
58412.0000
58433.0000
58575.0000
58737.0000
58383.0000
55525.0000
56989.0000
60623.0000
55168.0000
61113.0000
mass
54000
56000
58000
60000
62000
64000
66000

FIG. 14A

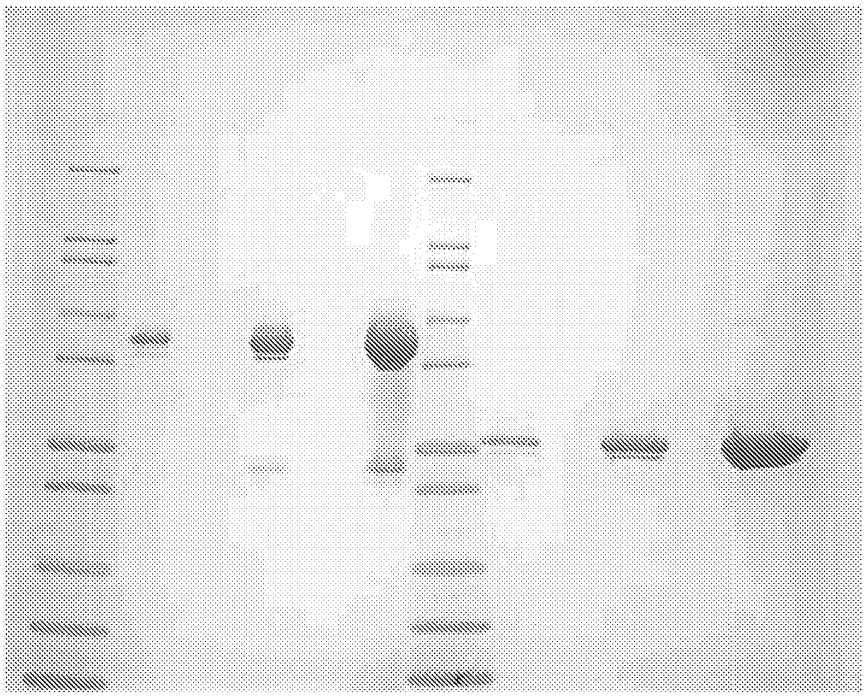


FIG. 14B

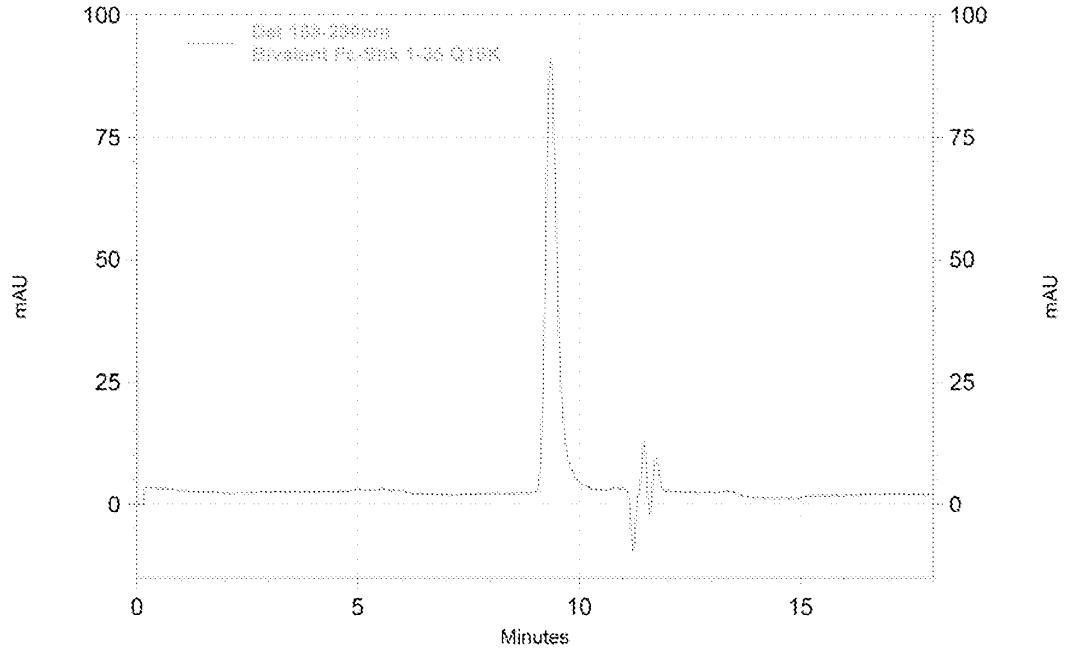


FIG. 14C

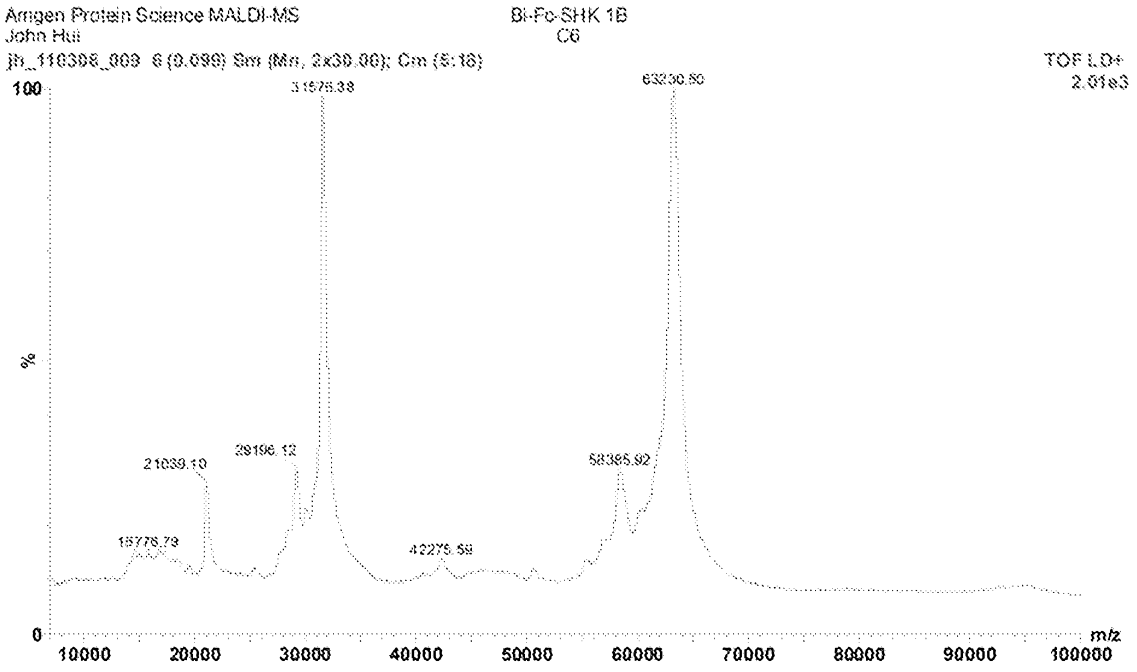

Amgen Protein Science MALDI-MS
John Hill
Bi-Fc-SHK 1B
C6
TOF LD+
2.01e3
100
%
0
21039.10
28196.12
31675.38
15778.79
42275.58
58385.92
63230.80
m/z
10000
20000
30000
40000
50000
60000
70000
80000
90000
100000

FIG. 15A

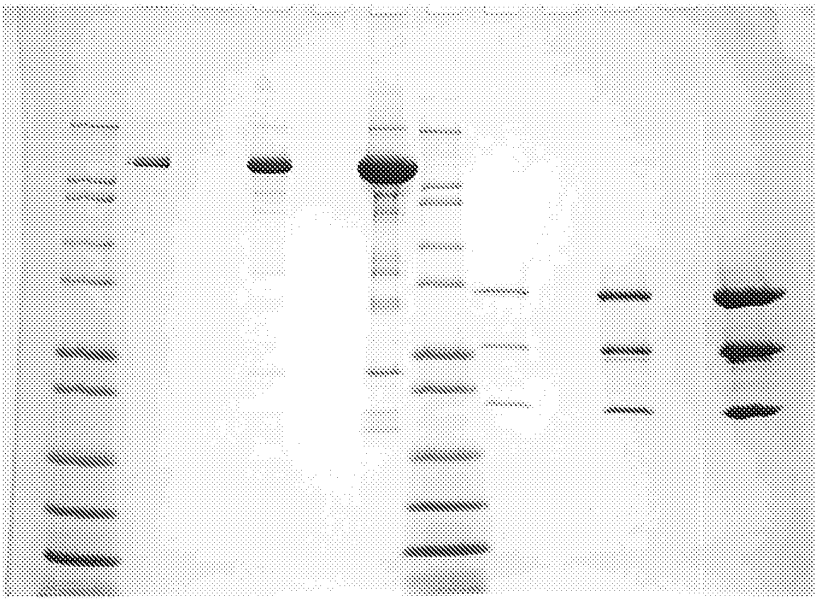


FIG. 15B

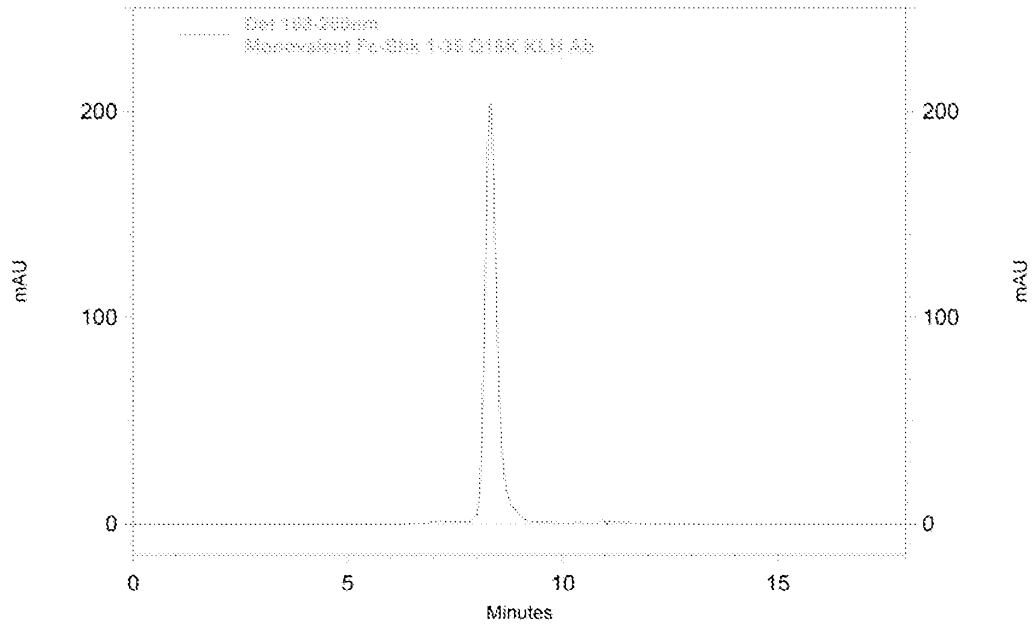


FIG. 15C

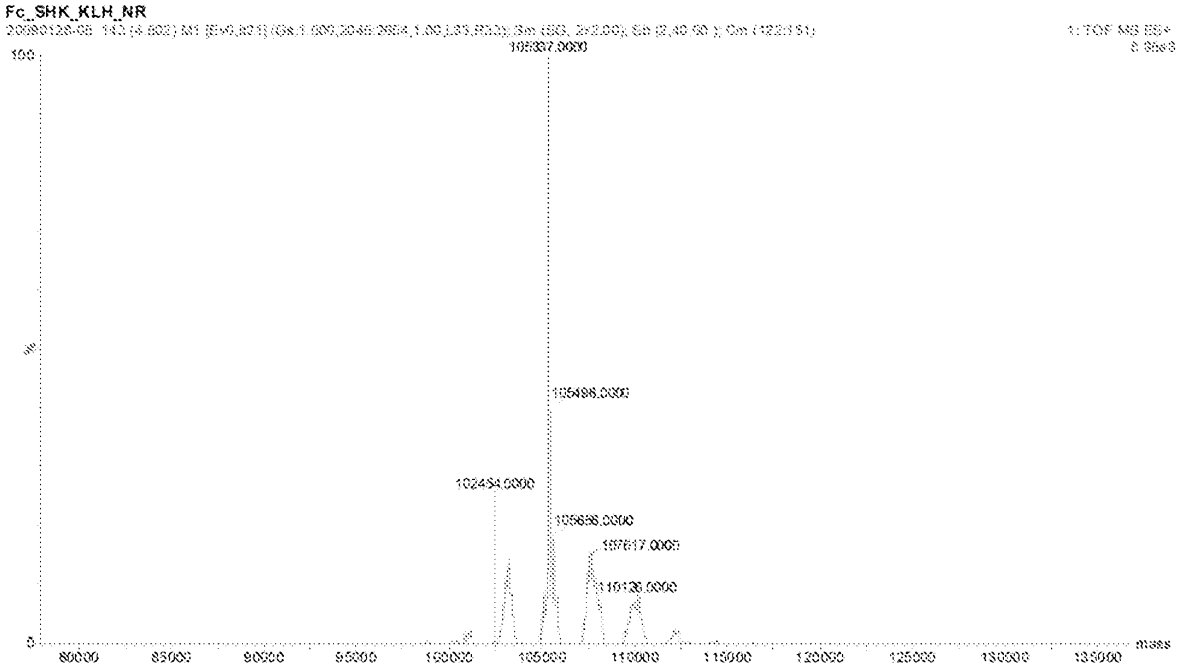

Fc_SHK_KLH_NR
105307.0000
105486.0000
102494.0000
105638.0000
107617.0000
110126.0000
100
%
0
80000
85000
90000
95000
100000
105000
110000
115000
120000
125000
130000
135000
mass

FIG. 16A

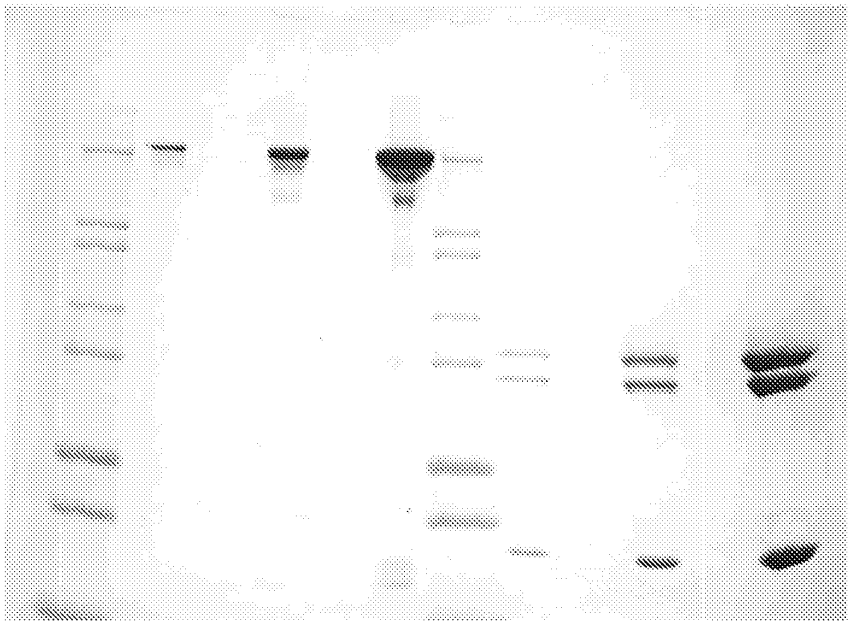


FIG. 16B

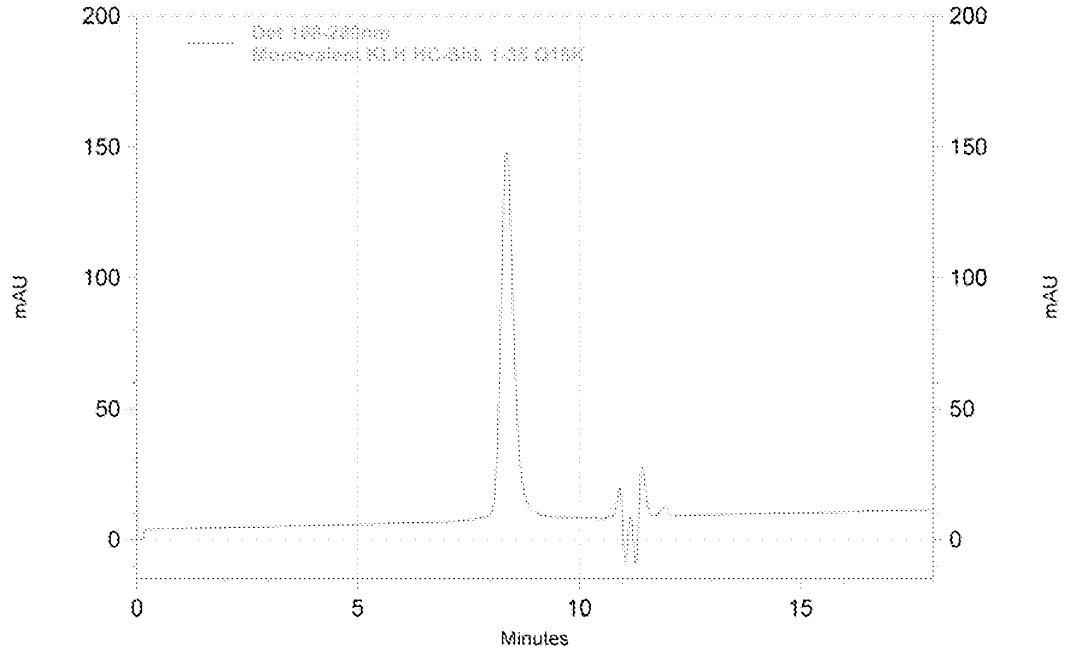


FIG. 16C

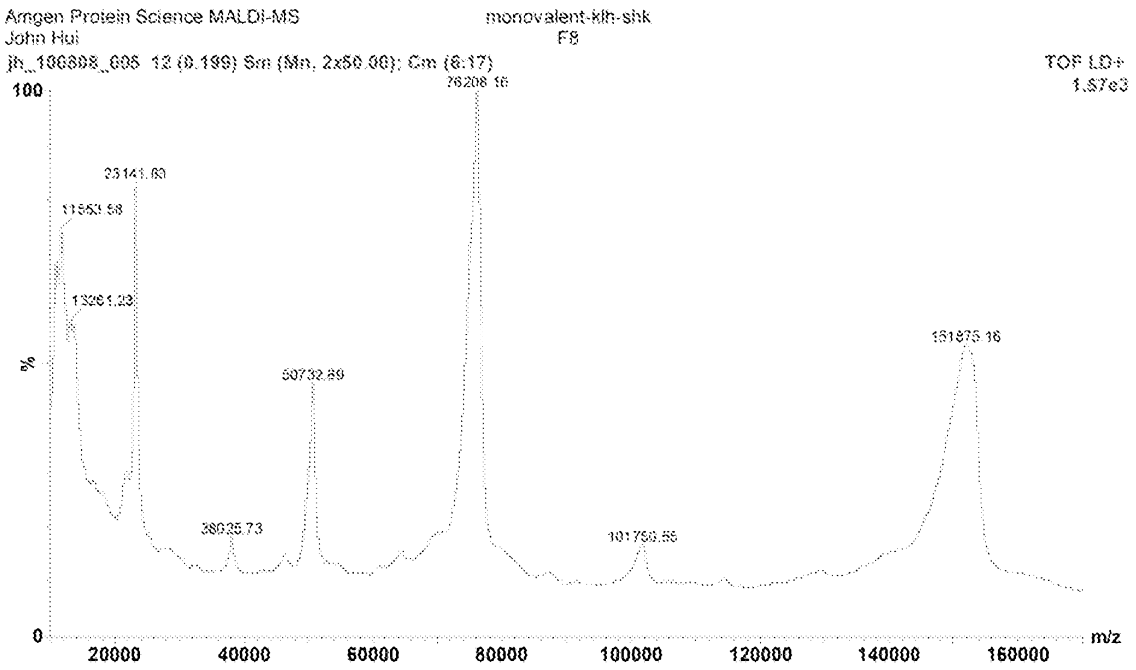

Amgen Protein Science MALDI-MS
John Hui
monovalent-klh-shk
FS
jh_106808_005 12 (0.199) Sm (Mn, 2x50.06); Cm (6:17)
TOF LD+
1.87e3
100
%
0
76208.16
23141.60
11563.58
13261.23
50732.89
38025.73
101756.55
181875.16
m/z
20000
40000
60000
80000
100000
120000
140000
160000

FIG. 17A

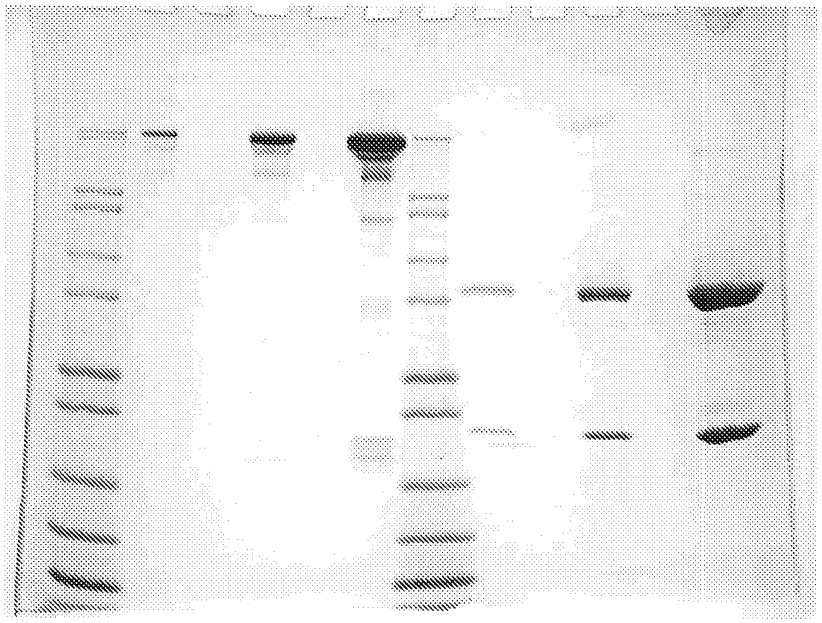


FIG. 17B

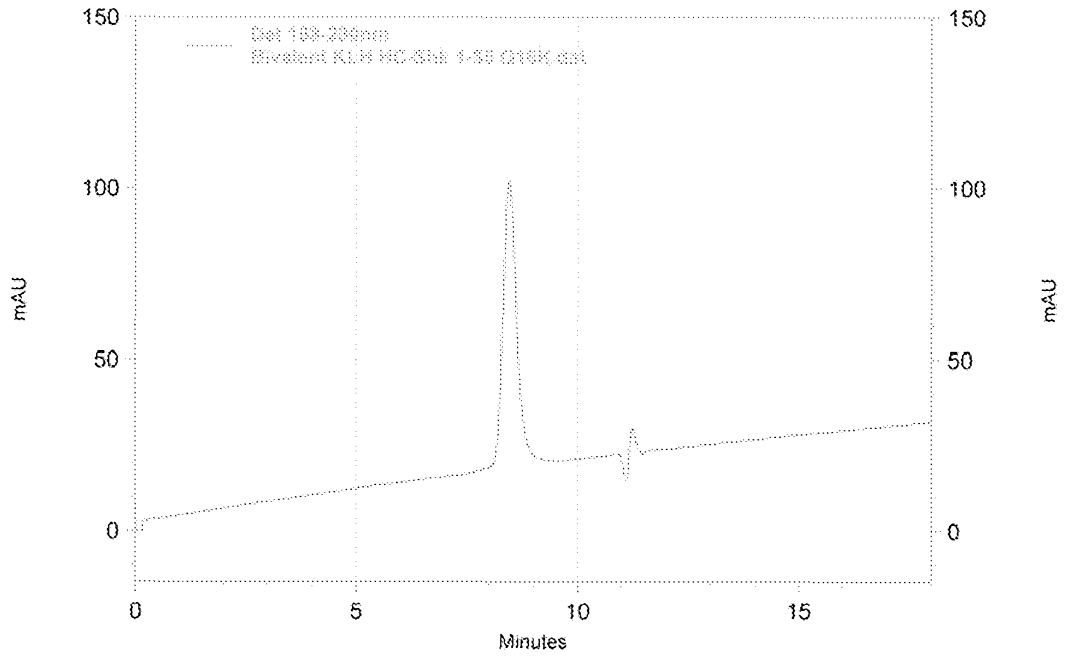


FIG. 17C

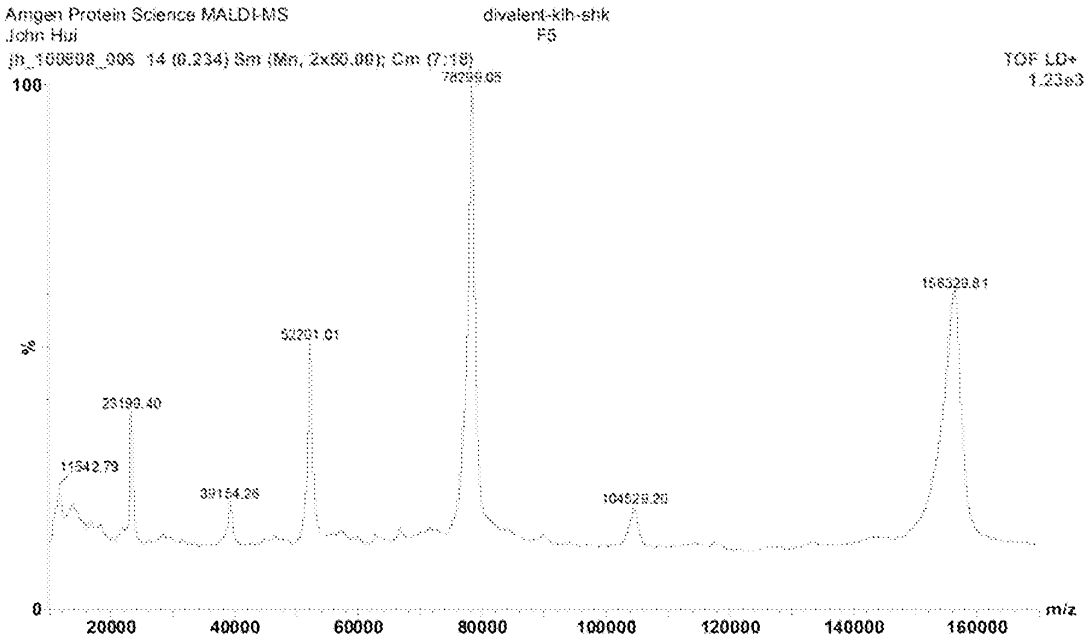

Amgen Protein Science MALDI-MS
John Hui
divalent-kih-shk
F5
TOF LD+
1.23e3
100
%
0
m/z
20000
40000
60000
80000
100000
120000
140000
160000
11542.79
23199.40
39154.26
52291.01
78299.05
104529.26
156329.81

FIG. 18A

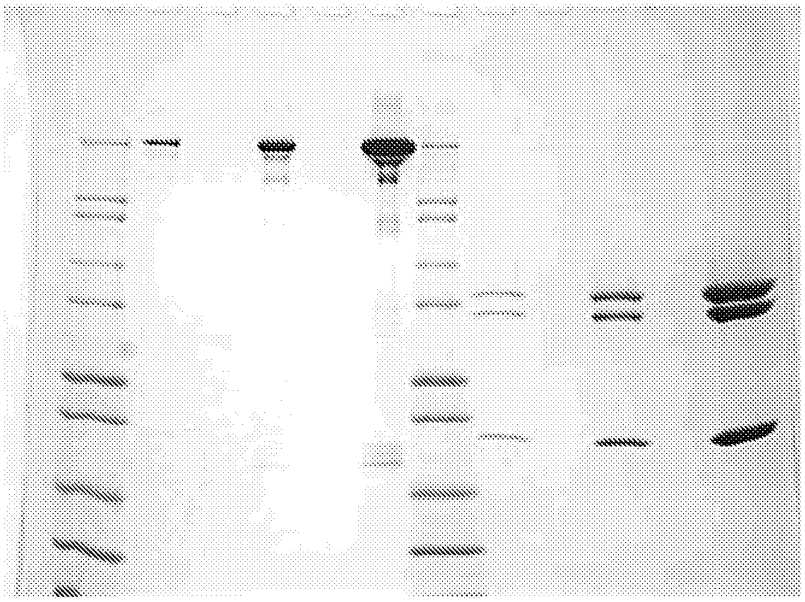


FIG. 18B

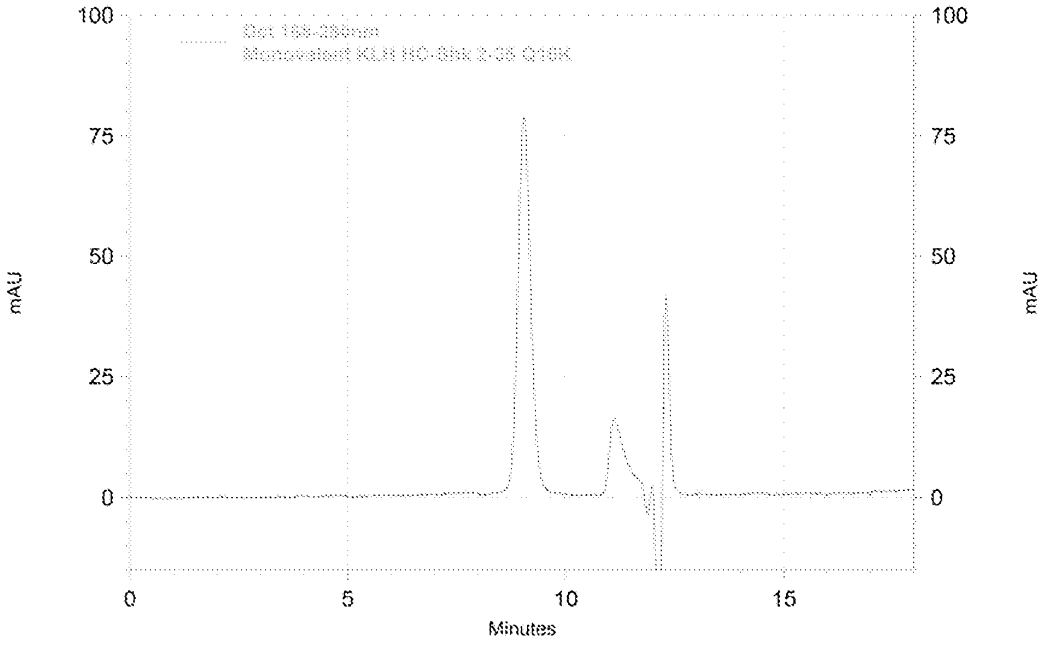


FIG. 18C

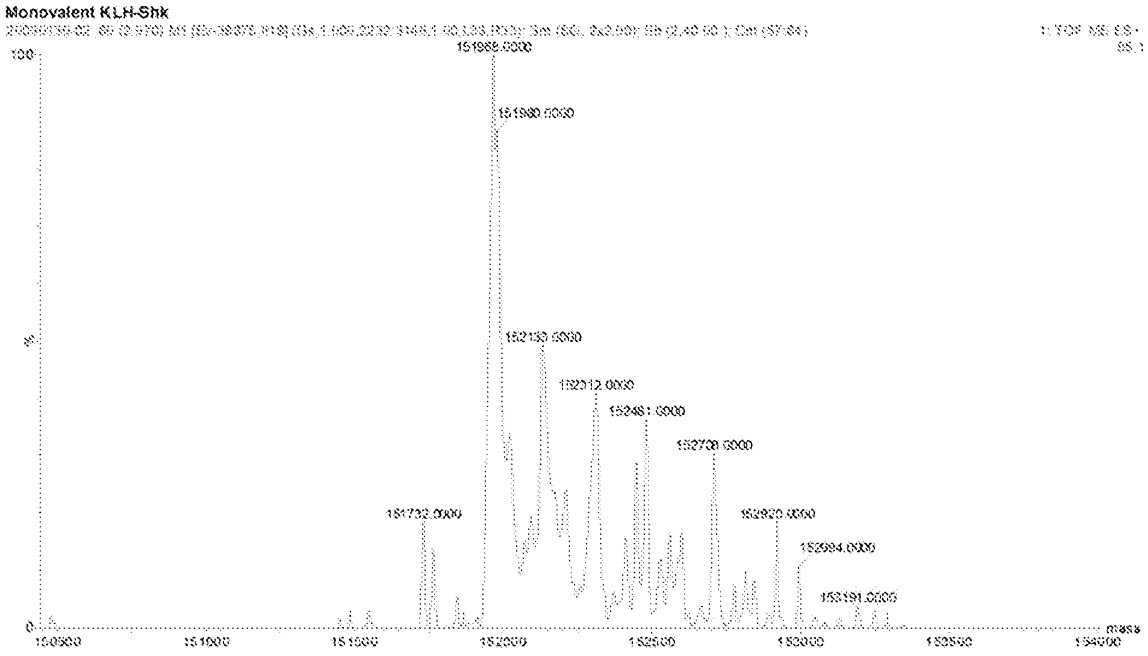

Monovalent KLH-Shk
100
%
0
151986.0000
151980.0000
152133.0000
152112.0000
152481.0000
152720.0000
151732.0000
152920.0000
152984.0000
153191.0000
151500
151900
152000
152500
153000
153500
154000
mass

FIG. 19A

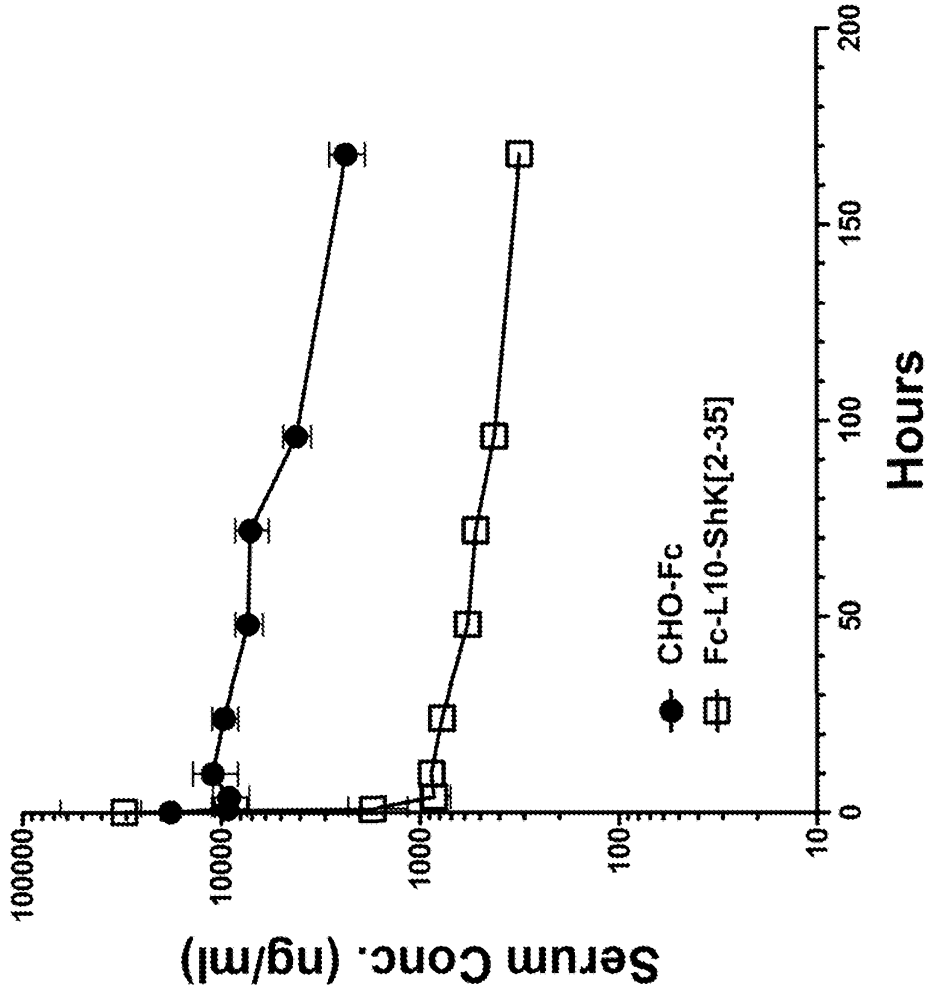

Serum Conc. (ng/ml)
100000
10000
1000
100
10
0
50
100
150
200
Hours
CHO-Fc
Fc-L10-ShK[2-35]

FIG. 19B

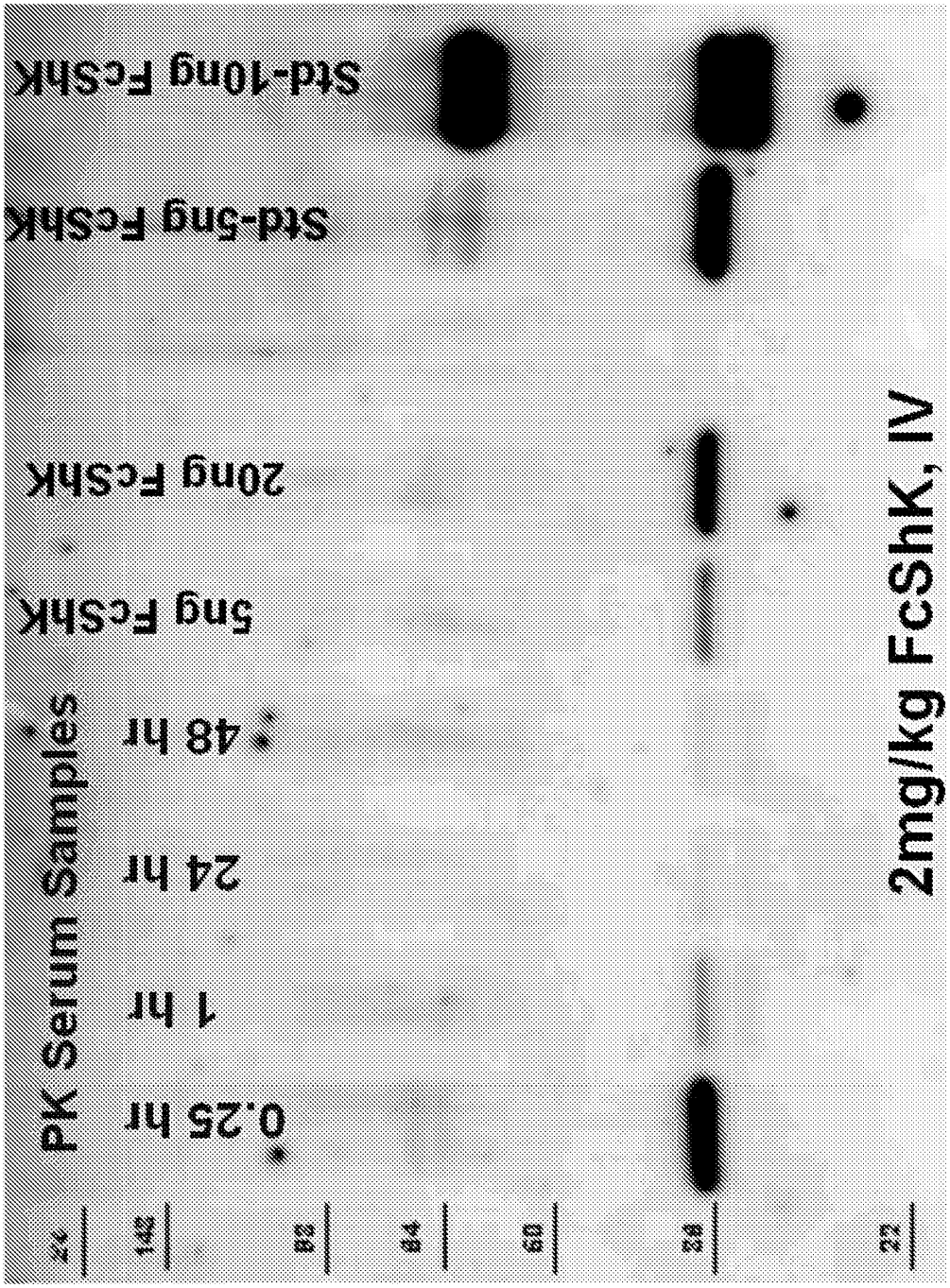

2mg/kg FcShK, IV
PK Serum Samples
0.25 hr
1 hr
24 hr
48 hr
5ng FcShK
20ng FcShK
Std-5ng FcShK
Std-10ng FcShK
148
98
64
50
36
22

FIG. 19C

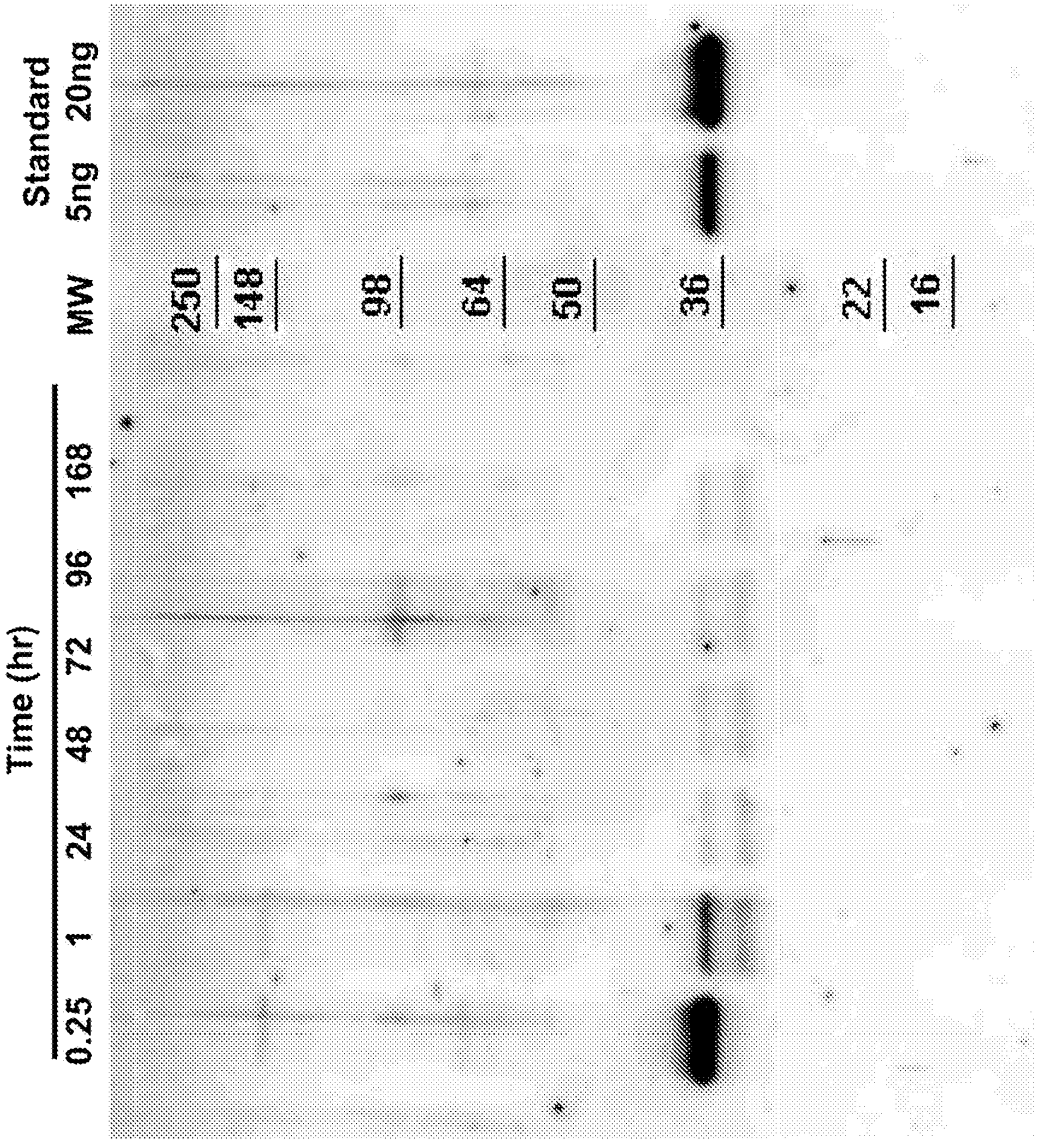

Time (hr)
0.25
1
24
48
72
96
168
MW
Standard
5ng
20ng
250
148
98
64
50
36
22
16

FIG. 19D

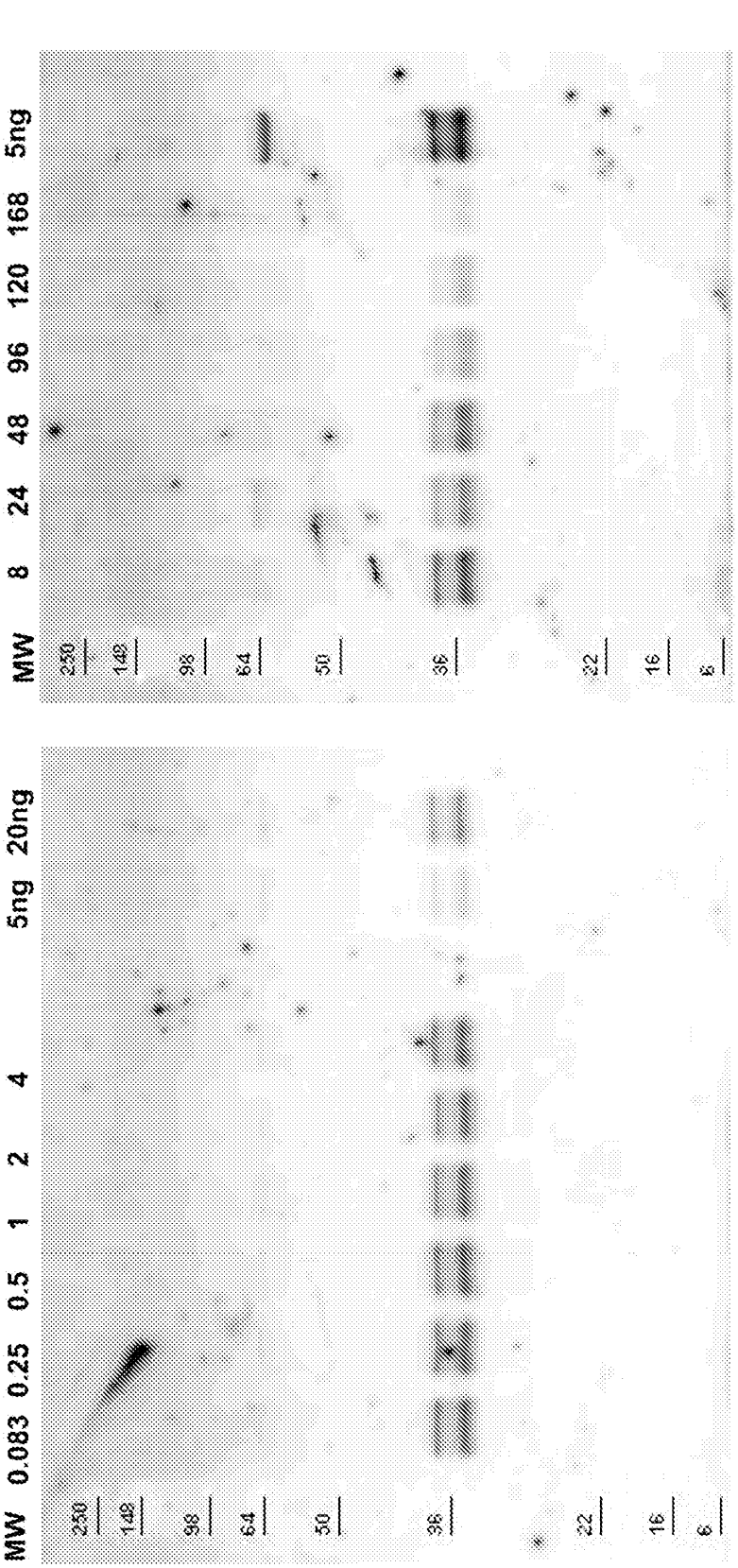

MW
0.083
0.25
0.5
1
2
4
Time (hr)
EIA
5ng
20ng
250
148
98
64
50
36
22
16
6
MW
8
24
48
96
120
168
Time (hr)
Direct Load
5ng
250
148
98
64
50
36
22
16
6

FIG. 20

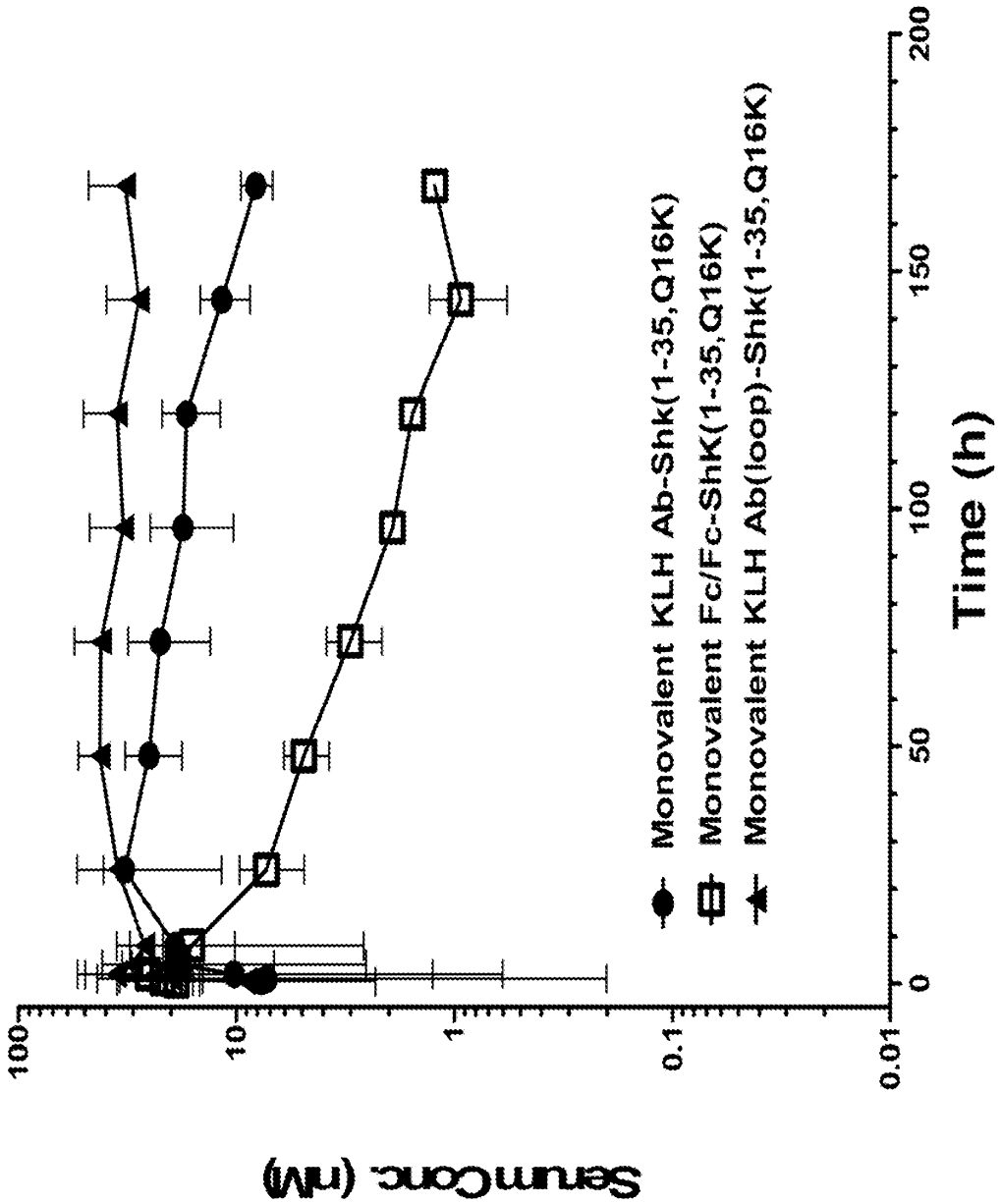

Serum Conc. (nM)
100
10
1
0.1
0.01
0
50
100
150
200
Time (h)
Monovalent KLH Ab-Shk(1-35,Q16K)
Monovalent Fc/Fc-ShK(1-35,Q16K)
Monovalent KLH Ab(loop)-Shk(1-35,Q16K)

FIG. 21

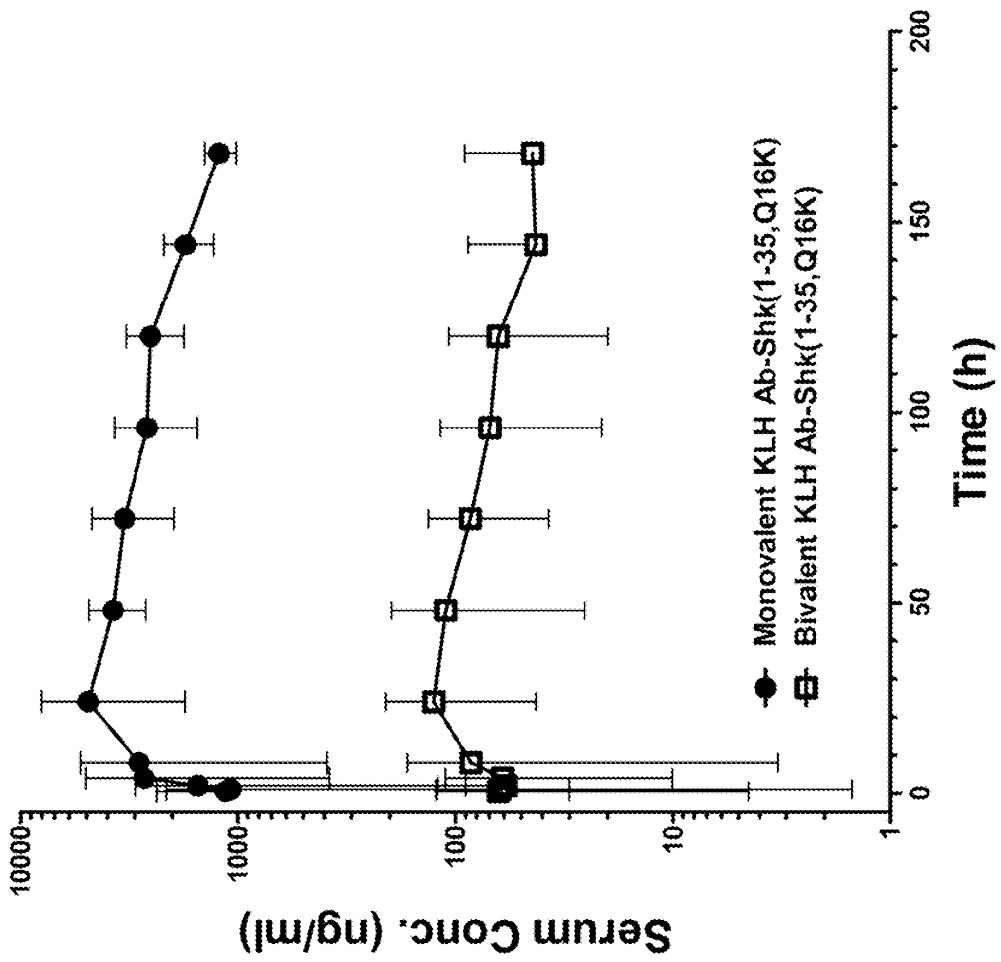

Serum Conc. (ng/ml)
10000
1000
100
10
1
0
50
100
150
200
Time (h)
Monovalent KLH Ab-Shk(1-35,Q16K)
Bivalent KLH Ab-Shk(1-35,Q16K)

FIG. 22

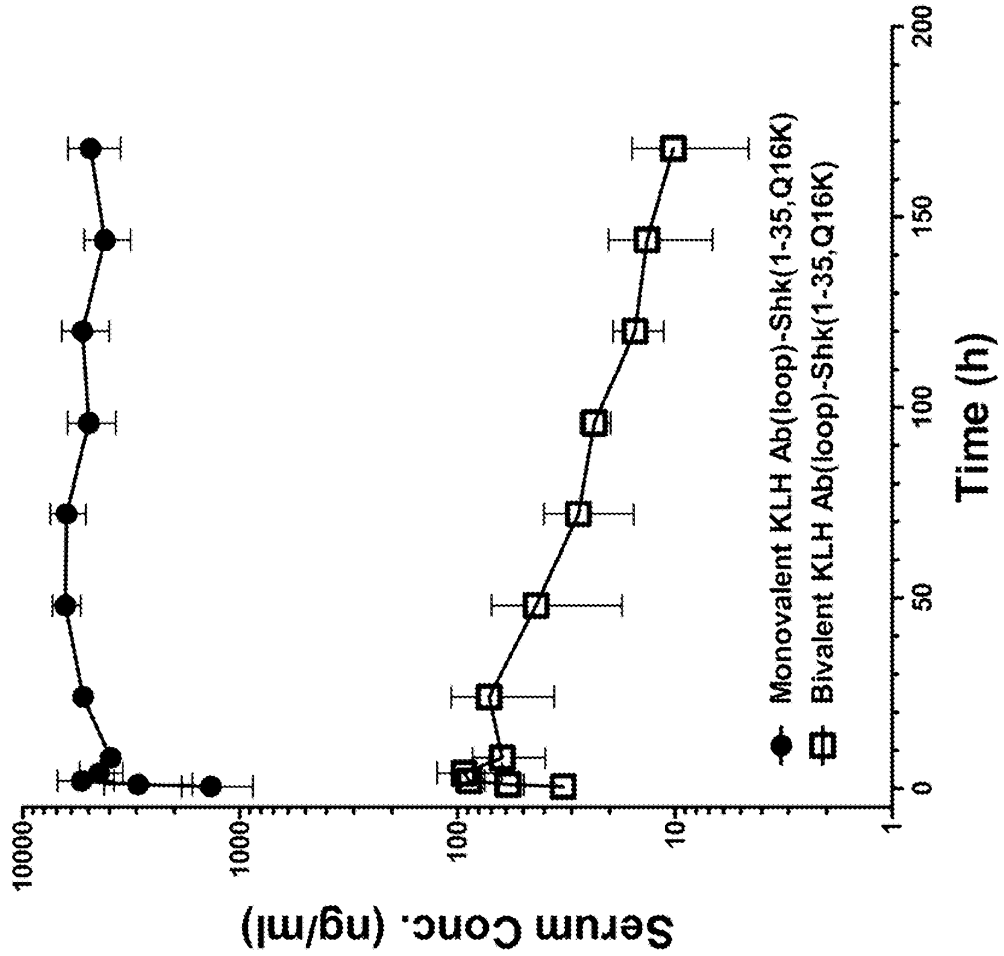

Serum Conc. (ng/ml)
10000
1000
100
10
1
0
50
100
150
200
Time (h)
Monovalent KLH Ab(loop)-Shk(1-35,Q16K)
Bivalent KLH Ab(loop)-Shk(1-35,Q16K)

FIG. 23

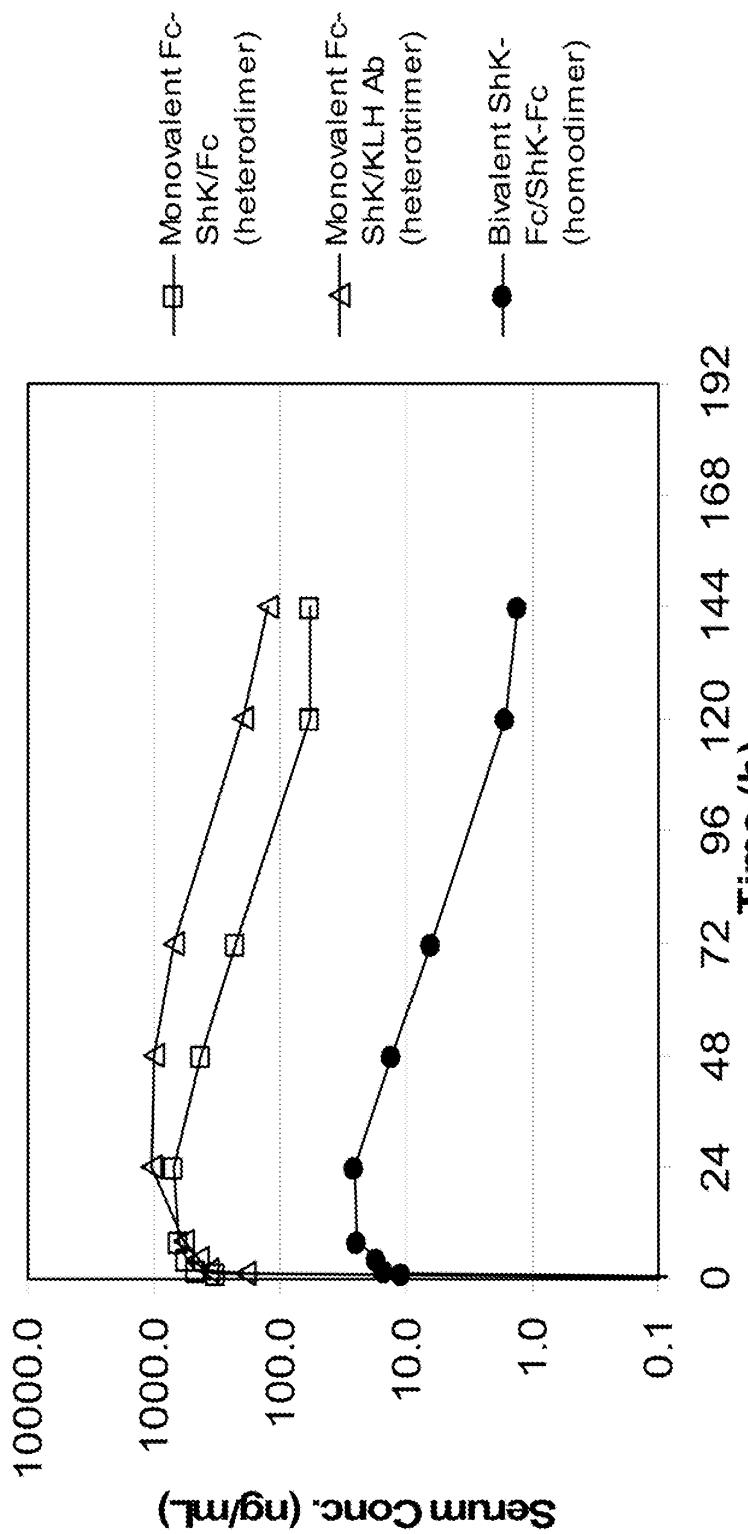

Monovalent Fc-ShK/Fc (heterodimer)
Monovalent Fc-ShK/KLH Ab (heterotrimer)
Bivalent ShK-Fc/ShK-Fc (homodimer)
Serum Conc. (ng/mL)
10000.0
1000.0
100.0
10.0
1.0
0.1
0
24
48
72
96
120
144
168
192
Time (h)

FIG. 24

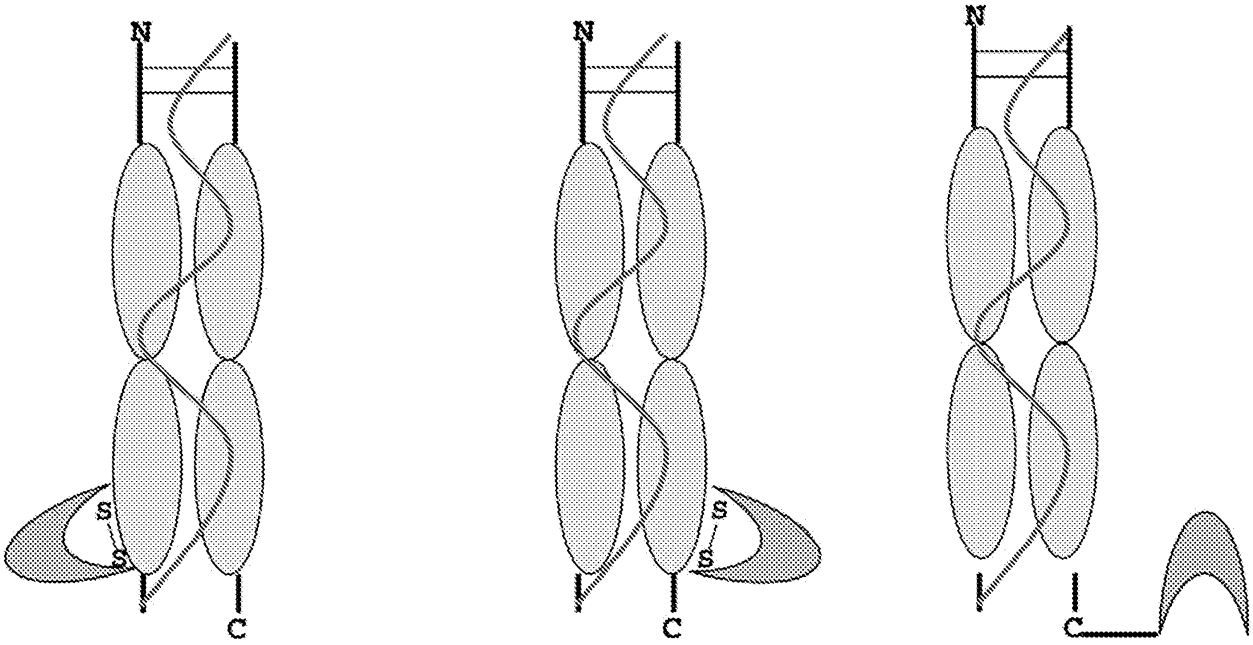


FIG. 25A

MDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY
KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELGGRSCIDTIPKSRC
TAFQCKHSMKYRLSFCRKTCGTCGGTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH
NHYTQKSLSLSPGK

FIG. 25B

MDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY
KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELGGRSCIDTIPK
SRCTAFQCKHSMKYRLSFCRKTCGTCGGTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM
HEALHNHYTQKSLSLSPGK

FIG. 25C

MDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY
KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF
YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSRSCIDTIPKSRCTAF
KCKHSMKYRLSFCRKTCGTCA

FIG. 26A

SDS-PAGE

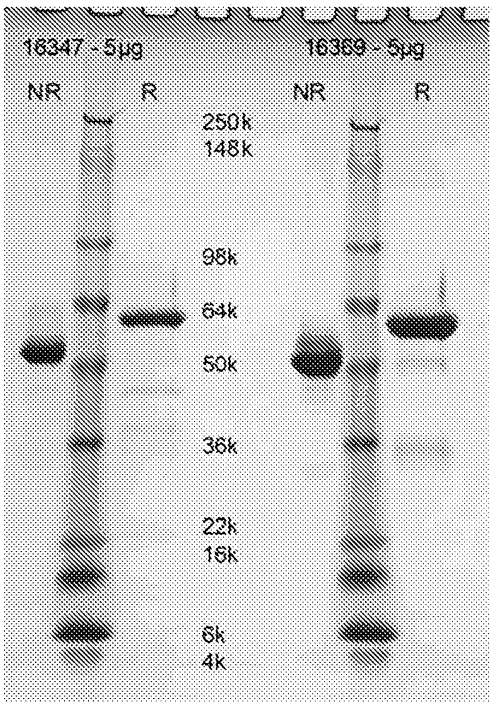


FIG. 26B

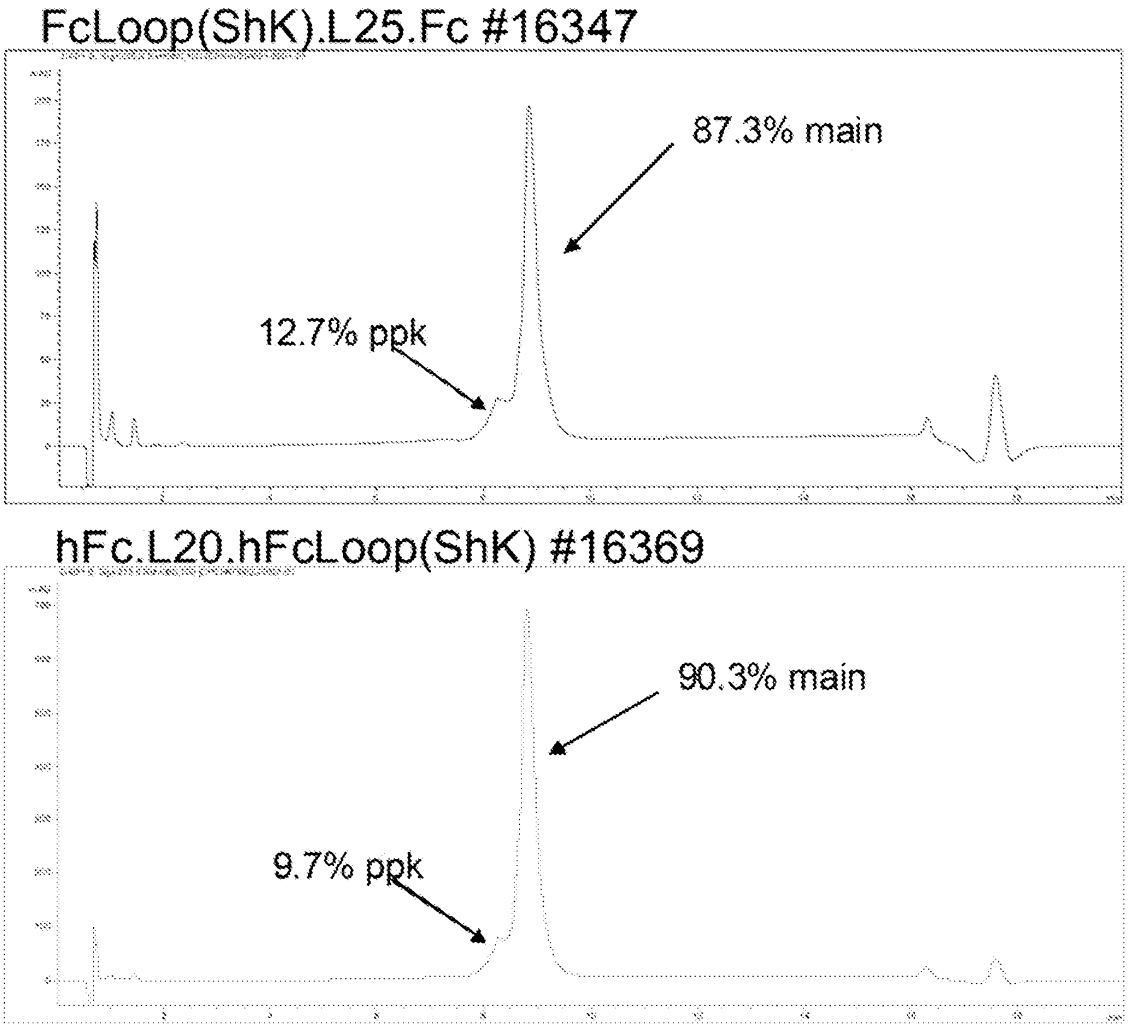

FcLoop(ShK).L25.Fc #16347
87.3% main
12.7% ppk
hFc.L20.hFcLoop(ShK) #16369
90.3% main
9.7% ppk

FIG. 27A

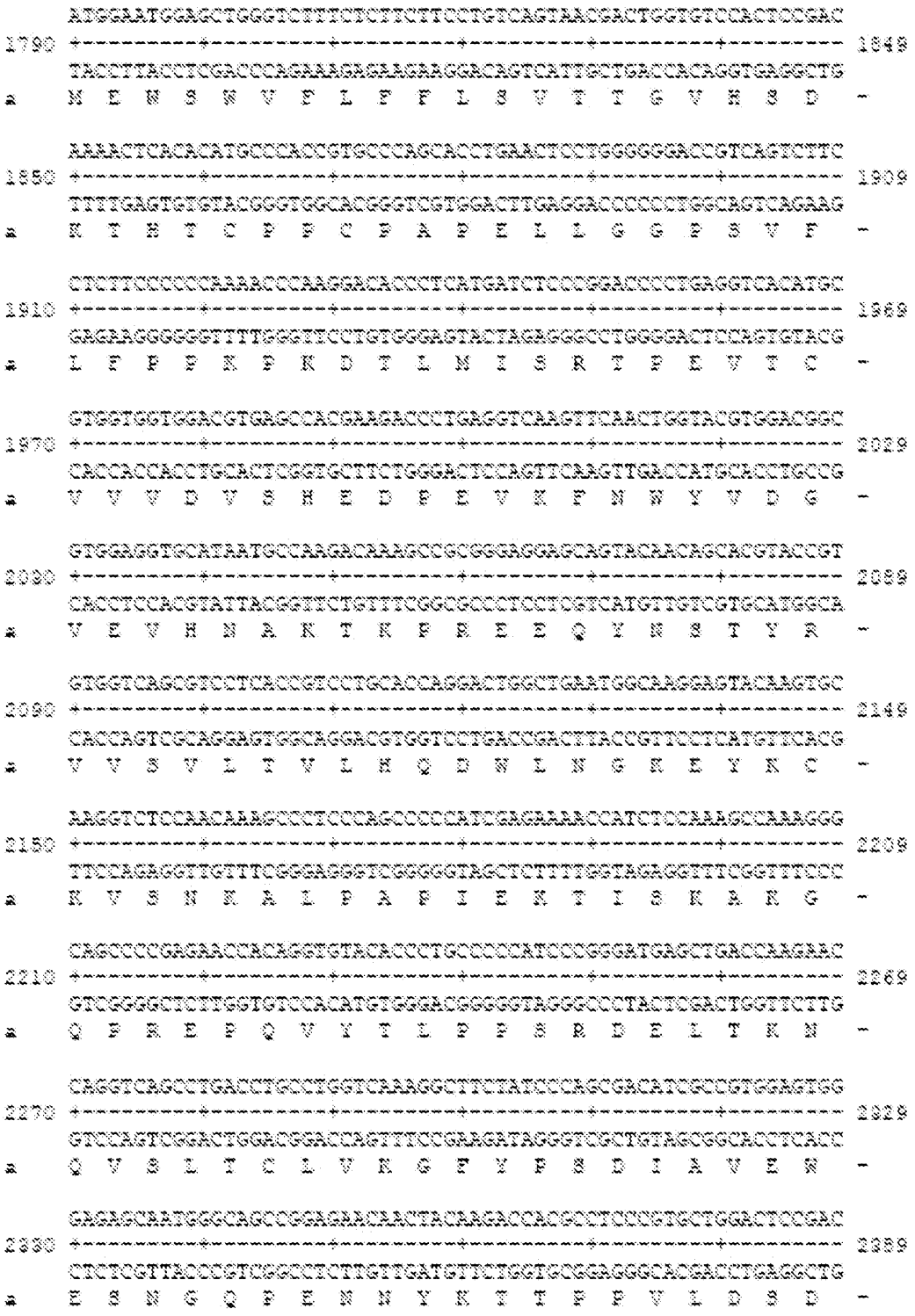

ATGGAATGGAGCTGGGTCTTTCTCTTCTTCCTGTCAGTAACGACTGGTGTCCACTCCGAC
1790 +---------+---------+---------+---------+---------+--------- 1848
TACCTTACCTCGACCCAGAAAGAGAAGAAGGACAGTCATTGCTGACCACAGGTGAGGCTG
M E W S W V F L F F L S V T T G V H S D -
AAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTC
1850 +---------+---------+---------+---------+---------+--------- 1909
TTTTGAGTGTGTACGGGTGGCACGGGTCGTGGACTTGAGGACCCCCCTGGCAGTCAGAAG
K T H T C P P C P A P E L L G G P S V F -
CTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGC
1910 +---------+---------+---------+---------+---------+--------- 1969
GAGAAGGGGGGTTTTGGGTTCCTGTGGGAGTACTAGAGGGCCTGGGGACTCCAGTGTACG
L F P P K P K D T L M I S R T P E V T C -
GTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGC
1970 +---------+---------+---------+---------+---------+--------- 2029
CACCACCACCTGCACTCGGTGCTTCTGGGACTCCAGTTCAAGTTGACCATGCACCTGCCG
V V V D V S H E D P E V K F N W Y V D G -
GTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGT
2030 +---------+---------+---------+---------+---------+--------- 2089
CACCTCCACGTATTACGGTTCTGTTTCGGCGCCCTCCTCGTCATGTTGTCGTGCATGGCA
V E V H N A K T K P R E E Q Y N S T Y R -
GTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC
2090 +---------+---------+---------+---------+---------+--------- 2149
CACCAGTCGCAGGAGTGGCAGGACGTGGTCCTGACCGACTTACCGTTCCTCATGTTCACG
V V S V L T V L H Q D W L N G K E Y K C -
AAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGG
2150 +---------+---------+---------+---------+---------+--------- 2209
TTCCAGAGGTTGTTTCGGGAGGGTCGGGGGTAGCTCTTTTGGTAGAGGTTTCGGTTTCCC
K V S N K A L P A P I E K T I S K A K G -
CAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAAC
2210 +---------+---------+---------+---------+---------+--------- 2269
GTCGGGGCTCTTGGTGTCCACATGTGGGACGGGGGTAGGGCCCTACTCGACTGGTTCTTG
Q P R E P Q V Y T L P P S R D E L T K N -
CAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGG
2270 +---------+---------+---------+---------+---------+--------- 2329
GTCCAGTCGGACTGGACGGACCAGTTTCCGAAGATAGGGTCGCTGTAGCGGCACCTCACC
Q V S L T C L V K G F Y P S D I A V E W -
GAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGAC
2330 +---------+---------+---------+---------+---------+--------- 2389
CTCTCGTTACCCGTCGGCCTCTTGTTGATGTTCTGGTGCGGAGGGCACGACCTGAGGCTG
E S N G Q P E N N Y K T T P P V L D S D -

FIG. 27B

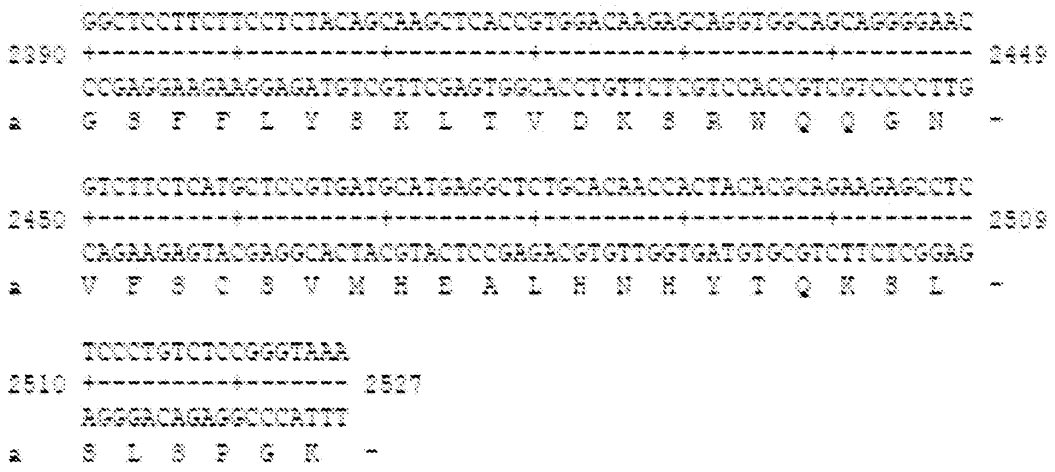

GGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAAC
2390 +---------+---------+---------+---------+---------+--------- 2449
CCGAGGAAGAAGGAGATGTCGTTCGAGTGGCACCTGTTCTCGTCCACCGTCGTCCCCTTG
a G S F F L Y S K L T V D K S R W Q Q G N -
GTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTC
2450 +---------+---------+---------+---------+---------+--------- 2509
CAGAAGAGTACGAGGCACTACGTACTCCGAGACGTGTTGGTGATGTGCGTCTTCTCGGAG
a V F S C S V M H E A L H N H Y T Q K S L -
TCCCTGTCTCCGGGTAAA
2510 +----------+------- 2527
AGGGACAGAGGCCCATTT
a S L S P G K -

FIG. 28A

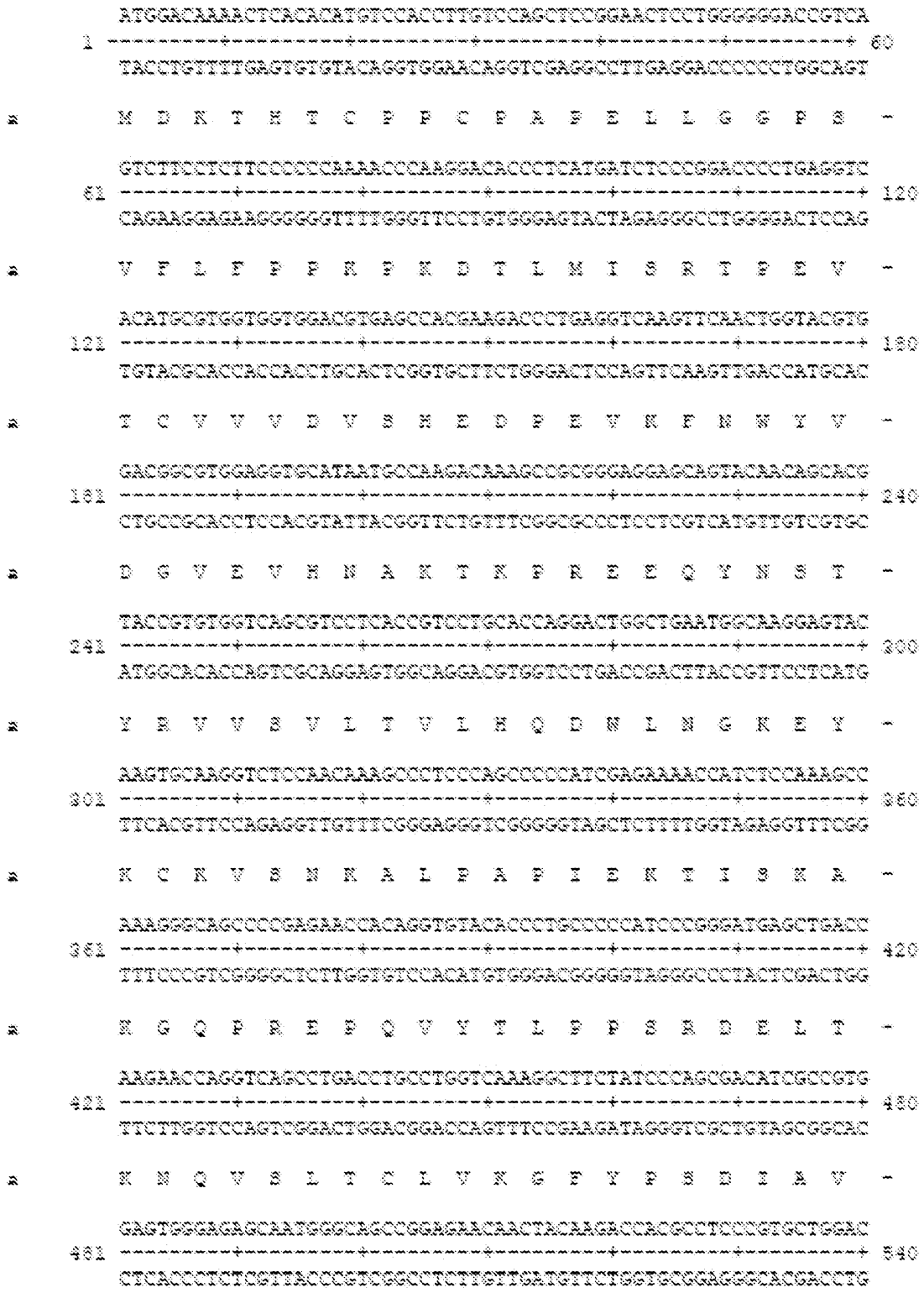

ATGGACAAAACTCACACATGTCCACCTTGTCCAGCTCCGGAACTCCTGGGGGGACCGTCA
1 ---------+---------+---------+---------+---------+---------+ 60
TACCTGTTTTGAGTGTGTACAGGTGGAACAGGTCGAGGCCTTGAGGACCCCCCTGGCAGT
M D K T H T C P P C P A P E L L G G P S -
GTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTC
61 ---------+---------+---------+---------+---------+---------+ 120
CAGAAGGAGAAGGGGGGTTTTGGGTTCCTGTGGGAGTACTAGAGGGCCTGGGGACTCCAG
V F L F P P K P K D T L M I S R T P E V -
ACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTG
121 ---------+---------+---------+---------+---------+---------+ 180
TGTACGCACCACCACCTGCACTCGGTGCTTCTGGGACTCCAGTTCAAGTTGACCATGCAC
T C V V V D V S H E D P E V K F N W Y V -
GACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACG
181 ---------+---------+---------+---------+---------+---------+ 240
CTGCCGCACCTCCACGTATTACGGTTCTGTTTCGGCGCCCTCCTCGTCATGTTGTCGTGC
D G V E V H N A K T K P R E E Q Y N S T -
TACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTAC
241 ---------+---------+---------+---------+---------+---------+ 300
ATGGCACACCAGTCGCAGGAGTGGCAGGACGTGGTCCTGACCGACTTACCGTTCCTCATG
Y R V V S V L T V L H Q D W L N G K E Y -
AAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCC
301 ---------+---------+---------+---------+---------+---------+ 360
TTCACGTTCCAGAGGTTGTTTCGGGAGGGTCGGGGGTAGCTCTTTTGGTAGAGGTTTCGG
K C K V S N K A L P A P I E K T I S K A -
AAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACC
361 ---------+---------+---------+---------+---------+---------+ 420
TTTCCCGTCGGGGGCTCTTGGTGTCCACATGTGGGACGGGGGTAGGGCCCTACTCGACTGG
K G Q P R E P Q V Y T L P P S R D E L T -
AAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTG
421 ---------+---------+---------+---------+---------+---------+ 480
TTCTTGGTCCAGTCGGACTGGACGGACCAGTTTCCGAAGATAGGGTCGCTGTAGCGGCAC
K N Q V S L T C L V K G F Y P S D I A V -
GAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGAC
481 ---------+---------+---------+---------+---------+---------+ 540
CTCACCCTCTCGTTACCCGTCGGCCTCTTGTTGATGTTCTGGTGCGGAGGGCACGACCTG

FIG. 28B

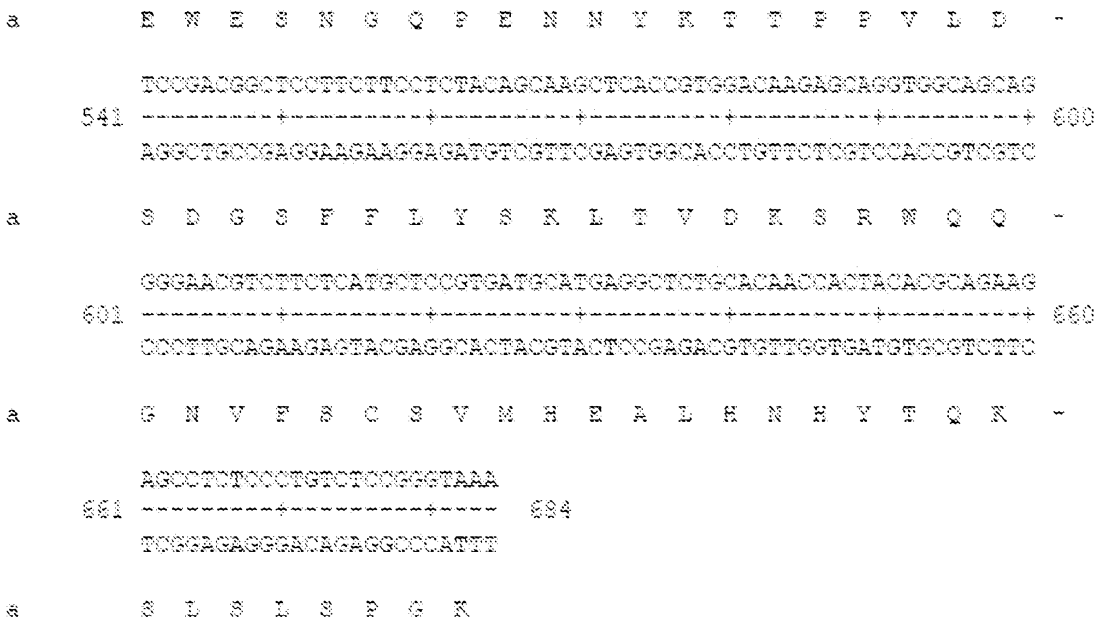

a E N E S N G Q P E N N Y K T T P P V L D -
TCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAG
541 ----------+----------+----------+----------+----------+----------+ 600
AGGCTGCCGAGGAAGAAGGAGATGTCGTTCGAGTGGCACCTGTTCTCGTCCACCGTCGTC
a S D G S F F L Y S K L T V D K S R W Q Q -
GGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAG
601 ----------+----------+----------+----------+----------+----------+ 660
CCCTTGCAGAAGAGTACGAGGCACTACGTACTCCGAGACGTGTTGGTGATGTGCGTCTTC
a G N V F S C S V M H E A L H N H Y T Q K -
AGCCTCTCCCTGTCTCCGGGTAAA
661 ----------+----------+----- 694
TCGGAGAGGGACAGAGGCCCATTT
a S L S L S P G K

FIG. 29A

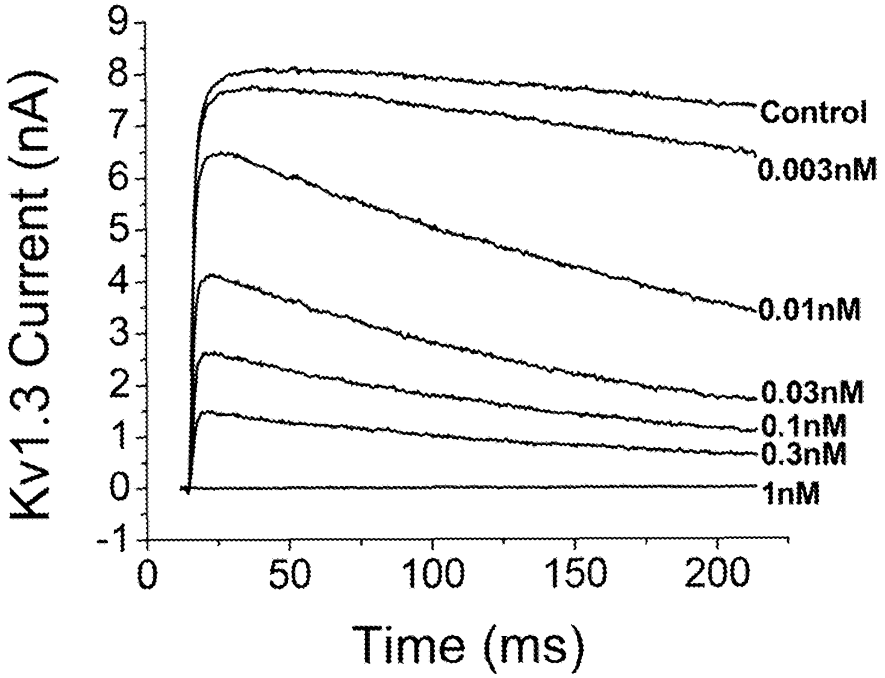


FIG. 29B

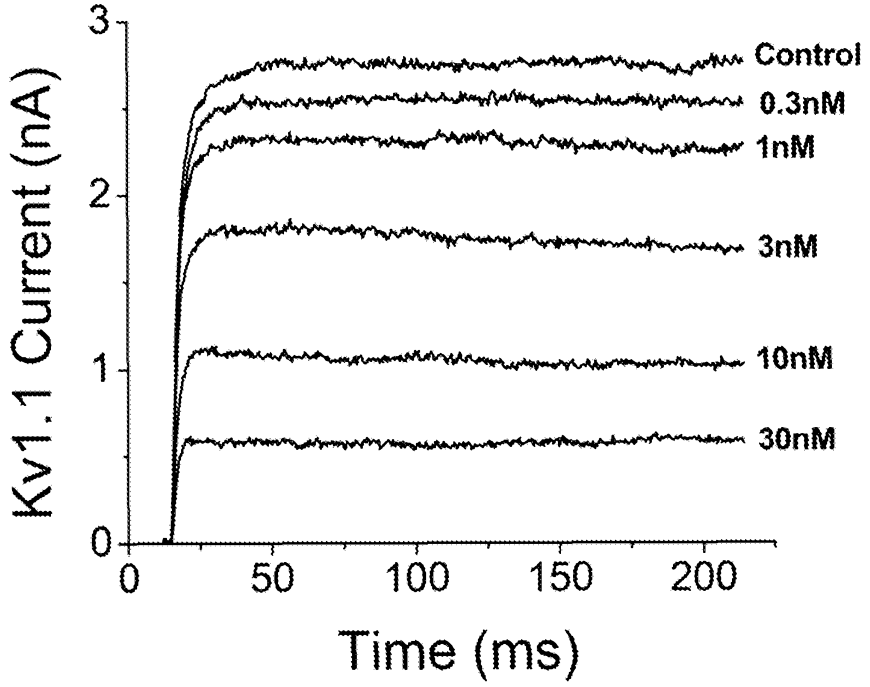


FIG. 30A

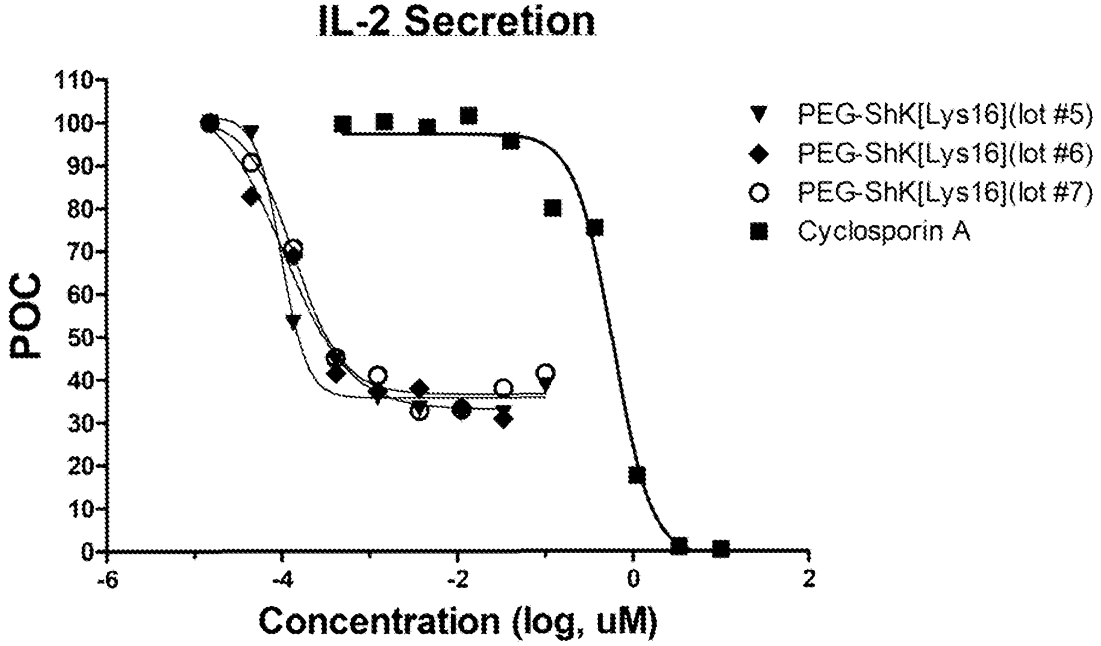


FIG. 30B

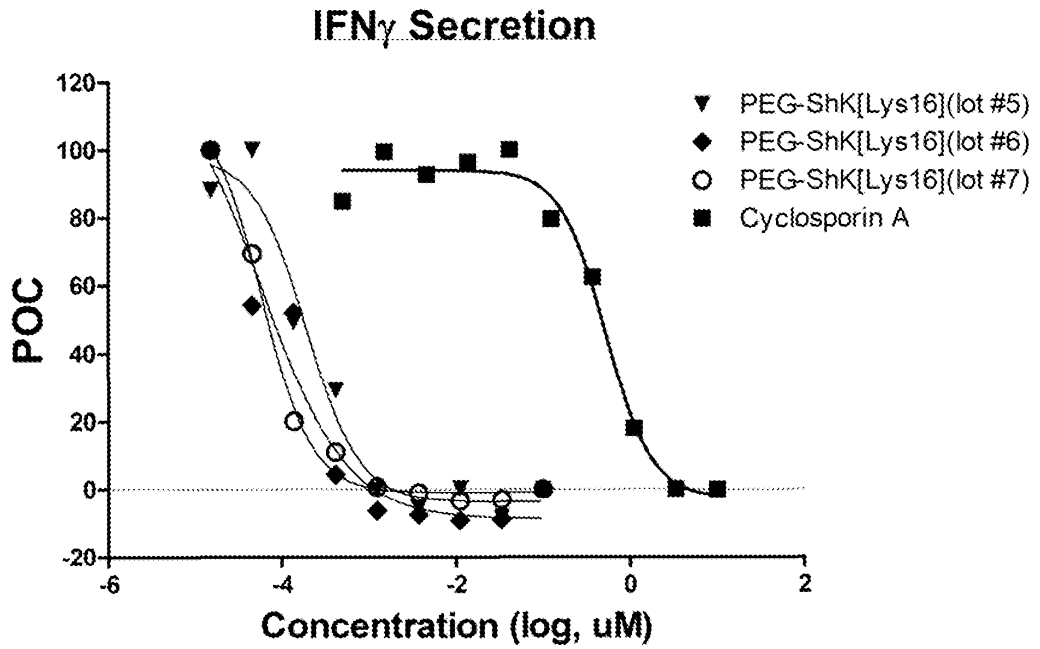


FIG. 31

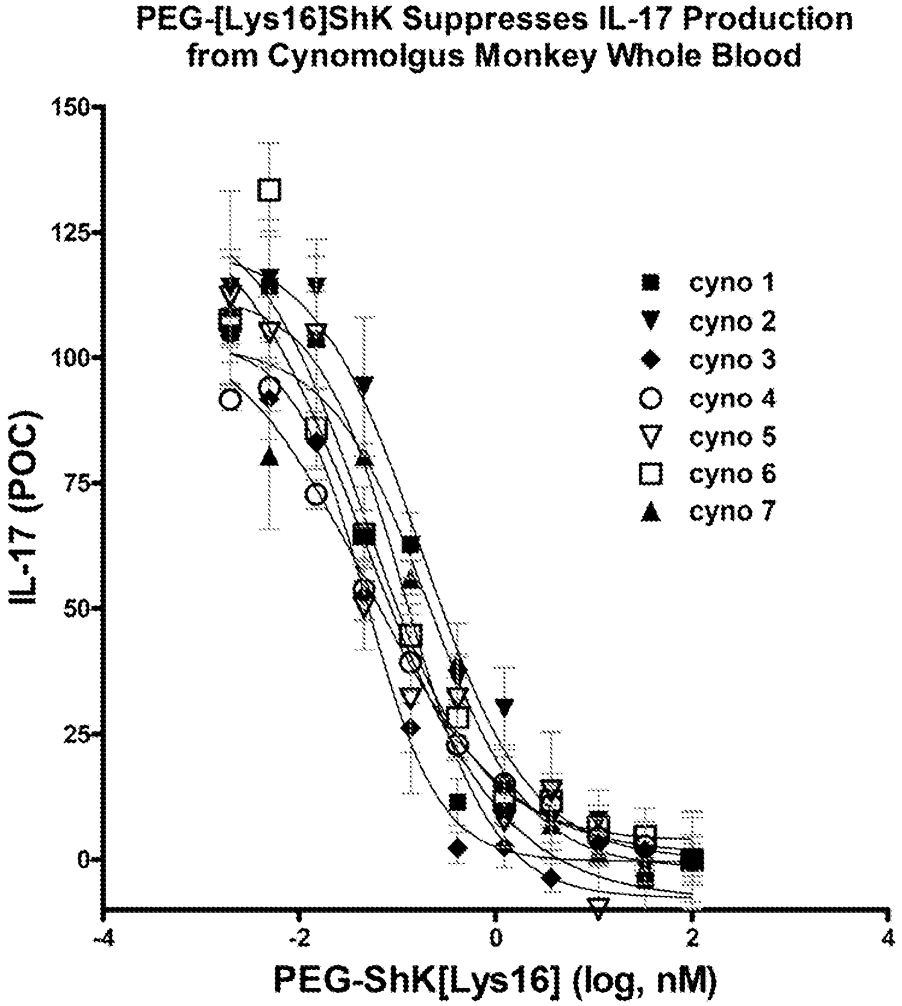

PEG-[Lys16]ShK Suppresses IL-17 Production
from Cynomolgus Monkey Whole Blood
IL-17 (POC)
150
125
100
75
50
25
0
-4
-2
0
2
4
PEG-ShK[Lys16] (log, nM)
cyno 1
cyno 2
cyno 3
cyno 4
cyno 5
cyno 6
cyno 7

FIG. 32

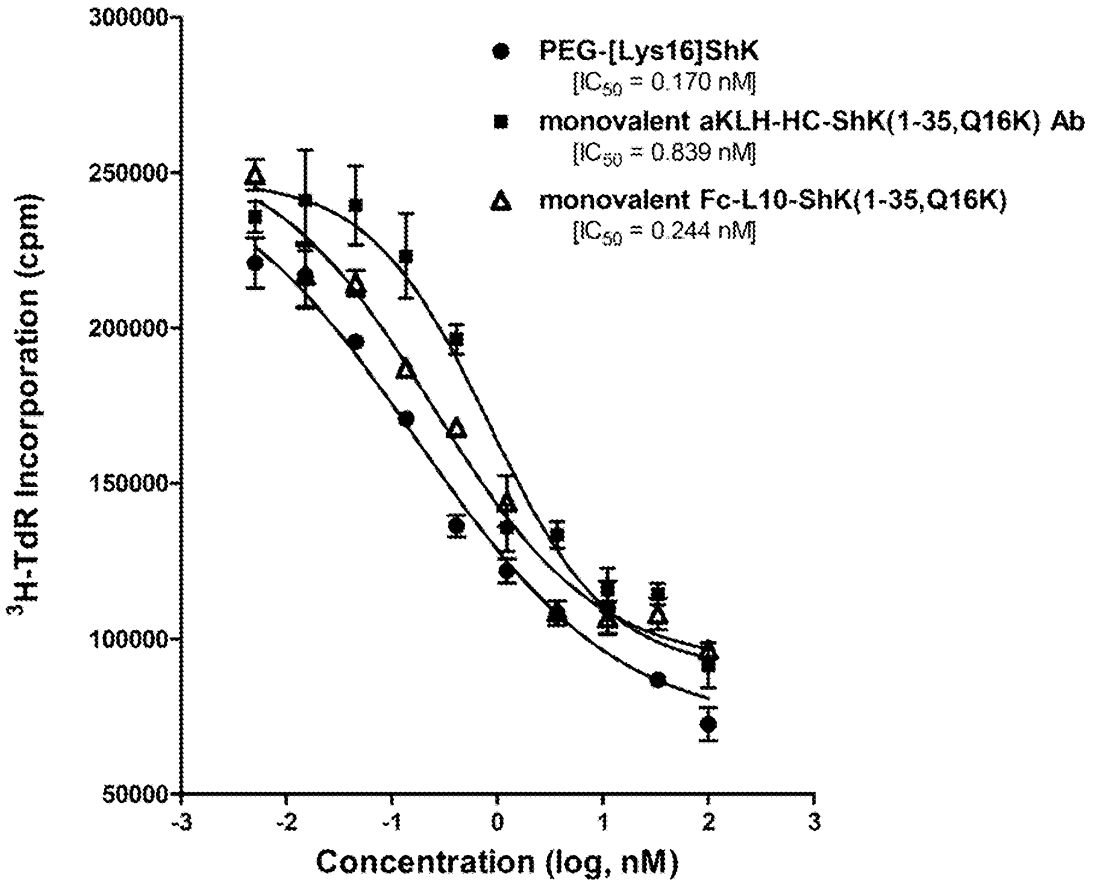

PEG-[Lys16]ShK
[IC50 = 0.170 nM]
monovalent aKLH-HC-ShK(1-35,Q16K) Ab
[IC50 = 0.839 nM]
monovalent Fc-L10-ShK(1-35,Q16K)
[IC50 = 0.244 nM]
3H-TdR Incorporation (cpm)
300000
250000
200000
150000
100000
50000
-3
-2
-1
0
1
2
3
Concentration (log, nM)

FIG. 33

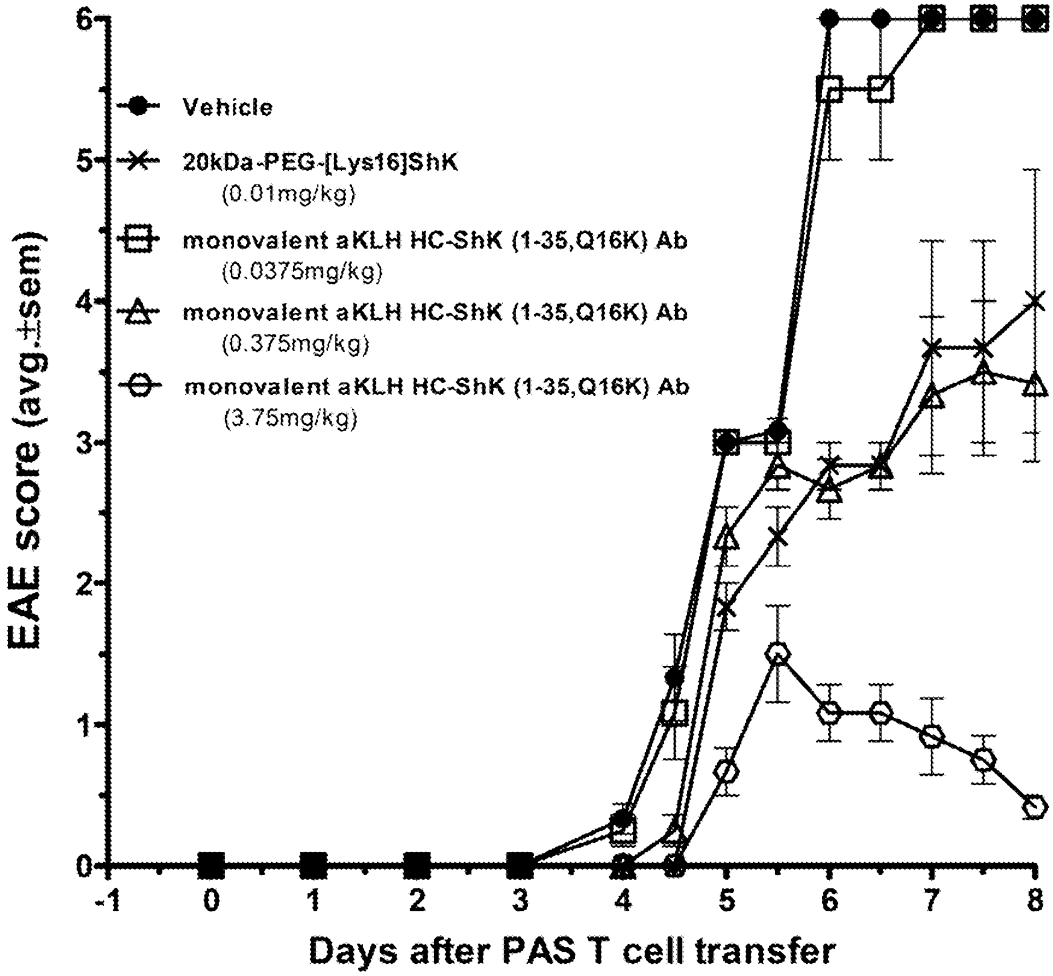

EAE score (avg.±sem)
Days after PAS T cell transfer
Vehicle
20kDa-PEG-[Lys16]ShK
(0.01mg/kg)
monovalent aKLH HC-ShK (1-35,Q16K) Ab
(0.0375mg/kg)
monovalent aKLH HC-ShK (1-35,Q16K) Ab
(0.375mg/kg)
monovalent aKLH HC-ShK (1-35,Q16K) Ab
(3.75mg/kg)
0
1
2
3
4
5
6
-1
0
1
2
3
4
5
6
7
8

FIG. 34A

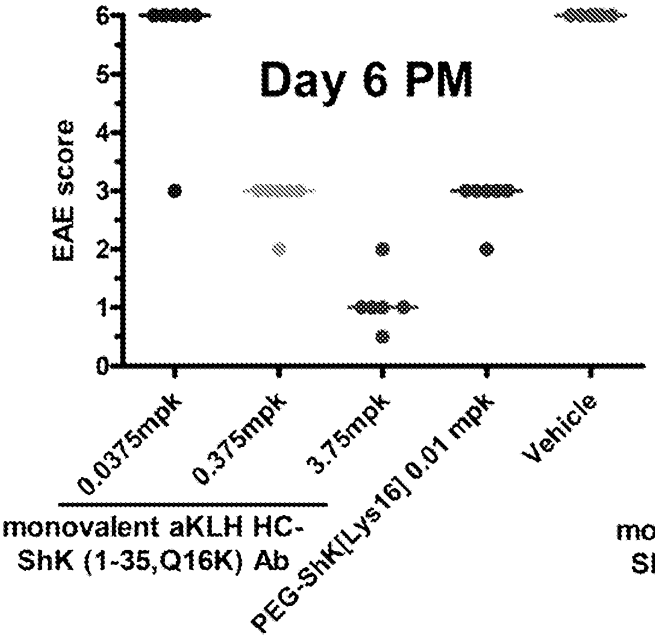


FIG. 34B

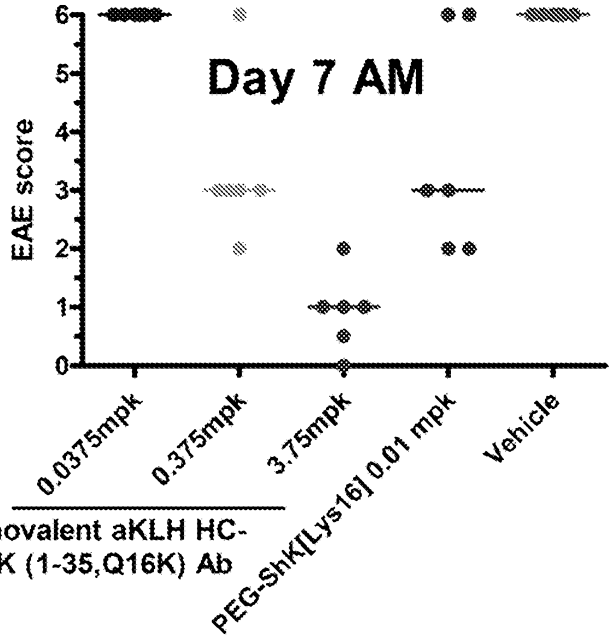


FIG. 34C

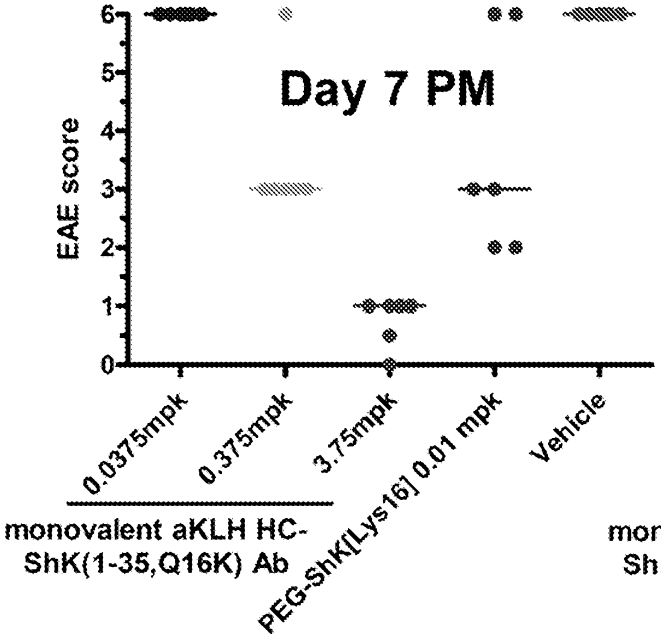


FIG. 34D

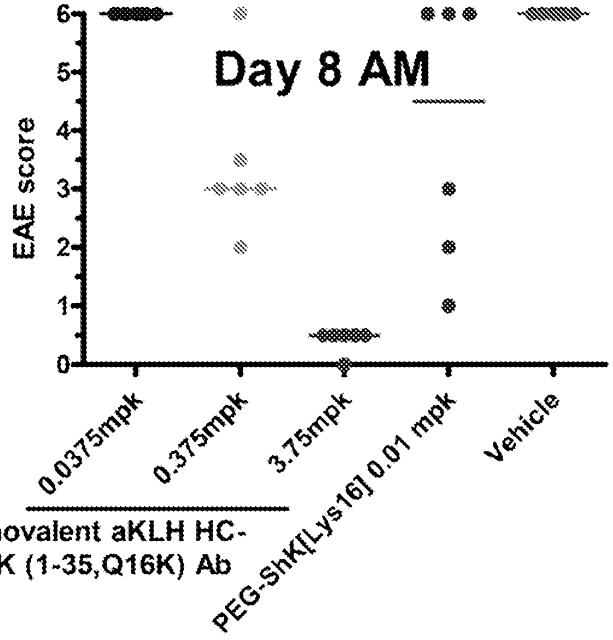


FIG. 35

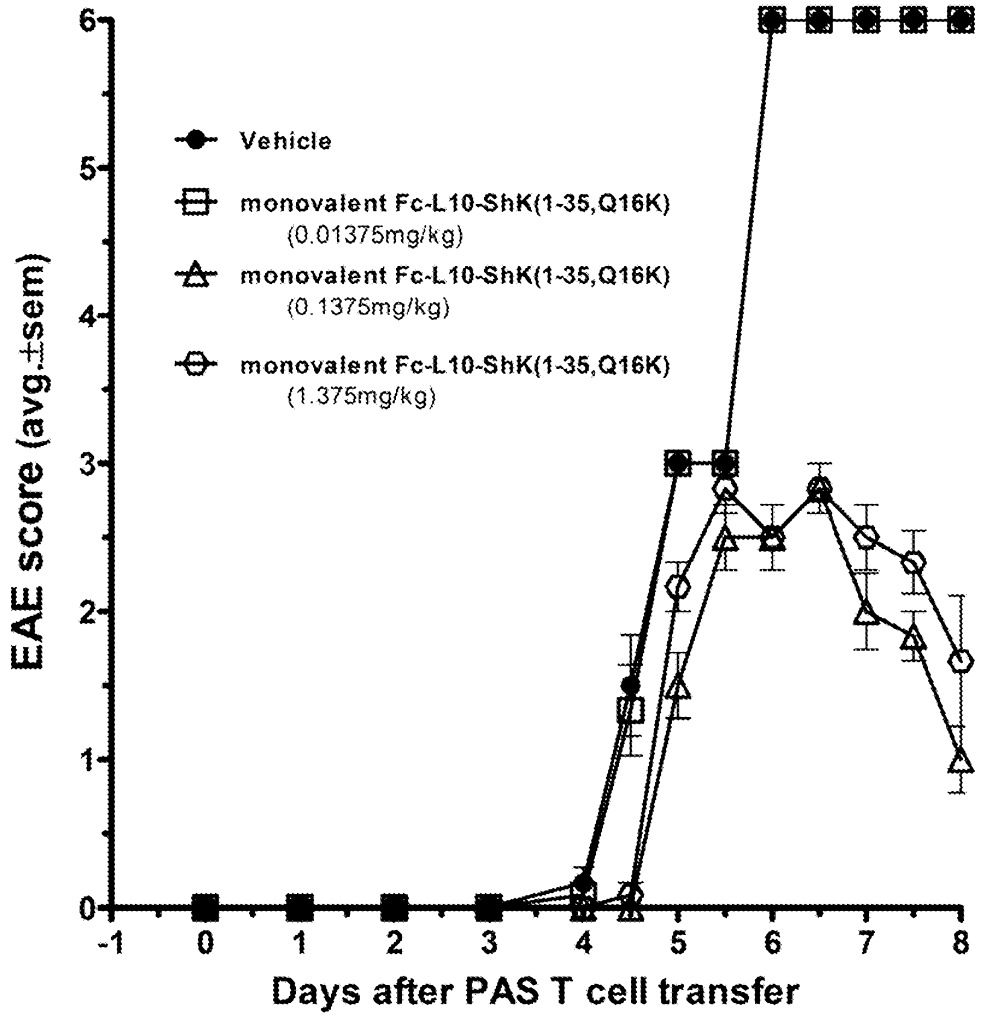

Vehicle
monovalent Fc-L10-ShK(1-35,Q16K)
(0.01375mg/kg)
monovalent Fc-L10-ShK(1-35,Q16K)
(0.1375mg/kg)
monovalent Fc-L10-ShK(1-35,Q16K)
(1.375mg/kg)
EAE score (avg.±sem)
Days after PAS T cell transfer
0
1
2
3
4
5
6
-1
0
1
2
3
4
5
6
7
8

FIG. 36A

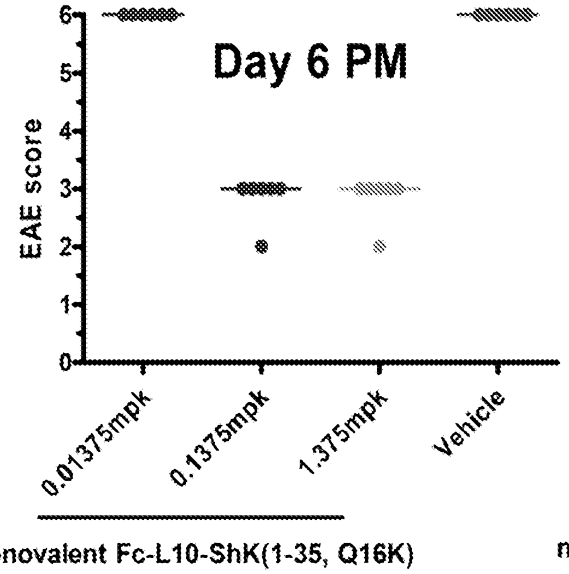


FIG. 36B

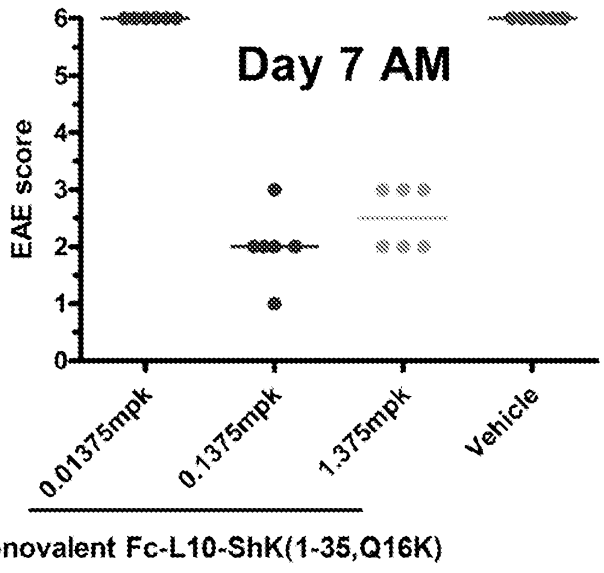


FIG. 36C

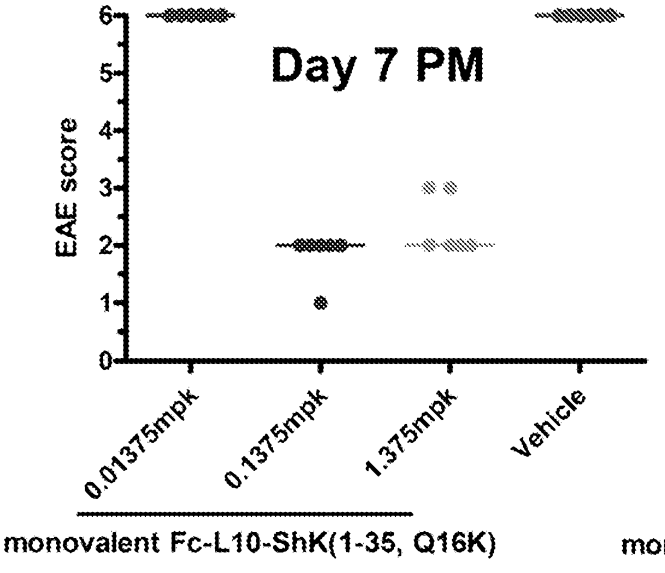
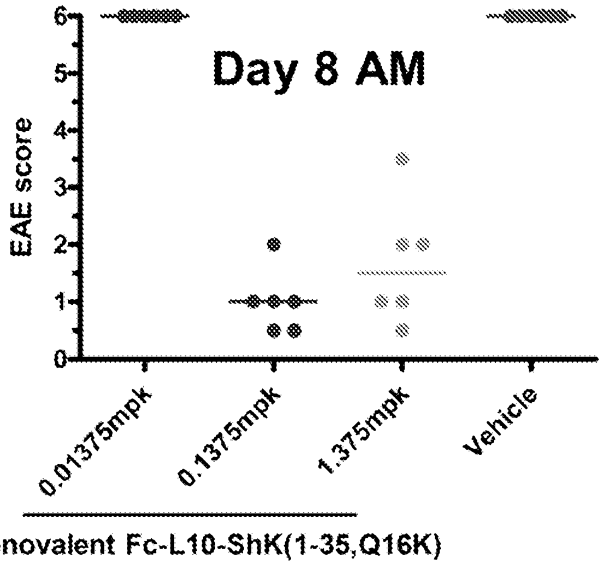


FIG. 36D



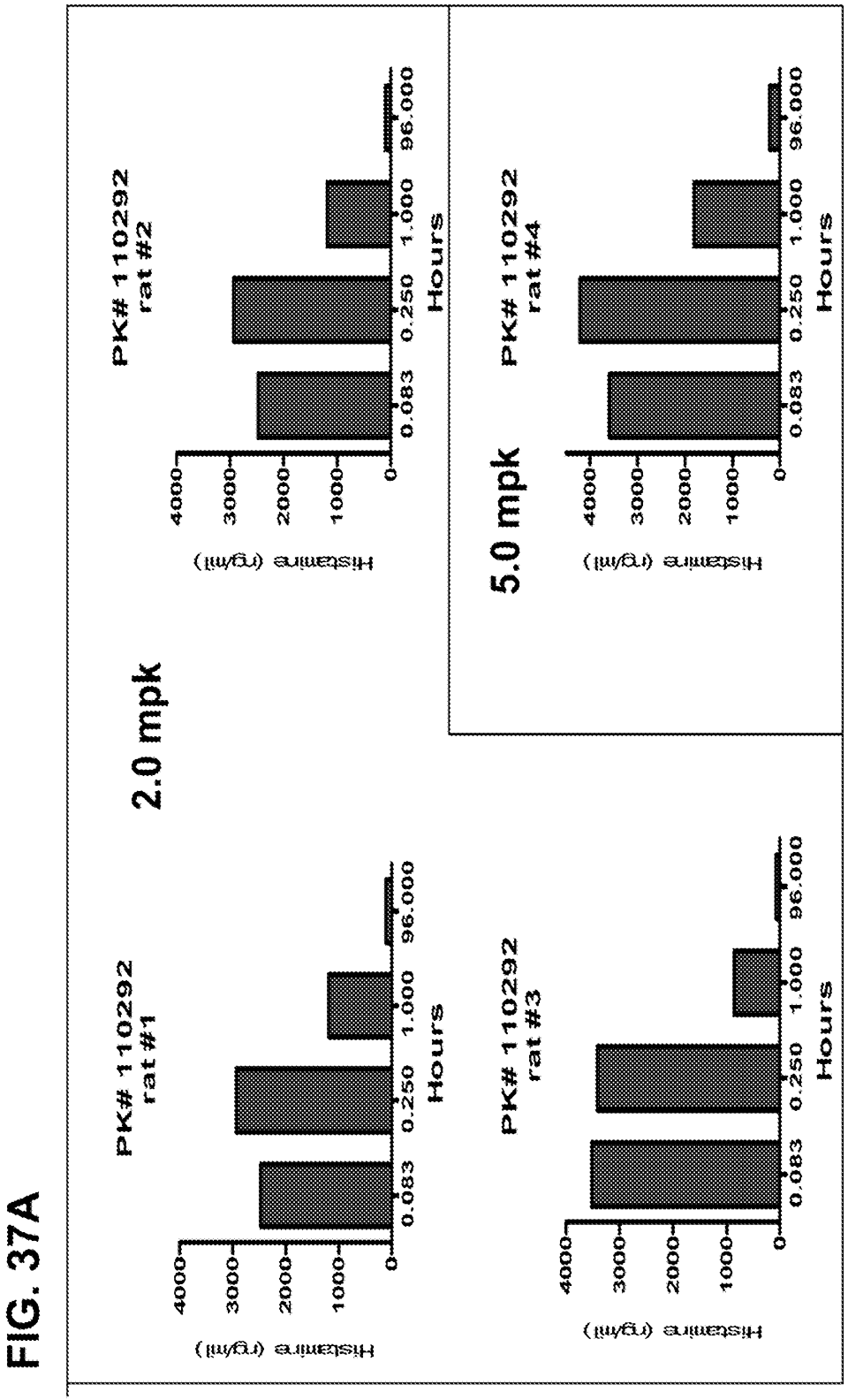


FIG. 37A

FIG. 37B

FIG. 38

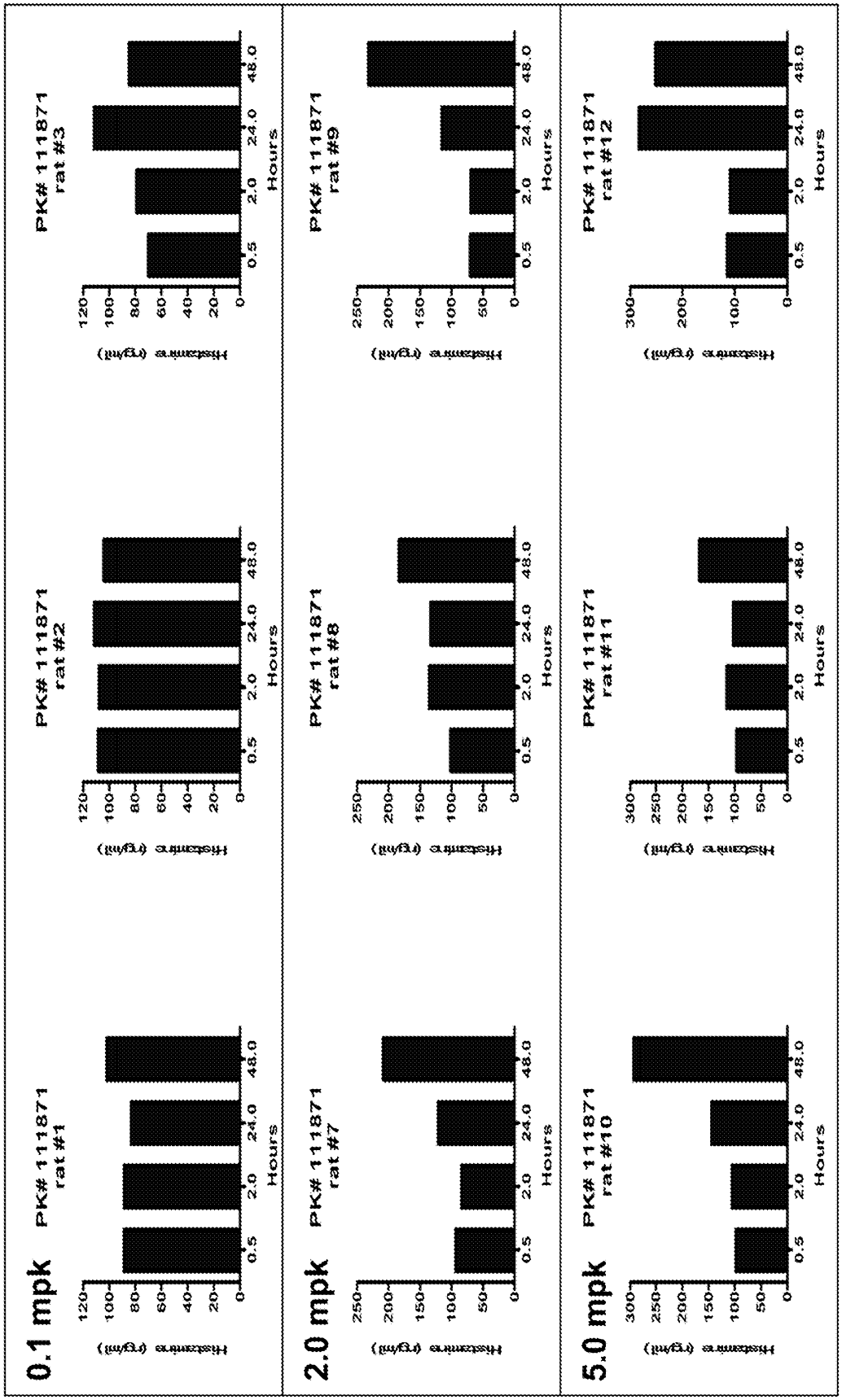

0.1 mpk
2.0 mpk
5.0 mpk
PK# 111871 rat #1
PK# 111871 rat #2
PK# 111871 rat #3
PK# 111871 rat #7
PK# 111871 rat #8
PK# 111871 rat #9
PK# 111871 rat #10
PK# 111871 rat #11
PK# 111871 rat #12
Histamine (ng/ml)
Hours
0.5
2.0
24.0
48.0

FIG. 39

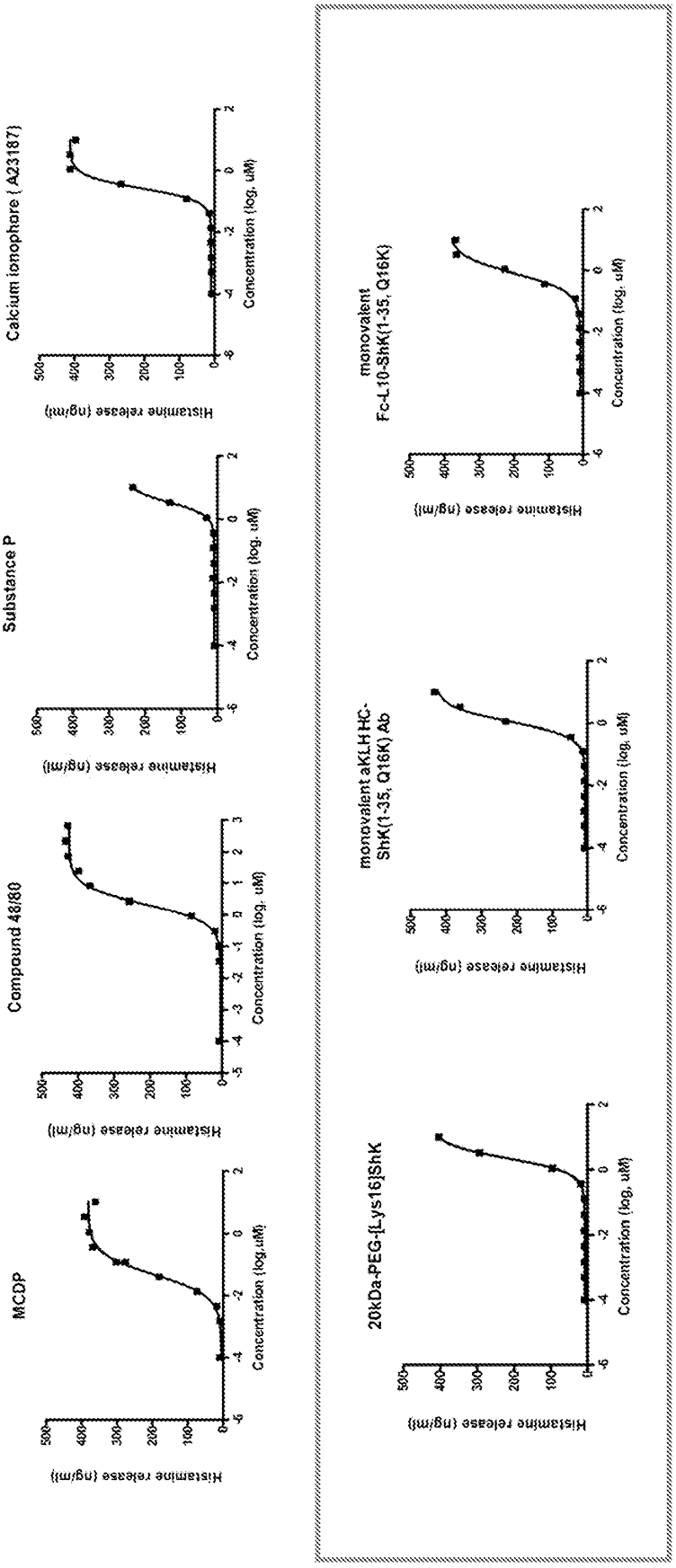
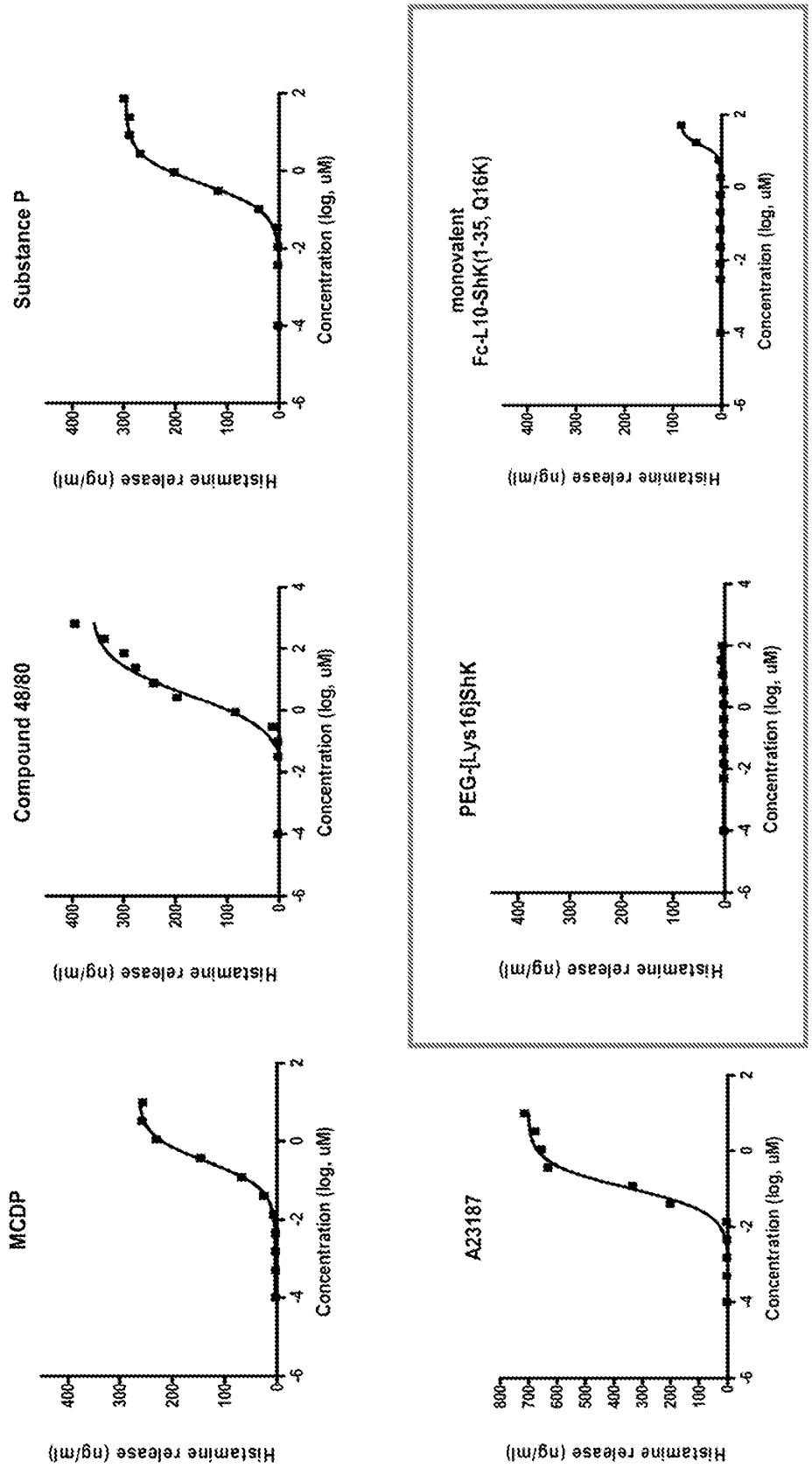

MCDP
Compound 48/80
Substance P
Calcium ionophore (A23187)
20kDa-PEG-[Lys16]ShK
monovalent aKLH HC-ShK(1-35, Q16K) Ab
monovalent Fc-L10-ShK(1-35, Q16K)
Histamine release (ng/ml)
Concentration (log, uM)

FIG. 40


MCDP
Histamine release (ng/ml)
Concentration (log, uM)
Compound 48/80
Histamine release (ng/ml)
Concentration (log, uM)
Substance P
Histamine release (ng/ml)
Concentration (log, uM)
A23187
Histamine release (ng/ml)
Concentration (log, uM)
PEG-[Lys16]ShK
Histamine release (ng/ml)
Concentration (log, uM)
monovalent
Fc-L10-ShK(1-35, Q16K)
Histamine release (ng/ml)
Concentration (log, uM)

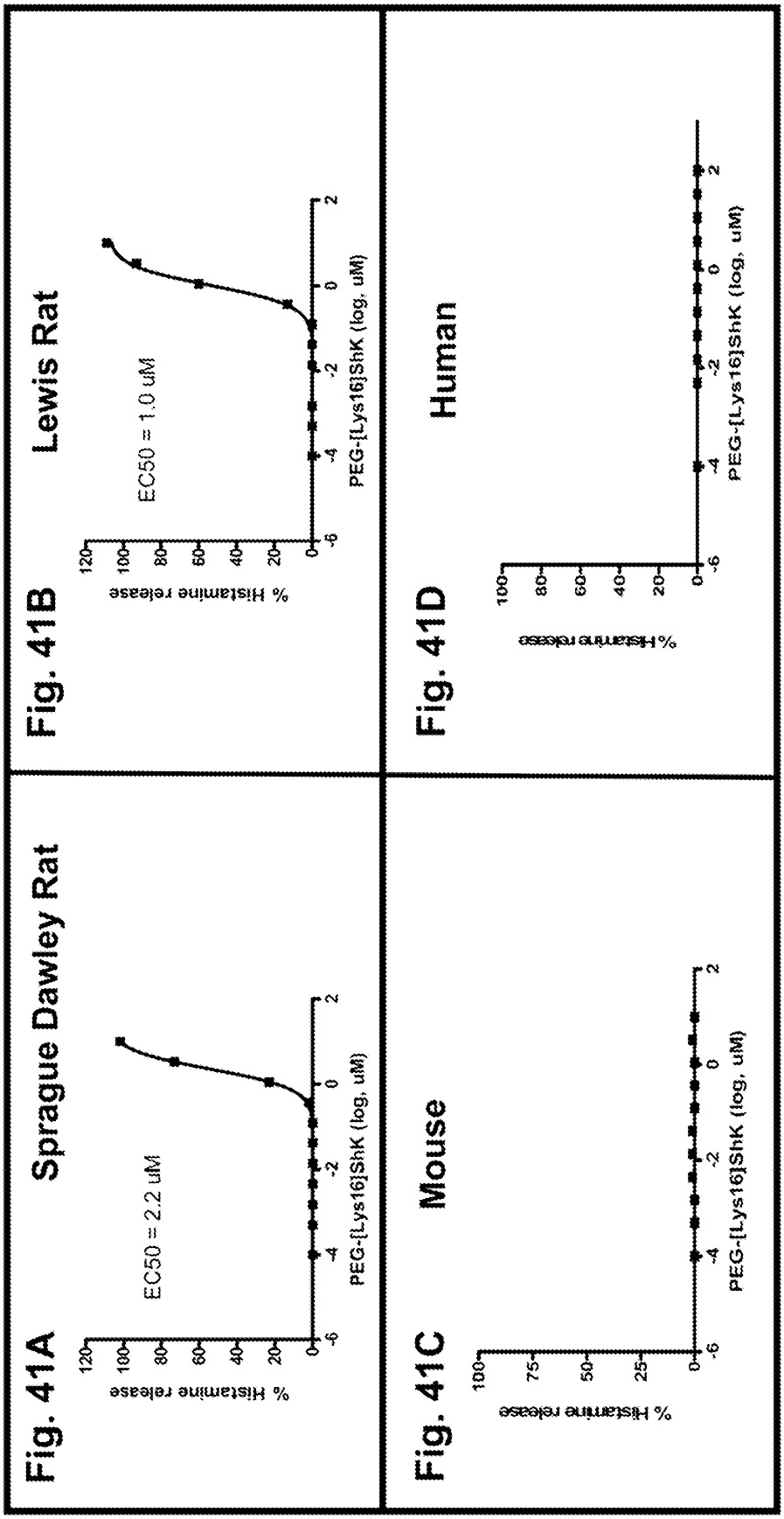


Fig. 41A
Sprague Dawley Rat
EC50 = 2.2 uM
% Histamine release
PEG-[Lys16]ShK (log, uM)
Fig. 41B
Lewis Rat
EC50 = 1.0 uM
% Histamine release
PEG-[Lys16]ShK (log, uM)
Fig. 41C
Mouse
% Histamine release
PEG-[Lys16]ShK (log, uM)
Fig. 41D
Human
% Histamine release
PEG-[Lys16]ShK (log, uM)

FIG. 42

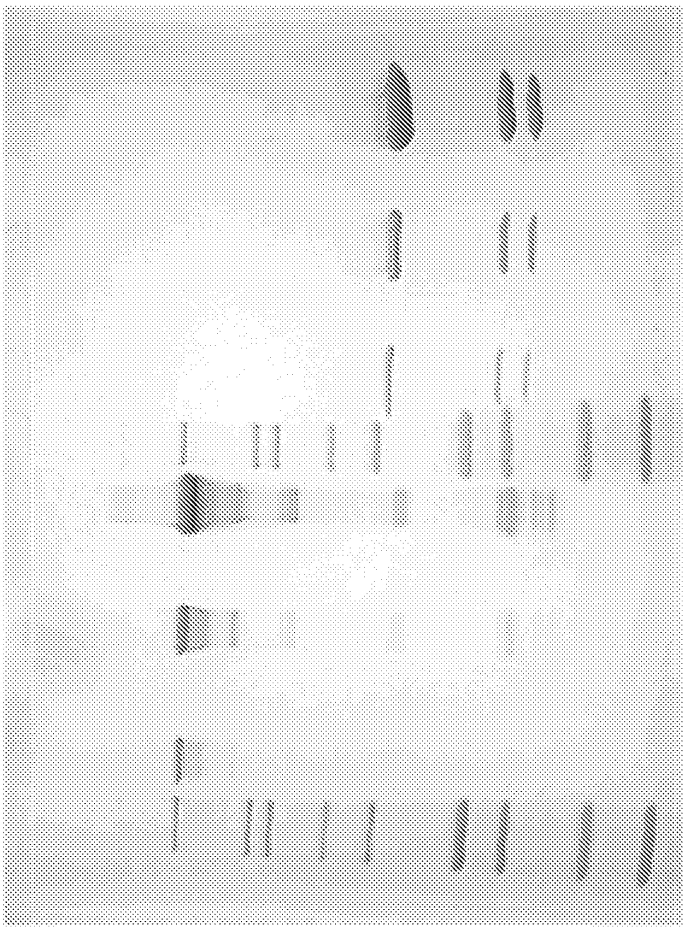


FIG. 43

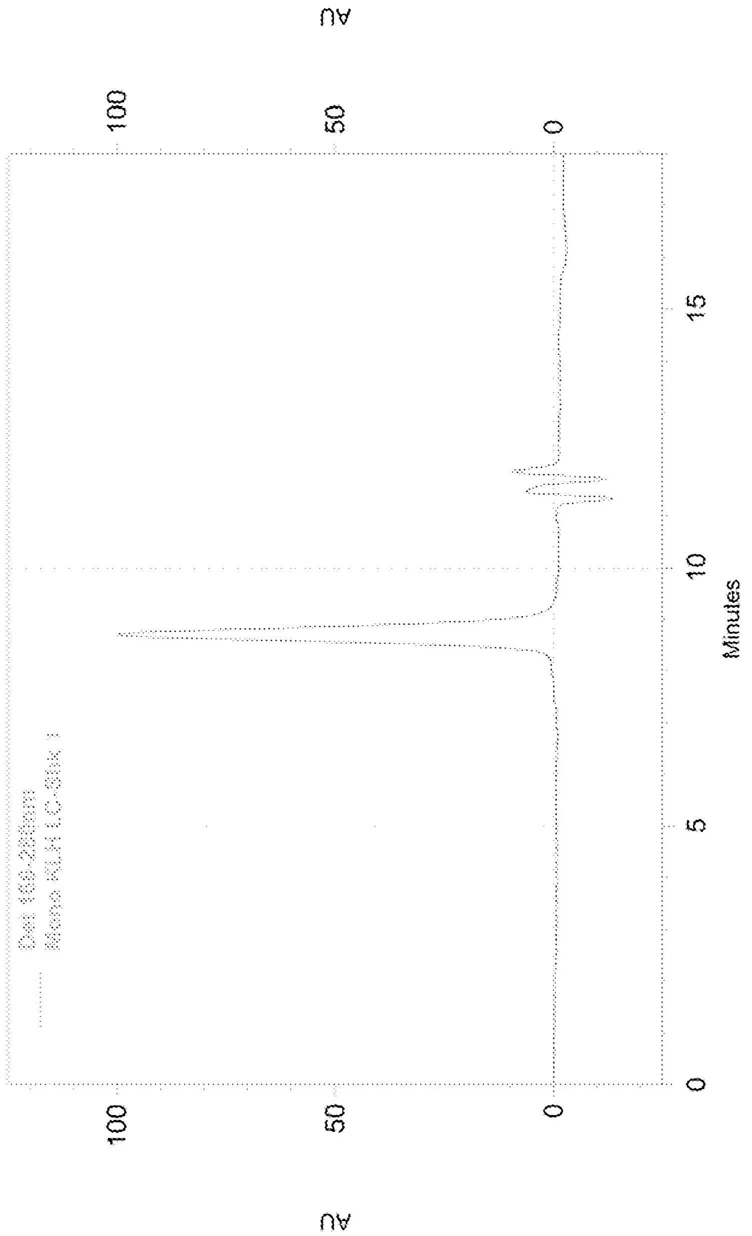


FIG. 44A

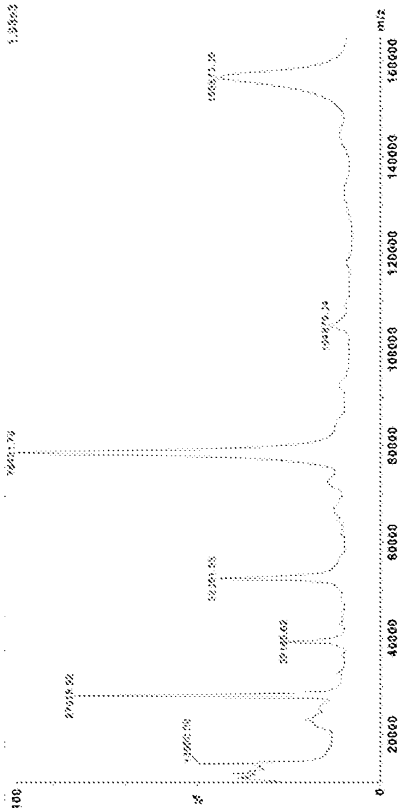


FIG. 44B

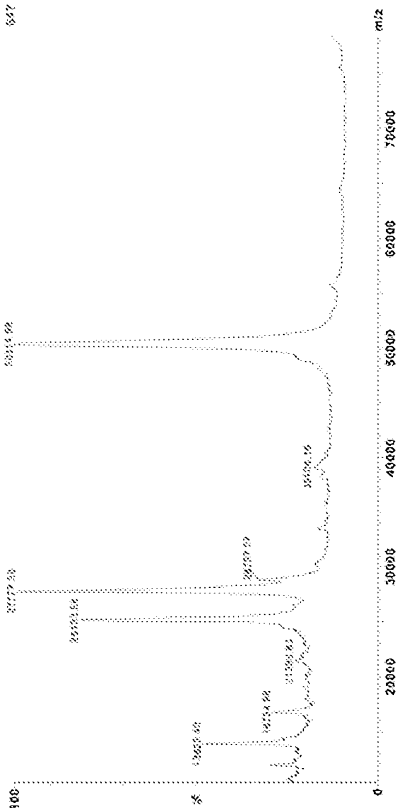


FIG. 45

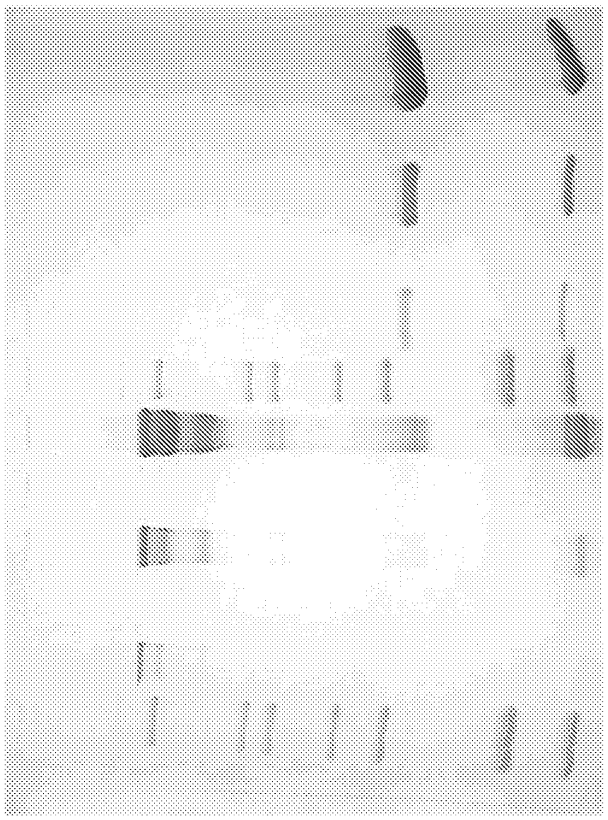


FIG. 46

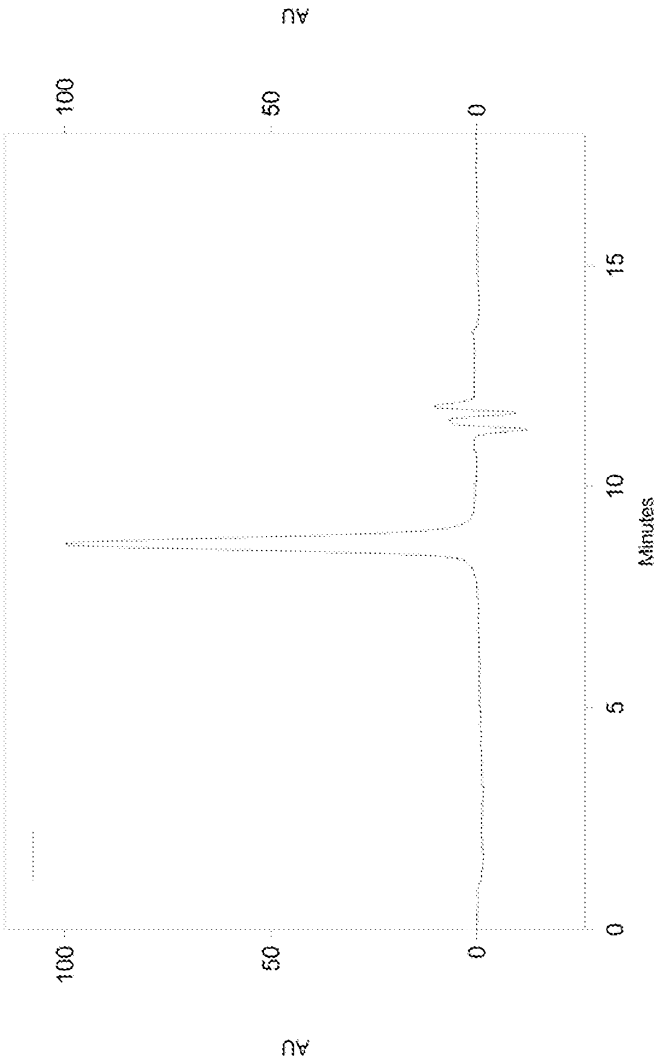


FIG. 47A

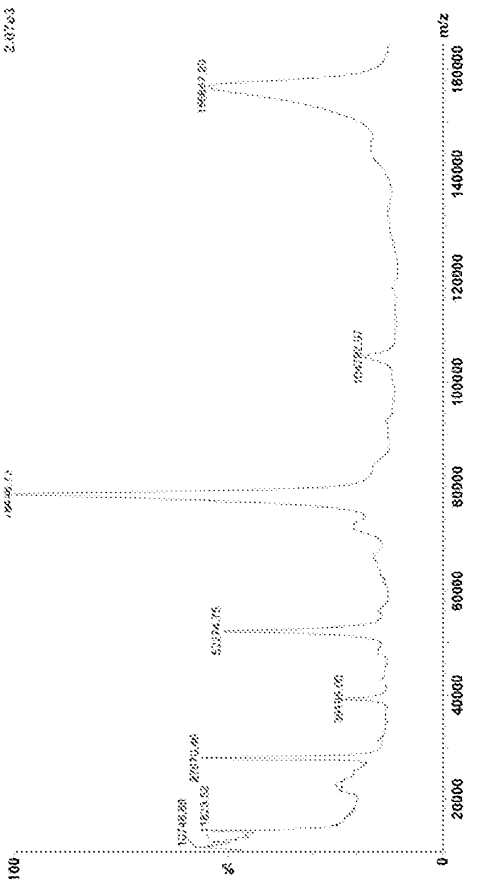


FIG. 47B

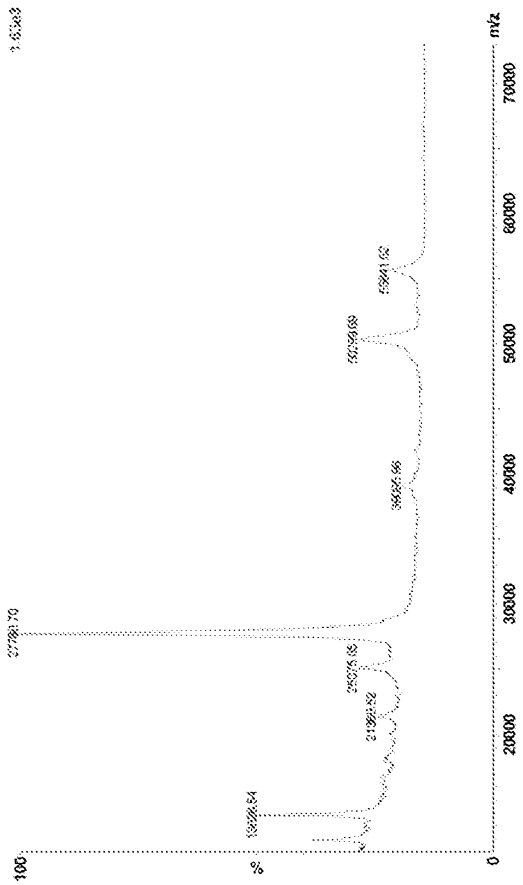


FIG. 48

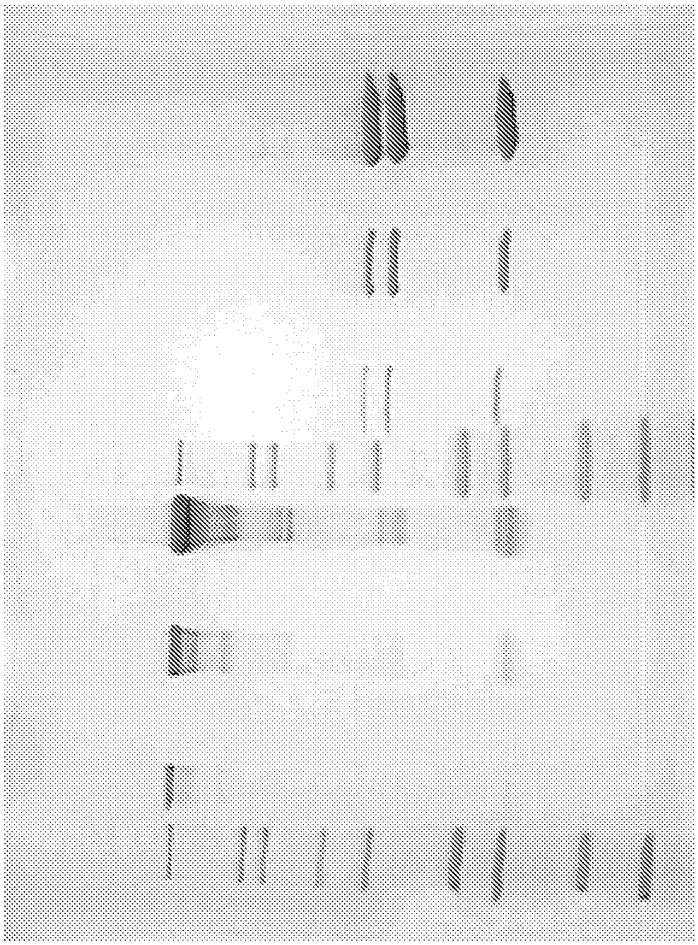


FIG. 49

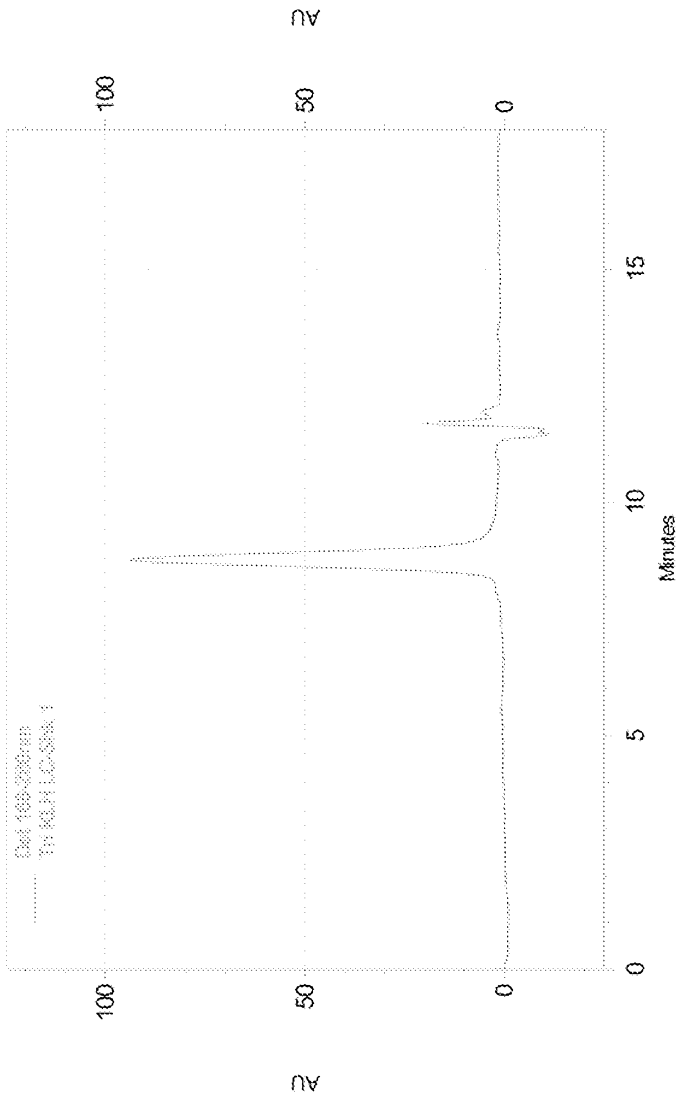


FIG. 50A

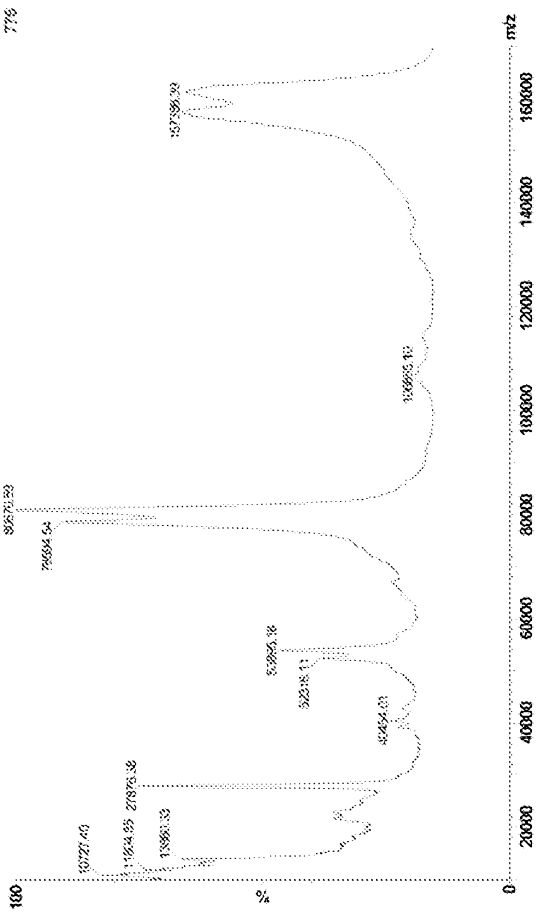


FIG. 50B

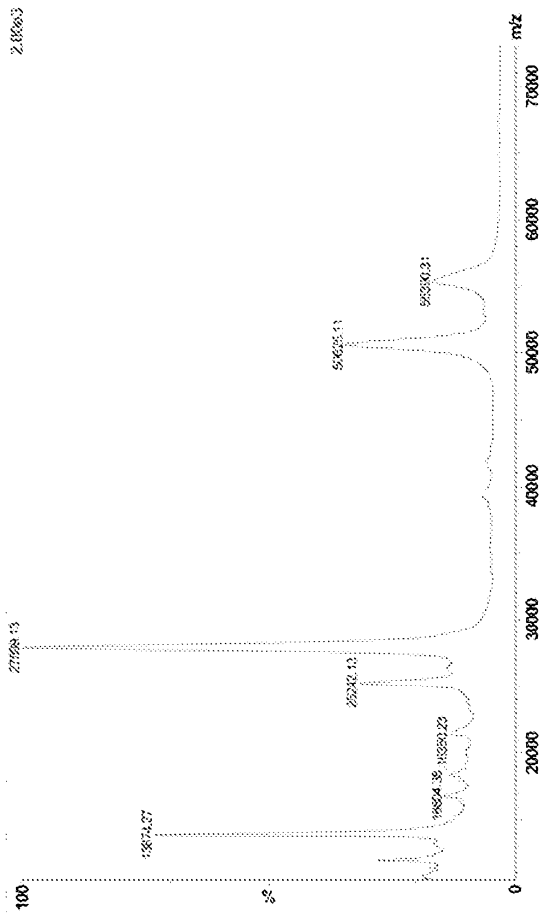


FIG. 51

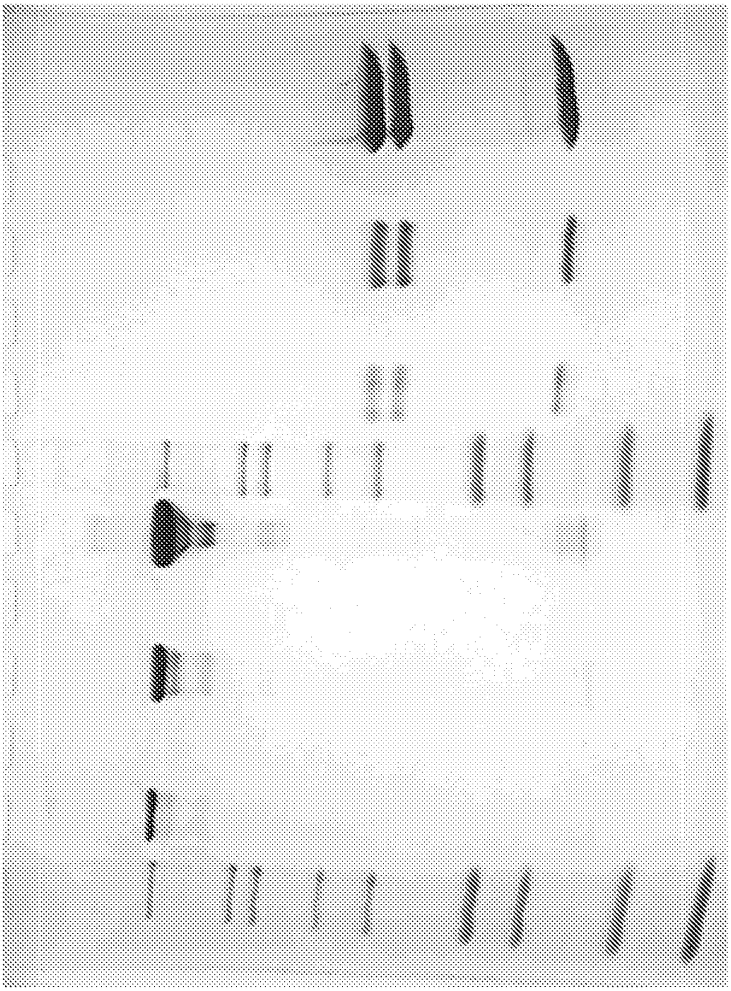


FIG. 52

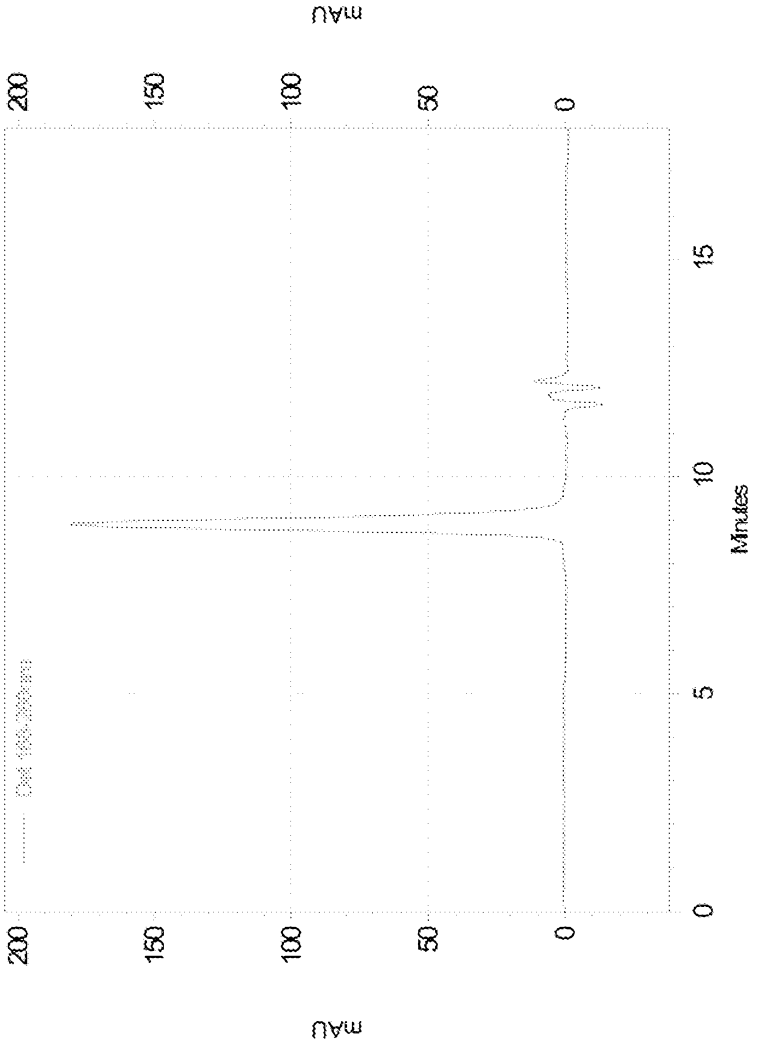


FIG. 53

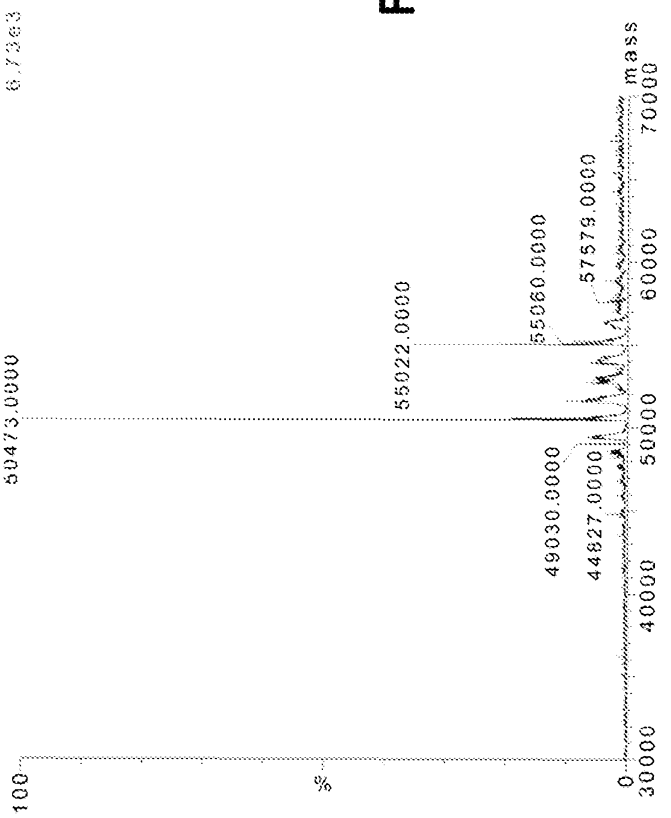


FIG. 54A

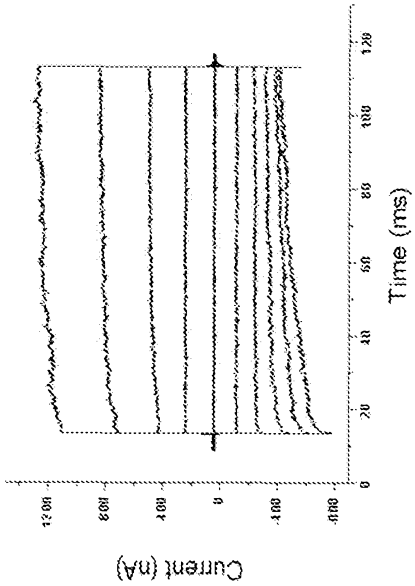
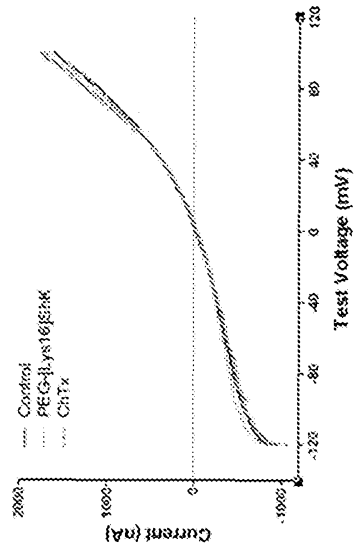


FIG. 54B



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<140> XX/XX XX/XXXXXX
<141> 2010-03-19

<150> 61/210,594
<151> 2009-03-20

<160> 482

<170> PatentIn version 3.4

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<212> PRT
<213> Stichodactyla helianthus

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1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 2
<211> 35
<212> PRT
<213> Radianthus magnifica

<400> 2

Arg Thr Cys Lys Asp Leu Ile Pro Val Ser Glu Cys Thr Asp Ile Arg
1 5 10 15

Cys Arg Thr Ser Met Lys Tyr Arg Leu Asn Leu Cys Arg Lys Thr Cys
20 25 30

Gly Ser Cys
35

<210> 3
<211> 35
<212> PRT
<213> Anemonia erythraea

<400> 3

Arg Ala Cys Lys Asp Tyr Leu Pro Lys Ser Glu Cys Thr Gln Phe Arg
1 5 10 15

Cys Arg Thr Ser Met Lys Tyr Lys Tyr Thr Asn Cys Lys Lys Thr Cys

20 25 30

Gly Thr Cys
35

<210> 4
<211> 38
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Toxin peptide analog

<220>
<221> MISC_FEATURE
<223> Carboxy-terminal residue is optionally amidated

<220>
<221> MISC_FEATURE
<222> (1)..(2)
<223> Xaa 1 and Xaa 2 are absent, or Xaa 1 is absent and Xaa 2 is Glu, Ser, Ala, or Thr, or Xaa 1 is Arg or Ala and Xaa 2 is Glu, Ser, Ala, or Thr

<220>
<221> DISULFID
<222> (3)..(35)

<220>
<221> MISC_FEATURE
<222> (4)..(4)
<223> Xaa 4 is an alkyl, basic, or acidic amino acid residue

<220>
<221> MISC_FEATURE
<222> (6)..(6)
<223> Xaa 6 is Thr, Tyr, Ala, or Leu

<220>
<221> MISC_FEATURE
<222> (7)..(7)
<223> Xaa 7 is Leu, Ile, Ala, or Lys

<220>
<221> MISC_FEATURE
<222> (8)..(8)
<223> Xaa 8 is Pro, Ala, Arg, Lys, 1-Nal, or Glu

<220>
<221> MISC_FEATURE
<222> (9)..(9)
<223> Xaa 9 is Lys, Ala, Val or or an acidic amino acid residue

<220>
<221> MISC_FEATURE
<222> (10)..(10)
<223> Xaa 10 is Ser, Glu, Arg, or Ala

<220>
<221> MISC_FEATURE
<222> (11)..(11)
<223> Xaa 11 is Arg, Glu, or Ala

<220>
<221> DISULFID

<222> (12)..(28)

<220>
<221> MISC_FEATURE
<222> (13)..(13)
<223> Xaa 13 is Thr, Ala, Arg, Lys, 1-Nal, or Glu

<220>
<221> MISC_FEATURE
<222> (14)..(14)
<223> Xaa 14 is Gln, Ala or an acidic amino acid reside

<220>
<221> MISC_FEATURE
<222> (15)..(15)
<223> Xaa 15 is an alkyl or aromatic amino acid residue

<220>
<221> MISC_FEATURE
<222> (16)..(16)
<223> Xaa 16 is a basic, alkyl, or aromatic amino acid residue other than Ala, Gln, Glu or Arg

<220>
<221> DISULFID
<222> (17)..(32)

<220>
<221> MISC_FEATURE
<222> (18)..(18)
<223> Xaa 18 is an Ala or an acidic or basic amino acid residue

<220>
<221> MISC_FEATURE
<222> (19)..(19)
<223> Xaa 19 is Thr, Ala or a basic amino acid residue

<220>
<221> MISC_FEATURE
<222> (20)..(20)
<223> Xaa 20 is Ser, Ala or a basic amino acid residue

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Xaa 21 is an alkyl or aromatic amino acid residue, other than Ala or Met

<220>
<221> MISC_FEATURE
<222> (22)..(22)
<223> Xaa 22 is Lys or Ala

<220>
<221> MISC_FEATURE
<222> (23)..(23)
<223> Xaa 23 is Tyr or Ala

<220>
<221> MISC_FEATURE
<222> (24)..(24)
<223> Xaa 24 is Arg, Lys, or Ala

<220>
<221> MISC_FEATURE
<222> (25)..(25)
<223> Xaa 25 is Tyr, Leu, or Ala

<220>
<221> MISC_FEATURE
<222> (26)..(26)
<223> Xaa 26 is Ser, Thr, Asn, Ala, or an aromatic amino acid residue

<220>
<221> MISC_FEATURE
<222> (27)..(27)
<223> Xaa 27 is Leu, Ala, Asn, or an aromatic amino acid residue

<220>
<221> MISC_FEATURE
<222> (29)..(29)
<223> Xaa 29 is 1-Nal, 2-Nal, Ala, or an basic amino acid residue

<220>
<221> MISC_FEATURE
<222> (30)..(30)
<223> Xaa 30 is Ala or an acidic or basic amino acid residue

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<222> (31)..(31)
<223> Xaa 31 is Thr, Ala or an aromatic amino acid residue

<220>
<221> MISC_FEATURE
<222> (33)..(33)
<223> Xaa 33 is Gly, Ala, Arg, Lys, 1-Nal, or Glu

<220>
<221> MISC_FEATURE
<222> (34)..(34)
<223> Xaa 34 is Thr, Ser, Ala, Lys, or an aromatic amino acid residue

<220>
<221> MISC_FEATURE
<222> (36)..(38)
<223> Xaa in positions 36-38 are each independently absent are independently a neutral, basic, acidic, or N-alkylated amino acid residue

<220>
<221> MOD_RES
<222> (38)..(38)
<223> Carboxy-terminal residue is optionally amidated

<400> 4

```
Xaa Xaa Cys Xaa Asp Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa
1               5                   10                  15


Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Cys
            20                  25                  30


Xaa Xaa Cys Xaa Xaa Xaa
        35
```

<210> 5
<211> 36
<212> PRT
<213> Actinia equina

<400> 5

Gly Cys Lys Asp Asn Phe Ser Ala Asn Thr Cys Lys His Val Lys Ala
1 5 10 15

Asn Asn Asn Cys Gly Ser Gln Lys Tyr Ala Thr Asn Cys Ala Lys Thr
20 25 30

Cys Gly Lys Cys
35

<210> 6
<211> 36
<212> PRT
<213> Anemonia sulcata

<400> 6

Ala Cys Lys Asp Asn Phe Ala Ala Ala Thr Cys Lys His Val Lys Glu
1 5 10 15

Asn Lys Asn Cys Gly Ser Gln Lys Tyr Ala Thr Asn Cys Ala Lys Thr
20 25 30

Cys Gly Lys Cys
35

<210> 7
<211> 37
<212> PRT
<213> Bunodosoma granulifera

<400> 7

Val Cys Arg Asp Trp Phe Lys Glu Thr Ala Cys Arg His Ala Lys Ser
1 5 10 15

Leu Gly Asn Cys Arg Thr Ser Gln Lys Tyr Arg Ala Asn Cys Ala Lys
20 25 30

Thr Cys Glu Leu Cys
35

<210> 8
<211> 35
<212> PRT
<213> Stichodactyla helianthus

<220>
<221> MISC_FEATURE
<223> Pegylated

<400> 8

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
        35

<210> 9
<211> 38
<212> PRT
<213> Orthochirus scrobiculosus

<400> 9

Gly Val Ile Ile Asn Val Lys Cys Lys Ile Ser Arg Gln Cys Leu Glu
1   5   10   15

Pro Cys Lys Lys Ala Gly Met Arg Phe Gly Lys Cys Met Asn Gly Lys
            20   25   30

Cys Ala Cys Thr Pro Lys
        35

<210> 10
<211> 35
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<213> ARTIFICIAL SEQUENCE

<220>
<223> HmK peptide

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norvaline

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 10

Arg Thr Cys Lys Asp Leu Ile Pro Val Ser Glu Cys Thr Asp Ile Lys
1   5   10   15

Cys Arg Thr Ser Xaa Lys Tyr Arg Leu Asn Leu Cys Arg Lys Thr Cys
            20   25   30

Gly Ser Cys
        35

<210> 11
<211> 35
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<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>

<221> MISC_FEATURE
<222> (16)..(16)
<223> 3-(1-naphthyl)alanine

<400> 11

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Xaa
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 12
<211> 35
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<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (26)..(26)
<223> 3-(1-naphthyl)alanine

<400> 12

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Xaa Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 13
<211> 35
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<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK[1-35, Q16K] polypeptide

<400> 13

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 14
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination toxin peptide analog (C-Terminal Amide)

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 14

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 15
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Position 21 Toxin Peptide Analog

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 15

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 16
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK PEGylated Toxin Peptide

<220>
<221> MISC_FEATURE
<222> (1)..(1)
<223> PEGylated

<400> 16

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1   5   10   15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 17
<211> 36
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK reference peptide

<220>
<221> MISC_FEATURE
<222> (1)..(1)
<223> L-phosphonotyrosine

<220>
<221> MISC_FEATURE
<222> (1)..(2)
<223> Between residues 1 and 2 is: {2-[2-Aminoethoxy]ethoxy}acetic acid

<400> 17

Xaa Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe
1   5   10   15

Gln Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr
            20   25   30

Cys Gly Thr Cys
        35

<210> 18
<211> 36
<212> PRT
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<220>
<223> ShK reference peptide

<220>
<221> MISC_FEATURE
<222> (1)..(2)
<223> Between residues 1 and 2 is: {2-[2-Aminoethoxy]ethoxy}acetic acid

<220>
<221> MISC_FEATURE
<222> (1)..(1)
<223> L-phosphonotyrosine

<220>
<221> MISC_FEATURE
<222> (22)..(22)
<223> Norleucine

<220>
<221> MOD_RES
<222> (36)..(36)
<223> AMIDATION

<400> 18

Xaa Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe
1   5   10   15

Gln Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr
            20   25   30

Cys Gly Thr Cys
        35

<210> 19
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 19

Arg Arg Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1   5   10   15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 20
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 20

Arg Ser Cys Arg Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1   5   10   15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
35

<210> 21
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 21

Arg Ser Cys Ile Arg Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 22
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 22

Arg Ser Cys Ile Asp Arg Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 23
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 23

Arg Ser Cys Ile Asp Thr Arg Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys

35

<210> 24
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 24

Arg Ser Cys Ile Asp Thr Ile Arg Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 25
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 25

Arg Ser Cys Ile Asp Thr Ile Pro Arg Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 26
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 26

Arg Ser Cys Ile Asp Thr Ile Pro Lys Arg Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 27
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 27

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Arg Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 28
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 28

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Arg Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 29
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 29

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Arg Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 30

<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 30

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Arg
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 31
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 31

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Arg His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 32
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 32

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys Arg Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 33
<211> 35
<212> PRT

<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 33

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Arg Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 34
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 34

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Arg Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 35
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 35

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Arg Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 36
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 36

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1   5   10   15

Cys Lys His Ser Met Lys Arg Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 37
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 37

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1   5   10   15

Cys Lys His Ser Met Lys Tyr Arg Arg Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 38
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 38

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1   5   10   15

Cys Lys His Ser Met Lys Tyr Arg Leu Arg Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 39
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 39

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Arg Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 40
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 40

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Arg Thr Cys
20 25 30

Gly Thr Cys
35

<210> 41
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 41

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Arg Cys
20 25 30

Gly Thr Cys
35

<210> 42
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 42

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Arg Thr Cys
35

<210> 43
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 43

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Arg Cys
35

<210> 44
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 44

Glu Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 45
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 45

Arg Glu Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln

1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 46
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 46

Arg Ser Cys Glu Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 47
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 47

Arg Ser Cys Ile Glu Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 48
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 48

Arg Ser Cys Ile Asp Glu Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 49
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 49

Arg Ser Cys Ile Asp Thr Glu Pro Lys Ser Arg Cys Thr Ala Phe Gln
1   5   10   15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 50
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 50

Arg Ser Cys Ile Asp Thr Ile Glu Lys Ser Arg Cys Thr Ala Phe Gln
1   5   10   15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 51
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 51

Arg Ser Cys Ile Asp Thr Ile Pro Glu Ser Arg Cys Thr Ala Phe Gln
1   5   10   15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys

20 25 30

Gly Thr Cys
35

<210> 52
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 52

Arg Ser Cys Ile Asp Thr Ile Pro Lys Glu Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 53
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 53

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Glu Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 54
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 54

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Glu Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 55
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptdie

<400> 55

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Glu Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 56
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 56

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Glu Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 57
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 57

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Glu
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys

35

<210> 58
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 58

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Glu His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 59
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 59

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys Glu Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 60
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 60

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Glu Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 61
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Shk Peptide

<400> 61

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Glu Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 62
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 62

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Glu Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 63
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 63

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Glu Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 64

<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 64

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Glu Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 65
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 65

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Glu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 66
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 66

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Glu Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 67
<211> 35
<212> PRT

<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 67

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Glu Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 68
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 68

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Glu Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 69
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 69

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Glu Thr Cys
20 25 30

Gly Thr Cys
35

<210> 70
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 70

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Glu Cys
20 25 30

Gly Thr Cys
35

<210> 71
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 71

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Glu Thr Cys
35

<210> 72
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 72

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Glu Cys
35

<210> 73
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (1)..(1)
<223> 3-(1-naphthyl)alanine

<400> 73

Xaa Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 74
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> 3-(1-naphthyl)alanine

<400> 74

Arg Xaa Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 75
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (4)..(4)
<223> 3-(1-naphthyl)alanine

<400> 75

Arg Ser Cys Xaa Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 76
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (5)..(5)
<223> 3-(1-naphthyl)alanine

<400> 76

Arg Ser Cys Ile Xaa Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1   5   10   15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 77
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (6)..(6)
<223> 3-(1-naphthyl)alanine

<400> 77

Arg Ser Cys Ile Asp Xaa Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1   5   10   15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 78
<211> 35
<212> PRT

<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (7)..(7)
<223> 3-(1-naphthyl)alanine

<400> 78

Arg Ser Cys Ile Asp Thr Xaa Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 79
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (8)..(8)
<223> 3-(1-naphthyl)alanine

<400> 79

Arg Ser Cys Ile Asp Thr Ile Xaa Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 80
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (9)..(9)
<223> 3-(1-naphthyl)alanine

<400> 80

Arg Ser Cys Ile Asp Thr Ile Pro Xaa Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 81
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (10)..(10)
<223> 3-(1-naphthyl)alanine

<400> 81

Arg Ser Cys Ile Asp Thr Ile Pro Lys Xaa Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 82
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (11)..(11)
<223> 3-(1-naphthyl)alanine

<400> 82

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Xaa Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 83
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (13)..(13)
<223> 3-(1-naphthyl)alanine

<400> 83

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Xaa Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 84
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (14)..(14)
<223> 3-(1-naphthyl)alanine

<400> 84

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Xaa Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 85
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE

<222> (15)..(15)
<223> 3-(1-naphthyl)alanine

<400> 85

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Xaa Gln
1   5   10   15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 86
<211> 20
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Linker sequence

<400> 86

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1   5   10   15

Gly Gly Gly Ser
            20

<210> 87
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (18)..(18)
<223> 3-(1-naphthyl)alanine

<400> 87

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1   5   10   15

Cys Xaa His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 88
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (19)..(19)
<223> 3-(1-naphthyl)alanine

<400> 88

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1   5   10   15

Cys Lys Xaa Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 89
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (20)..(20)
<223> 3-(1-naphthyl)alanine

<400> 89

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1   5   10   15

Cys Lys His Xaa Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 90
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptdie

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> 3-(1-naphthyl)alanine

<400> 90

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln

1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 91
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (22)..(22)
<223> 3-(1-naphthyl)alanine

<400> 91

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Xaa Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 92
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (23)..(23)
<223> 3-(1-naphthyl)alanine

<400> 92

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Xaa Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 93

<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (24)..(24)
<223> 3-(1-naphthyl)alanine

<400> 93

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Xaa Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 94
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (25)..(25)
<223> 3-(1-naphthyl)alanine

<400> 94

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Xaa Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 95
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (27)..(27)
<223> 3-(1-naphthyl)alanine

<400> 95

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Xaa Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 96
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (29)..(29)
<223> 3-(1-naphthyl)alanine

<400> 96

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Xaa Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 97
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (30)..(30)
<223> 3-(1-naphthyl)alanine

<400> 97

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Xaa Thr Cys
20 25 30

Gly Thr Cys

35

<210> 98
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShKpeptide

<220>
<221> MISC_FEATURE
<222> (31)..(31)
<223> 3-(1-naphthyl)alanine

<400> 98

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Xaa Cys
20 25 30

Gly Thr Cys
35

<210> 99
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (33)..(33)
<223> 3-(1-naphthyl)alanine

<400> 99

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Xaa Thr Cys
35

<210> 100
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (34)..(34)
<223> 3-(1-naphthyl)alanine

<400> 100

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Xaa Cys
35

<210> 101
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 101

Ala Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 102
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 102

Arg Ala Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 103
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 103

Arg Ser Cys Ala Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 104
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 104

Arg Ser Cys Ile Ala Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 105
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 105

Arg Ser Cys Ile Asp Ala Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 106
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 106

Arg Ser Cys Ile Asp Thr Ala Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 107
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 107

Arg Ser Cys Ile Asp Thr Ile Ala Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 108
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 108

Arg Ser Cys Ile Asp Thr Ile Pro Ala Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 109
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 109

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ala Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 110
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 110

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Ala Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 111
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Shk peptide

<400> 111

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Ala Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 112
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 112

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Ala Gln

1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 113
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Shk peptide

<400> 113

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Ala
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 114
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 114

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Ala His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 115
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 115

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys Ala Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 116
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 116

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1   5   10   15

Cys Lys His Ala Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 117
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 117

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1   5   10   15

Cys Lys His Ser Ala Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 118
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 118

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1   5   10   15

Cys Lys His Ser Met Ala Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys

20 25 30

Gly Thr Cys
35

<210> 119
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 119

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Ala Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 120
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 120

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Ala Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 121
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 121

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Ala Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 122
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 122

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ala Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 123
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 123

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Ala Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 124
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 124

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Ala Lys Thr Cys
20 25 30

Gly Thr Cys

35

<210> 125
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 125

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Ala Thr Cys
20 25 30

Gly Thr Cys
35

<210> 126
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 126

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Ala Cys
20 25 30

Gly Thr Cys
35

<210> 127
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 127

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Ala Thr Cys
35

<210> 128
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 128

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Ala Cys
35

<210> 129
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 129

Lys Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 130
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 130

Arg Lys Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 131

<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 131

Arg Ser Cys Lys Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 132
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 132

Arg Ser Cys Ile Lys Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 133
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 133

Arg Ser Cys Ile Asp Lys Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 134
<211> 35
<212> PRT

<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 134

Arg Ser Cys Ile Asp Thr Lys Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 135
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 135

Arg Ser Cys Ile Asp Thr Ile Lys Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 136
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 136

Arg Ser Cys Ile Asp Thr Ile Pro Lys Lys Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 137
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 137

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Lys Cys Thr Ala Phe Gln
1   5   10   15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 138
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 138

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Lys Ala Phe Gln
1   5   10   15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 139
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptdie

<400> 139

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Lys Phe Gln
1   5   10   15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 140
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 140

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Lys Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 141
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 141

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys Lys Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 142
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 142

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Lys Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 143
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 143

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Lys Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 144
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 144

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Lys Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 145
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 145

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Lys Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 146
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 146

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln

1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Lys Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 147
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 147

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Lys Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 148
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 148

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Lys Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 149
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 149

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Lys Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 150
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 150

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1   5   10   15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Lys Cys
            20   25   30

Gly Thr Cys
        35

<210> 151
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 151

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1   5   10   15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Lys Thr Cys
        35

<210> 152
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<400> 152

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1   5   10   15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys

20 25 30

Gly Lys Cys
35

<210> 153
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Shk peptide

<220>
<221> MISC_FEATURE
<222> (16)..(16)
<223> Ornithine

<400> 153

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Xaa
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 154
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (16)..(16)
<223> alpha, gamma-diaminobutyric acid

<400> 154

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Xaa
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 155
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (16)..(16)
<223> 3-amino-6-hydroxy-2-piperidone

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 155

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Xaa
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 156
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 156

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Asn
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 157
<211> 35

<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 157

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe His
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 158
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK PEGylated toxin peptide analog

<220>
<221> MISC_FEATURE
<222> (1)..(1)
<223> PEGylated

<400> 158

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 159
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK PEGylated toxin peptide analog

<220>
<221> MISC_FEATURE
<222> (1)..(1)
<223> PEGylated

<220>
<221> MISC_FEATURE
<222> (16)..(16)
<223> 3-(1-naphthyl)alanine

<400> 159

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Xaa
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 160
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK PEGylated toxin peptide analog

<220>
<221> MISC_FEATURE
<222> (1)..(1)
<223> PEGylated

<220>
<221> MISC_FEATURE
<222> (27)..(27)
<223> 3-(1-naphthyl)alanine

<400> 160

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Xaa Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 161
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK PEGylated toxin peptide analog

<220>
<221> MISC_FEATURE

<222> (1)..(1)
<223> PEGylated

<400> 161

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Ala Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 162
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> PEGylated Toxin Peptide Analog

<220>
<221> MISC_FEATURE
<222> (1)..(1)
<223> PEGylated

<220>
<221> MISC_FEATURE
<222> (27)..(27)
<223> 3-(1-naphthyl)alanine

<400> 162

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Xaa Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 163
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> PEGylated Toxin Peptide Analog

<220>
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<223> PEGylated

<220>
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<222> (35)..(35)
<223> AMIDATION

<400> 163

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 164
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<212> PRT
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<220>
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<220>
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<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 164

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 165
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
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<222> (21)..(21)
<223> Norvaline

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 165

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 166
<211> 35
<212> PRT
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<220>
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<220>
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<220>
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<222> (35)..(35)
<223> AMIDATION

<400> 166

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Gln Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 167
<211> 35
<212> PRT
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<220>
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<220>
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<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 167

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Ala Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 168
<211> 35
<212> PRT
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<220>
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<220>
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<222> (21)..(21)
<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 168

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Glu His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 169
<211> 35
<212> PRT
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<220>
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<220>
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<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 169

Arg Ser Cys Glu Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1   5   10   15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 170
<211> 36
<212> PRT
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<220>
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<220>
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<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<400> 170

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1   5   10   15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys Ala
        35

<210> 171

<211> 36
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<220>
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<220>
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<220>
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<222> (21)..(21)
<223> Norvaline

<400> 171

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys Ala
35

<210> 172
<211> 36
<212> PRT
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<222> (21)..(21)
<223> Norvaline

<400> 172

Arg Ser Cys Glu Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys Ala
35

<210> 173
<211> 36
<212> PRT

<213> ARTIFICIAL SEQUENCE

<220>
<223> PEGylated Toxin Peptide Analog

<220>
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<223> PEGylated

<400> 173

Arg Ser Cys Glu Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Gln Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys Ala
35

<210> 174
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
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<220>
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<222> (16)..(16)
<223> PEGylated

<400> 174

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 175
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination toxin peptide analog (free acid)

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<400> 175

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 176
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination toxin peptide analog (free acid)

<400> 176

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Ala Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 177
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination toxin peptide analog (free acid)

<400> 177

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Arg His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 178
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination toxin peptide analog (free acid)

<400> 178

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys

1 5 10 15

Cys Lys His Ser Ala Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 179
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination toxin peptide analog (free acid)

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<400> 179

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 180
<211> 35
<212> PRT
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<220>
<223> Combination toxin peptide analog (free acid)

<220>
<221> MISC_FEATURE
<222> (27)..(27)
<223> 3-(1-naphthyl)alanine

<400> 180

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Xaa Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 181

<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination toxin peptide analog (free acid)

<400> 181

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Lys Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 182
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination toxin peptide analog (free acid)

<400> 182

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Arg Thr Cys
20 25 30

Gly Thr Cys
35

<210> 183
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination toxin peptide analog (free acid)

<400> 183

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Glu Thr Cys
20 25 30

Gly Thr Cys
35

<210> 184
<211> 35
<212> PRT

<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination toxin peptide analog (free acid)

<220>
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<222> (27)..(27)
<223> 3-(1-naphthyl)alanine

<400> 184

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Xaa Cys Lys Arg Thr Cys
20 25 30

Gly Thr Cys
35

<210> 185
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination toxin peptide analog (free acid)

<400> 185

Arg Ser Cys Glu Asp Thr Ile Pro Lys Arg Arg Cys Thr Ala Ala Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Arg Thr Cys
20 25 30

Gly Thr Cys
35

<210> 186
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 186

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Ala Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys

20 25 30

Gly Thr Cys
35

<210> 187
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
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<222> (27)..(27)
<223> 3-(1-naphthyl)alanine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 187

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Xaa Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 188
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 188

Arg Ser Cys Glu Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 189
<211> 35
<212> PRT
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<220>
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<220>
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<222> (35)..(35)
<223> AMIDATION

<400> 189

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Arg Thr Cys
20 25 30

Gly Thr Cys
35

<210> 190
<211> 35
<212> PRT
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<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
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<222> (35)..(35)
<223> AMIDATION

<400> 190

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Glu Thr Cys
20 25 30

Gly Thr Cys
35

<210> 191
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
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<223> 3-(1-naphthyl)alanine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 191

Arg Ser Cys Glu Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Xaa Phe Cys Lys Arg Thr Cys
20 25 30

Gly Thr Cys
35

<210> 192
<211> 35
<212> PRT
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<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 192

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Tyr Phe Cys Lys Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 193
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MISC_FEATURE
<222> (26)..(26)
<223> 3-(2-naphthyl)alanine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 193

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Xaa Phe Cys Lys Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 194
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
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<222> (21)..(21)
<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 194

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Glu Thr Cys
20 25 30

Gly Thr Cys
35

<210> 195
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 195

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Glu His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys

20 25 30

Gly Thr Cys
35

<210> 196
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
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<220>
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<222> (21)..(21)
<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 196

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Arg Thr Cys
20 25 30

Gly Thr Cys
35

<210> 197
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
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<220>
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<222> (21)..(21)
<223> Norleucine

<220>
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<222> (26)..(26)
<223> 3-(1-naphthyl)alanine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 197

Arg Ser Cys Glu Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Xaa Phe Cys Lys Arg Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 198
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 198

Arg Ser Cys Glu Asp Thr Ile Pro Glu Ser Arg Cys Thr Ala Phe Lys
1   5   10   15

Cys Arg His Ser Met Lys Tyr Arg Leu Ser Phe Cys Lys Arg Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 199
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 199

Arg Ser Cys Glu Asp Thr Ile Pro Lys Glu Arg Cys Thr Ala Phe Lys
1   5   10   15

Cys Arg His Ser Met Lys Tyr Arg Leu Ser Phe Cys Lys Arg Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 200
<211> 35

<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 200

Arg Ser Cys Glu Asp Thr Ile Pro Glu Glu Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Arg His Ser Met Lys Tyr Arg Leu Ser Phe Cys Lys Arg Thr Cys
20 25 30

Gly Thr Cys
35

<210> 201
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 201

Arg Ser Cys Glu Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Lys Arg Thr Cys
20 25 30

Gly Thr Cys
35

<210> 202
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 202

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Trp Cys Lys Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 203
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 203

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Lys Arg Thr Cys
20 25 30

Gly Thr Cys
35

<210> 204
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 204

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1   5   10   15

Cys Glu His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 205
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 205

Arg Ser Cys Glu Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1   5   10   15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 206
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 206

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Ala Lys
1   5   10   15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 207
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MISC_FEATURE
<222> (27)..(27)
<223> 3-(2-naphthyl)alanine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 207

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1   5   10   15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Xaa Cys Lys Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 208
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 208

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1   5   10   15

Cys Arg His Ser Met Lys Tyr Arg Leu Ser Phe Cys Lys Arg Thr Cys
            20   25   30

Gly Thr Cys

35

<210> 209
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 209

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Phe Phe Cys Lys Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 210
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 210

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Lys Ala Thr Cys
20 25 30

Gly Thr Cys
35

<210> 211
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 211

Arg Ser Cys Glu Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Arg His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Lys Arg Thr Cys
20 25 30

Gly Thr Cys
35

<210> 212
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 212

Arg Ser Cys Glu Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Arg His Ser Met Lys Tyr Arg Leu Ser Phe Cys Lys Arg Thr Cys
20 25 30

Gly Thr Cys
35

<210> 213
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 213

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Lys Arg Thr Cys
20 25 30

Gly Thr Cys
35

<210> 214
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 214

Arg Ser Cys Glu Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Ala Lys
1 5 10 15

Cys Glu His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 215
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
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<222> (21)..(21)
<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 215

Arg Ser Cys Glu Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Glu His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 216
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
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<222> (21)..(21)
<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 216

Arg Ser Cys Glu Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Ala Lys
1   5   10   15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 217
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
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<222> (21)..(21)
<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 217

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Ala Lys
1   5   10   15

Cys Glu His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 218
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 218

Arg Ser Cys Glu Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1   5   10   15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Arg Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 219
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 219

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1   5   10   15

Cys Glu His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Arg Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 220
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 220

Arg Ser Cys Glu Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1   5   10   15

Cys Glu His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Arg Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 221
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 221

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Ala Lys
1   5   10   15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Arg Thr Cys
            20   25   30

Gly Thr Cys
35

<210> 222
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
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<222> (21)..(21)
<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 222

Arg Ser Cys Ile Asp Thr Ile Pro Glu Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 223
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
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<222> (21)..(21)
<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 223

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Ala Lys
1 5 10 15

Cys Glu His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Arg Thr Cys
20 25 30

Gly Thr Cys
35

<210> 224
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 224

Arg Ser Cys Glu Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Ala Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Arg Thr Cys
20 25 30

Gly Thr Cys
35

<210> 225
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 225

Arg Ser Cys Ile Asp Thr Ile Pro Lys Glu Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 226
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MISC_FEATURE
<222> (26)..(26)
<223> 3-(1-naphthyl)alanine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 226

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Xaa Phe Cys Lys Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 227
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<220>
<221> MISC_FEATURE
<222> (26)..(26)
<223> 3-(1-naphthyl)alanine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 227

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Xaa Phe Cys Lys Arg Thr Cys
20 25 30

Gly Thr Cys

35

<210> 228
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 228

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Tyr Cys Lys Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 229
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 229

Arg Ser Cys Glu Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Thr Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 230
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 230

Arg Ser Cys Glu Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1   5   10   15

Cys Lys His Ser Ser Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 231
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 231

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1   5   10   15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Lys Ala Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 232
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norvaline

<220>
<221> MOD_RES

<222> (35)..(35)
<223> AMIDATION

<400> 232

Arg Ser Cys Glu Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1   5   10   15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 233
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Aminobutyric acid

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 233

Arg Ser Cys Glu Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1   5   10   15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 234
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Combination Toxin Peptide Analog {C-Terminal Amide}

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 234

Arg Ser Cys Glu Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1   5   10   15

Cys Lys His Ser Gln Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 235
<211> 36
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Toxin Peptide Analog with C-Terminal extension

<400> 235

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1   5   10   15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys Ala
        35

<210> 236
<211> 36
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Toxin Peptide Analog with C-Terminal extension

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<400> 236

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1   5   10   15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys Ala
        35

<210> 237
<211> 36
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Toxin Peptide Analog with C-Terminal extension

<400> 237

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Asn Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys Ala
35

<210> 238
<211> 36
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Toxin Peptide Analog with C-Terminal extension

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norvaline

<400> 238

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys Ala
35

<210> 239
<211> 36
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Toxin Peptide Analog with C-Terminal extension

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norvaline

<220>
<221> MOD_RES
<222> (36)..(36)
<223> AMIDATION

<400> 239

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys

20 25 30

Gly Thr Cys Ala
35

<210> 240
<211> 36
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Toxin Peptide Analog with C-Terminal extension

<400> 240

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Gln Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys Ala
35

<210> 241
<211> 36
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Toxin Peptide Analog with C-Terminal extension

<400> 241

Arg Ser Cys Glu Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Gln Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys Ala
35

<210> 242
<211> 36
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Toxin Peptide Analog with C-Terminal extension

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norvaline

<400> 242

Arg Ser Cys Glu Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys

1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys Ala
35

<210> 243
<211> 36
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Toxin Peptide Analog with C-Terminal extension

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<400> 243

Arg Ser Cys Glu Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys Ala
35

<210> 244
<211> 36
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Toxin Peptide Analog with C-Terminal extension

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<400> 244

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys Glu
35

<210> 245

<211> 36
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Toxin Peptide Analog with C-Terminal extension

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<400> 245

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys Tyr
35

<210> 246
<211> 36
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Toxin Peptide Analog with C-Terminal extension

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<400> 246

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys Val
35

<210> 247
<211> 36
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
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<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norvaline

<220>
<221> MISC_FEATURE
<222> (36)..(36)
<223> Beta Ala

<400> 247

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys Xaa
35

<210> 248
<211> 36
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Toxin Peptide Analog with C-Terminal extension {and C-Terminal Amide}

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norvaline

<220>
<221> MOD_RES
<222> (36)..(36)
<223> AMIDATION

<400> 248

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys Asp
35

<210> 249
<211> 36
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Toxin Peptide Analog with C-Terminal extension {and C-Terminal Amide}

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norvaline

<220>
<221> MOD_RES
<222> (36)..(36)
<223> AMIDATION

<400> 249

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys Glu
35

<210> 250
<211> 36
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Toxin Peptide Analog with C-Terminal extension {and C-Terminal Amide}

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<220>
<221> MOD_RES
<222> (36)..(36)
<223> AMIDATION

<400> 250

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys Asp
35

<210> 251
<211> 36
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Toxin Peptide Analog with C-Terminal extension {and C-Terminal Amide}

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norvaline

<220>
<221> MOD_RES
<222> (36)..(36)
<223> AMIDATION

<400> 251

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys Ser
35

<210> 252
<211> 36
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Toxin Peptide Analog with C-Terminal extension {and C-Terminal Amide}

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norvaline

<220>
<221> MOD_RES
<222> (36)..(36)
<223> AMIDATION

<400> 252

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys Thr
35

<210> 253
<211> 36
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Toxin Peptide Analog with C-Terminal extension {and C-Terminal Amide}

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norvaline

<220>
<221> MISC_FEATURE
<222> (36)..(36)
<223> Aminoisobutyric acid

<220>
<221> MOD_RES
<222> (36)..(36)
<223> AMIDATION

<400> 253

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys Xaa
35

<210> 254
<211> 36
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Toxin Peptide Analog with C-Terminal extension {and C-Terminal Amide}

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norvaline

<220>
<221> MOD_RES
<222> (36)..(36)
<223> AMIDATION

<400> 254

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys Ala
35

<210> 255
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Position 21 Toxin Peptide Analog

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norleucine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 255

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 256

<400> 256
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<210> 257
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Position 21 Toxin Peptide Analog

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Methinine oxide

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 257

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 258
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>

<223> Position 21 Toxin Peptide Analog

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 258

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1   5   10   15

Cys Lys His Ser Ser Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 259
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Position 21 Toxin Peptide Analog

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 259

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1   5   10   15

Cys Lys His Ser Thr Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 260
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Position 21 Toxin Peptide Analog

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Norvaline

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 260

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 261
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Position 21 Toxin Peptide Analog

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 261

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Gln Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 262
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Position 21 Toxin Peptide Analog

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 262

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser His Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys

35

<210> 263
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Position 21 Toxin Peptide Analog

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 263

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Tyr Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 264
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Position 21 Toxin Peptide Analog

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 264

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Asn Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 265
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Position 21 Toxin Peptide Analog

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 265

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1   5   10   15

Cys Lys His Ser Val Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 266
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Position 21 Toxin Peptide Analog

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 266

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1   5   10   15

Cys Lys His Ser Leu Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 267
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Position 21 Toxin Peptide Analog

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 267

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1   5   10   15

Cys Lys His Ser Trp Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys

20 25 30

Gly Thr Cys
35

<210> 268
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Position 21 Toxin Peptide Analog

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Aminobutyric acid

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 268

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 269
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Position 21 Toxin Peptide Analog

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Cyclohexylalanine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 269

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 270
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Position 21 Toxin Peptide Analog

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> Cyclohexylglycine

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 270

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 271
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Position 21 Toxin Peptide Analog

<220>
<221> MISC_FEATURE
<222> (21)..(21)
<223> 4-hydroxyproline (or hydroxyproline)

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 271

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys

35

<210> 272
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Position 21 Toxin Peptide Analog

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 272

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Phe Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 273
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Position 21 Toxin Peptide Analog

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 273

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Ile Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 274
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Position 21 Toxin Peptide Analog

<220>
<221> MOD_RES
<222> (35)..(35)
<223> AMIDATION

<400> 274

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1   5   10   15

Cys Lys His Ser Asp Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 275
<211> 83
<212> PRT
<213> Anemonia erythraea

<220>
<221> SIGNAL
<222> (1)..(83)
<223> Putative signal sequence

<400> 275

Met Lys Gly Gln Met Ile Ile Cys Leu Val Leu Ile Ala Leu Cys Met
1   5   10   15

Ser Val Val Val Met Ala Gln Asn Leu Arg Ala Glu Glu Leu Glu Lys
            20   25   30

Ala Asn Pro Lys Asp Glu Arg Val Arg Ser Phe Glu Arg Asn Gln Lys
        35   40   45

Arg Ala Cys Lys Asp Tyr Leu Pro Lys Ser Glu Cys Thr Gln Phe Arg
    50   55   60

Cys Arg Thr Ser Met Lys Tyr Lys Tyr Thr Asn Cys Lys Lys Thr Cys
65   70   75   80

Gly Thr Cys

<210> 276
<211> 74
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Hmk peptide sequence

<400> 276

Met Lys Ser Gln Met Ile Ala Ala Val Leu Leu Ile Ala Phe Cys Leu
1   5   10   15

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Cys Val Val Val Thr Ala Arg Met Glu Leu Gln Asp Val Glu Asp Met
            20                  25                  30

Glu Asn Gly Phe Gln Lys Arg Arg Thr Cys Lys Asp Leu Ile Pro Val
        35                  40                  45

Ser Glu Cys Thr Asp Ile Arg Cys Arg Thr Ser Met Lys Tyr Arg Leu
    50                  55                  60

Asn Leu Cys Arg Lys Thr Cys Gly Ser Cys
65                  70

<210>  277
<211>  738
<212>  DNA
<213>  Homo sapiens

<220>
<221>  CDS
<222>  (1)..(738)

<400>  277
atg gaa tgg agc tgg gtc ttt ctc ttc ttc ctg tca gta acg act ggt       48
Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu Ser Val Thr Thr Gly
1               5                   10                  15

gtc cac tcc gac aaa act cac aca tgc cca ccg tgc cca gca cct gaa       96
Val His Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
            20                  25                  30

ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac      144
Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
        35                  40                  45

acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac      192
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
    50                  55                  60

gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc      240
Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
65                  70                  75                  80

gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac      288
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
                85                  90                  95

agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg      336
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
            100                 105                 110

ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca      384
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
        115                 120                 125

gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa      432
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
    130                 135                 140

cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac      480
Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
145                 150                 155                 160
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cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc      528
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
                165                 170                 175

gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc      576
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
            180                 185                 190

acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag      624
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
        195                 200                 205

ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc      672
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
    210                 215                 220

tcc gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc      720
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
225                 230                 235                 240

tcc ctg tct ccg ggt aaa                                             738
Ser Leu Ser Pro Gly Lys
                245


<210>  278
<211>  246
<212>  PRT
<213>  Homo sapiens

<400>  278

Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu Ser Val Thr Thr Gly
1               5                   10                  15


Val His Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
            20                  25                  30


Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
        35                  40                  45


Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
    50                  55                  60


Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
65                  70                  75                  80


Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
                85                  90                  95


Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
            100                 105                 110


Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
        115                 120                 125


Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
    130                 135                 140
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Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
145 150 155 160

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
165 170 175

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
180 185 190

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
195 200 205

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
210 215 220

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
225 230 235 240

Ser Leu Ser Pro Gly Lys
245

<210> 279
<211> 6
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Linker sequence

<220>
<221> MISC_FEATURE
<222> (1)..(2)
<223> Xaa in positions 1-2 are each independently any amino acid residue

<220>
<221> MISC_FEATURE
<222> (4)..(5)
<223> Xaa in positions 4-5 are each independently any amino acid residue

<400> 279

Xaa Xaa Asn Xaa Xaa Gly
1 5

<210> 280
<211> 8
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptidyl linker

<400> 280

Gly Gly Gly Gly Gly Gly Gly Gly
1 5

<210> 281
<211> 5
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptidyl linker

<400> 281

Gly Gly Gly Gly Gly
1   5

<210> 282
<211> 7
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptidyl linker

<400> 282

Gly Gly Gly Gly Gly Gly Gly
1   5

<210> 283
<211> 5
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptidyl linker

<400> 283

Gly Gly Gly Gly Ser
1   5

<210> 284
<211> 6
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptidyl linker

<400> 284

Gly Gly Gly Gly Gly Lys
1   5

<210> 285
<211> 7
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptidyl linker

<400> 285

Gly Gly Gly Gly Gly Lys Arg
1   5

<210> 286
<211> 8
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptidyl linker

<400> 286

Gly Gly Gly Lys Gly Gly Gly Gly
1   5

<210> 287
<211> 8
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptidyl linker

<400> 287

Gly Gly Gly Asn Gly Ser Gly Gly
1   5

<210> 288
<211> 8
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptidyl linker

<400> 288

Gly Gly Gly Cys Gly Gly Gly Gly
1   5

<210> 289
<211> 5
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptidyl linker

<400> 289

Gly Pro Asn Gly Gly
1   5

<210> 290
<211> 8
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptidyl linker

<400> 290

Gly Gly Gly Lys Gly Gly Gly Gly
1 5

<210> 291
<211> 5
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptidyl linker

<400> 291

Gly Gly Gly Gly Ser
1 5

<210> 292
<211> 10
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptidyl linker

<400> 292

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10

<210> 293
<211> 25
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptidyl linker

<400> 293

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1 5 10 15

Gly Gly Gly Ser Gly Gly Gly Gly Ser
20 25

<210> 294
<211> 6
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptide linker

<400> 294

Gly Gly Glu Gly Gly Gly
1 5

<210> 295
<211> 8

<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptide linker

<400> 295

Gly Gly Glu Glu Glu Gly Gly Gly
1   5

<210> 296
<211> 5
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptide linker

<400> 296

Gly Glu Glu Glu Gly
1   5

<210> 297
<211> 4
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptide linker

<400> 297

Gly Glu Glu Glu
1

<210> 298
<211> 6
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptide linker

<400> 298

Gly Gly Asp Gly Gly Gly
1   5

<210> 299
<211> 7
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptide linker

<400> 299

Gly Gly Asp Asp Asp Gly Gly
1   5

<210> 300
<211> 5
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptide linker

<400> 300

Gly Asp Asp Asp Gly
1   5

<210> 301
<211> 4
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptide linker

<400> 301

Gly Asp Asp Asp
1

<210> 302
<211> 21
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptide linker

<400> 302

Gly Gly Gly Gly Ser Asp Asp Ser Asp Glu Gly Ser Asp Gly Glu Asp
1   5   10   15

Gly Gly Gly Gly Ser
            20

<210> 303
<211> 5
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptide linker

<400> 303

Trp Glu Trp Glu Trp
1   5

<210> 304
<211> 5
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptide linker

<400> 304

Phe Glu Phe Glu Phe
1 5

<210> 305
<211> 6
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptide linker

<400> 305

Glu Glu Glu Trp Trp Trp
1 5

<210> 306
<211> 6
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptide linker

<400> 306

Glu Glu Glu Phe Phe Phe
1 5

<210> 307
<211> 7
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptide linker

<400> 307

Trp Trp Glu Glu Glu Trp Trp
1 5

<210> 308
<211> 7
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptide linker

<400> 308

Phe Phe Glu Glu Glu Phe Phe
1 5

<210> 309
<211> 6
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>

<223> Peptide linker

<220>
<221> MISC_FEATURE
<222> (1)..(2)
<223> Xaa in positions 1-2 are each independently any amino acid residue

<220>
<221> MISC_FEATURE
<222> (4)..(5)
<223> Xaa in positions 4-5 are each independently any amino acid residue

<400> 309

Xaa Xaa Tyr Xaa Xaa Gly
1 5

<210> 310
<211> 6
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptide linker

<220>
<221> MISC_FEATURE
<222> (1)..(2)
<223> Xaa in positions 1-2 are each independently any amino acid residue

<220>
<221> MISC_FEATURE
<222> (4)..(5)
<223> Xaa in positions 4-5 are each independently any amino acid residue

<400> 310

Xaa Xaa Ser Xaa Xaa Gly
1 5

<210> 311
<211> 6
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Peptide linker

<220>
<221> MISC_FEATURE
<222> (1)..(2)
<223> Xaa in positions 1-2 are each independently any amino acid residue

<220>
<221> MISC_FEATURE
<222> (4)..(5)
<223> Xaa in positions 4-5 are each independently any amino acid residue

<400> 311

Xaa Xaa Thr Xaa Xaa Gly
1 5

<210> 312
<211> 22
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Flexible peptide linker

<400> 312

Gly Ser Gly Ser Ala Thr Gly Gly Ser Gly Ser Thr Ala Ser Ser Gly
1 5 10 15

Ser Gly Ser Ala Thr His
20

<210> 313
<211> 22
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Flexible peptide linker

<400> 313

His Gly Ser Gly Ser Ala Thr Gly Gly Ser Gly Ser Thr Ala Ser Ser
1 5 10 15

Gly Ser Gly Ser Ala Thr
20

<210> 314
<211> 19
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Flexible peptide linker

<400> 314

Ala Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys
1 5 10 15

Ala Gly Gly

<210> 315
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Reference peptide

<220>
<221> MISC_FEATURE
<222> (1)..(1)
<223> Nalpha branched PEGylated

<400> 315

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys
35

<210> 316
<211> 36
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> PEGylated Toxin Peptide Analog

<220>
<221> MISC_FEATURE
<222> (1)..(1)
<223> 20kDa PEG-[Lys16]Shk-Ala

<400> 316

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
1 5 10 15

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
20 25 30

Gly Thr Cys Ala
35

<210> 317
<211> 35
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK peptide

<220>
<221> MISC_FEATURE
<222> (22)..(22)
<223> Diaminopropionic acid

<400> 317

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln
1 5 10 15

Cys Lys His Ser Met Xaa Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
            20   25   30

Gly Thr Cys
        35

<210> 318
<211> 19
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> modifed VH21signal peptide

<400> 318

Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu Ser Val Thr Thr Gly
1   5   10   15

Val His Ser

<210> 319
<211> 30
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 319
catgaattcc ccaccatgga atggagctgg   30

<210> 320
<211> 38
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 320
cacggtgggc actcgacttt gcgctcggag tggacacc   38

<210> 321
<211> 132
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK[2-35] with N-terminal linker extension

<400> 321
ggaggaggag gatccggagg aggaggaagc agctgcatcg acaccatccc caagagccgc   60

tgcaccgcct tccagtgcaa gcacagcatg aagtaccgcc tgagcttctg ccgcaagacc   120

tgcggcacct gc   132

<210> 322

<211> 44
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK[2-35] with N-terminal linker extension

<400> 322

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Cys Ile Asp Thr Ile
1 5 10 15

Pro Lys Ser Arg Cys Thr Ala Phe Gln Cys Lys His Ser Met Lys Tyr
20 25 30

Arg Leu Ser Phe Cys Arg Lys Thr Cys Gly Thr Cys
35 40

<210> 323
<211> 37
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 323
gtccactccg agcgcaaagt cgagtgccca ccgtgcc 37

<210> 324
<211> 30
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 324
tcctcctcct ttacccggag acagggagag 30

<210> 325
<211> 29
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 325
gctgcaccgc cttcaagtgc aagcacagc 29

<210> 326
<211> 29
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 326
gctgtgcttg cacttgaagg cggtgcagc 29

<210> 327
<211> 132
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK[2-35, K16] with N-terminal linker extension

<400> 327
ggaggaggag gatccggagg aggaggaagc agctgcatcg acaccatccc caagagccgc 60

tgcaccgcct tcaagtgcaa gcacagcatg aagtaccgcc tgagcttctg ccgcaagacc 120

tgcggcacct gc 132

<210> 328
<211> 44
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK[2-35, Q16K] with N-terminal linker extension

<400> 328

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Cys Ile Asp Thr Ile
1 5 10 15

Pro Lys Ser Arg Cys Thr Ala Phe Lys Cys Lys His Ser Met Lys Tyr
20 25 30

Arg Leu Ser Phe Cys Arg Lys Thr Cys Gly Thr Cys
35 40

<210> 329
<211> 28
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 329
ccgggtaaag gaggaggagg atccggag 28

<210> 330
<211> 24
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 330
catgcggccg ctcattagca ggtg 24

<210> 331
<211> 648
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> Coding sequence of fragment of immunoglobulin Fc domain of human IgG2

<400> 331
gcaccacctg tggcaggacc gtcagtcttc ctcttccccc caaaacccaa ggacaccctc 60

atgatctccc ggacccctga ggtcacgtgc gtggtggtgg acgtgagcca cgaagacccc 120

gaggtccagt tcaactggta cgtggacggc gtggaggtgc ataatgccaa gacaaagcca 180

cgggaggagc agttcaacag cacgttccgt gtggtcagcg tcctcaccgt tgtgcaccag 240

gactggctga acggcaagga gtacaagtgc aaggtctcca acaaaggcct cccagccccc 300

atcgagaaaa ccatctccaa aaccaaaggg cagccccgag aaccacaggt gtacaccctg 360

cccccatccc gggaggagat gaccaagaac caggtcagcc tgacctgcct ggtcaaaggc 420

ttctacccca gcgacatcgc cgtggagtgg gagagcaatg ggcagccgga gaacaactac 480

aagaccacac ctcccatgct ggactccgac ggctccttct tcctctacag caagctcacc 540

gtggacaaga gcaggtggca gcaggggaac gtcttctcat gctccgtgat gcatgaggct 600

ctgcacaacc actacacgca gaagagcctc tccctgtctc cgggtaaa 648

<210> 332
<211> 216
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<223> Fragment of immunoglobulin Fc domain of human IgG2

<400> 332

Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
1 5 10 15

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
20 25 30

Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
35 40 45

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
50 55 60

Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln
65 70 75 80

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
85 90 95

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro
100 105 110

```
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr
        115                 120                 125

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
    130                 135                 140

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
145                 150                 155                 160

Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
                165                 170                 175

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
            180                 185                 190

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
        195                 200                 205

Ser Leu Ser Leu Ser Pro Gly Lys
    210                 215


<210>  333
<211>  511
<212>  PRT
<213>  ARTIFICIAL SEQUENCE

<220>
<223>  IgG2-HC-L10-ShK[1-35, Q16K] fusion polypeptide

<400>  333

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1               5                   10                  15

Leu Arg Gly Ala Arg Cys Gln Val Gln Leu Val Gln Ser Gly Ala Glu
            20                  25                  30

Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly
        35                  40                  45

Tyr Thr Phe Thr Gly Tyr His Met His Trp Val Arg Gln Ala Pro Gly
    50                  55                  60

Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr
65                  70                  75                  80

Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr
                85                  90                  95

Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp
            100                 105                 110
```

```
Thr Ala Val Tyr Tyr Cys Ala Arg Asp Arg Gly Ser Tyr Tyr Trp Phe
        115                 120                 125

Asp Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr
    130                 135                 140

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser
145                 150                 155                 160

Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
                165                 170                 175

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
            180                 185                 190

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
        195                 200                 205

Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys
    210                 215                 220

Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu
225                 230                 235                 240

Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala
                245                 250                 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
            260                 265                 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        275                 280                 285

Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    290                 295                 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe
305                 310                 315                 320

Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly
                325                 330                 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile
            340                 345                 350

Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val
        355                 360                 365

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
    370                 375                 380
```

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385 390 395 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
405 410 415

Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
420 425 430

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
435 440 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
450 455 460

Pro Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Arg Ser Cys Ile
465 470 475 480

Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys Cys Lys His Ser
485 490 495

Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys Gly Thr Cys
500 505 510

<210> 334
<211> 290
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> VH21SP-IgG2-Fc-L10-ShK(1-35, Q16K) Fusion polypeptide

<400> 334

Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu Ser Val Thr Thr Gly
1 5 10 15

Val His Ser Glu Arg Lys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro
20 25 30

Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
35 40 45

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
50 55 60

Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
65 70 75 80

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
85 90 95

Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu
100 105 110

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala
115 120 125

Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro
130 135 140

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
145 150 155 160

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
165 170 175

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
180 185 190

Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
195 200 205

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
210 215 220

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
225 230 235 240

Leu Ser Pro Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Arg
245 250 255

Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln Cys
260 265 270

Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys Gly
275 280 285

Thr Cys
290

<210> 335
<211> 30
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 335
cataagcttc ccaccatgga atggagctgg 30

<210> 336
<211> 31
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>

<223> Primer sequence

<400> 336
catggatcct catttacccg gagacaggga g 31

<210> 337
<211> 245
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<223> Immunoglobulin Fc domain of human IgG2

<400> 337

Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu Ser Val Thr Thr Gly
1 5 10 15

Val His Ser Glu Arg Lys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro
20 25 30

Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
35 40 45

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
50 55 60

Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
65 70 75 80

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
85 90 95

Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu
100 105 110

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala
115 120 125

Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro
130 135 140

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
145 150 155 160

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
165 170 175

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
180 185 190

Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
195 200 205

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
210 215 220

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
225 230 235 240

Leu Ser Pro Gly Lys
245

<210> 338
<211> 236
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> aKLH 120.6 kappa LC

<400> 338

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

Leu Arg Gly Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
20 25 30

Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
35 40 45

Gln Gly Ile Arg Asn Asp Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys
50 55 60

Ala Pro Lys Arg Leu Ile Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val
65 70 75 80

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr
85 90 95

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln
100 105 110

His Asn Ser Tyr Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile
115 120 125

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
130 135 140

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
145 150 155 160

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
165 170 175

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
180 185 190

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
195 200 205

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
210 215 220

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 339
<211> 467
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> aKLH 120.6 IgG2 HC

<400> 339

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

Leu Arg Gly Ala Arg Cys Gln Val Gln Leu Val Gln Ser Gly Ala Glu
20 25 30

Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly
35 40 45

Tyr Thr Phe Thr Gly Tyr His Met His Trp Val Arg Gln Ala Pro Gly
50 55 60

Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr
65 70 75 80

Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr
85 90 95

Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp
100 105 110

Thr Ala Val Tyr Tyr Cys Ala Arg Asp Arg Gly Ser Tyr Tyr Trp Phe
115 120 125

Asp Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr
130 135 140

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser
145 150 155 160

Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
165 170 175

```
Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
            180                 185                 190

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
        195                 200                 205

Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys
    210                 215                 220

Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu
225                 230                 235                 240

Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala
                245                 250                 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
            260                 265                 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        275                 280                 285

Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    290                 295                 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe
305                 310                 315                 320

Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly
                325                 330                 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile
            340                 345                 350

Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val
        355                 360                 365

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
    370                 375                 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385                 390                 395                 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                405                 410                 415

Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            420                 425                 430

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        435                 440                 445
```

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
450 455 460

Pro Gly Lys
465

<210> 340
<211> 290
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> IgG2 Fc-L10-ShK(1-35)

<400> 340

Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu Ser Val Thr Thr Gly
1 5 10 15

Val His Ser Glu Arg Lys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro
20 25 30

Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
35 40 45

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
50 55 60

Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
65 70 75 80

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
85 90 95

Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu
100 105 110

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala
115 120 125

Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro
130 135 140

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
145 150 155 160

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
165 170 175

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
180 185 190

```
Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
        195                 200                 205

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
    210                 215                 220

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
225                 230                 235                 240

Leu Ser Pro Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Arg
                245                 250                 255

Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln Cys
            260                 265                 270

Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys Gly
        275                 280                 285

Thr Cys
    290
```

<210> 341
<211> 511
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> aKLH 120.6 IgG2-ShK fusion

<400> 341

```
Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1               5                   10                  15

Leu Arg Gly Ala Arg Cys Gln Val Gln Leu Val Gln Ser Gly Ala Glu
            20                  25                  30

Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly
        35                  40                  45

Tyr Thr Phe Thr Gly Tyr His Met His Trp Val Arg Gln Ala Pro Gly
    50                  55                  60

Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr
65                  70                  75                  80

Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr
                85                  90                  95

Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp
            100                 105                 110

Thr Ala Val Tyr Tyr Cys Ala Arg Asp Arg Gly Ser Tyr Tyr Trp Phe
        115                 120                 125
```

```
Asp Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr
    130                 135                 140

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser
145                 150                 155                 160

Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
                165                 170                 175

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
            180                 185                 190

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
        195                 200                 205

Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys
    210                 215                 220

Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu
225                 230                 235                 240

Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala
                245                 250                 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
            260                 265                 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        275                 280                 285

Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    290                 295                 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe
305                 310                 315                 320

Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly
                325                 330                 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile
            340                 345                 350

Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val
        355                 360                 365

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
    370                 375                 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385                 390                 395                 400
```

```
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                405                 410                 415

Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            420                 425                 430

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        435                 440                 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    450                 455                 460

Pro Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Arg Ser Cys Ile
465                 470                 475                 480

Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln Cys Lys His Ser
                485                 490                 495

Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys Gly Thr Cys
            500                 505                 510


<210>  342
<211>  511
<212>  PRT
<213>  ARTIFICIAL SEQUENCE

<220>
<223>  aKLH 120.6 IgG2-ShK[1-35, Q16K] fusion

<400>  342

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1               5                   10                  15

Leu Arg Gly Ala Arg Cys Gln Val Gln Leu Val Gln Ser Gly Ala Glu
            20                  25                  30

Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly
        35                  40                  45

Tyr Thr Phe Thr Gly Tyr His Met His Trp Val Arg Gln Ala Pro Gly
    50                  55                  60

Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr
65                  70                  75                  80

Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr
                85                  90                  95

Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp
            100                 105                 110
```

```
Thr Ala Val Tyr Tyr Cys Ala Arg Asp Arg Gly Ser Tyr Tyr Trp Phe
        115                 120                 125

Asp Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr
    130                 135                 140

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser
145                 150                 155                 160

Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
                165                 170                 175

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
            180                 185                 190

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
        195                 200                 205

Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys
    210                 215                 220

Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu
225                 230                 235                 240

Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala
                245                 250                 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
            260                 265                 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        275                 280                 285

Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    290                 295                 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe
305                 310                 315                 320

Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly
                325                 330                 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile
            340                 345                 350

Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val
        355                 360                 365

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
    370                 375                 380
```

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385 390 395 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
405 410 415

Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
420 425 430

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
435 440 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
450 455 460

Pro Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Arg Ser Cys Ile
465 470 475 480

Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys Cys Lys His Ser
485 490 495

Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys Gly Thr Cys
500 505 510

<210> 343
<211> 471
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> aKLH 120.6 IgG1 HC

<400> 343

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

Leu Arg Gly Ala Arg Cys Gln Val Gln Leu Val Gln Ser Gly Ala Glu
20 25 30

Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly
35 40 45

Tyr Thr Phe Thr Gly Tyr His Met His Trp Val Arg Gln Ala Pro Gly
50 55 60

Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr
65 70 75 80

Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr
85 90 95

Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp
100 105 110

```
Thr Ala Val Tyr Tyr Cys Ala Arg Asp Arg Gly Ser Tyr Tyr Trp Phe
        115                 120                 125

Asp Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr
    130                 135                 140

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
145                 150                 155                 160

Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
                165                 170                 175

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
            180                 185                 190

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
        195                 200                 205

Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
    210                 215                 220

Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu
225                 230                 235                 240

Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
                245                 250                 255

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
            260                 265                 270

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
        275                 280                 285

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
    290                 295                 300

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
305                 310                 315                 320

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
                325                 330                 335

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
            340                 345                 350

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
        355                 360                 365

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys
    370                 375                 380
```

```
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
385                 390                 395                 400

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
                405                 410                 415

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
            420                 425                 430

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
        435                 440                 445

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
    450                 455                 460

Leu Ser Leu Ser Pro Gly Lys
465                 470


<210>  344
<211>  510
<212>  PRT
<213>  ARTIFICIAL SEQUENCE

<220>
<223>  aKLH 120.6 IgG1-loop-ShK

<400>  344

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1               5                   10                  15

Leu Arg Gly Ala Arg Cys Gln Val Gln Leu Val Gln Ser Gly Ala Glu
            20                  25                  30

Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly
        35                  40                  45

Tyr Thr Phe Thr Gly Tyr His Met His Trp Val Arg Gln Ala Pro Gly
    50                  55                  60

Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr
65                  70                  75                  80

Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr
                85                  90                  95

Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp
            100                 105                 110

Thr Ala Val Tyr Tyr Cys Ala Arg Asp Arg Gly Ser Tyr Tyr Trp Phe
        115                 120                 125
```

```
Asp Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr
    130                 135                 140

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
145                 150                 155                 160

Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
                165                 170                 175

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
            180                 185                 190

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
        195                 200                 205

Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
    210                 215                 220

Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu
225                 230                 235                 240

Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
                245                 250                 255

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
            260                 265                 270

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
        275                 280                 285

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
    290                 295                 300

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
305                 310                 315                 320

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
                325                 330                 335

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
            340                 345                 350

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
        355                 360                 365

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Gly Gly
    370                 375                 380

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
385                 390                 395                 400
```

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
405 410 415

Gly Thr Cys Gly Gly Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
420 425 430

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
435 440 445

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
450 455 460

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
465 470 475 480

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
485 490 495

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
500 505 510

<210> 345
<211> 289
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> VH21SP-IgG2-Fc-L10-ShK(1-35, Q16K) Fusion polypeptide

<400> 345

Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu Ser Val Thr Thr Gly
1 5 10 15

Val His Ser Glu Arg Lys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro
20 25 30

Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
35 40 45

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
50 55 60

Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
65 70 75 80

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
85 90 95

Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu
100 105 110

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala
115 120 125

```
Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro
    130                 135                 140

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
145                 150                 155                 160

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
                165                 170                 175

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
            180                 185                 190

Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
        195                 200                 205

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
    210                 215                 220

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
225                 230                 235                 240

Leu Ser Pro Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser
                245                 250                 255

Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln Cys Lys
            260                 265                 270

His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys Gly Thr
        275                 280                 285

Cys
```

<210> 346
<211> 289
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> VH21SP-IgG2-Fc-L10-ShK(1-35, Q16K) Fusion polypeptide

<400> 346

```
Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu Ser Val Thr Thr Gly
1               5                   10                  15

Val His Ser Glu Arg Lys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro
            20                  25                  30

Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
        35                  40                  45
```

```
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
    50                  55                  60

Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
65                  70                  75                  80

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
                85                  90                  95

Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu
            100                 105                 110

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala
        115                 120                 125

Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro
    130                 135                 140

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
145                 150                 155                 160

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
                165                 170                 175

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
            180                 185                 190

Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
        195                 200                 205

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
    210                 215                 220

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
225                 230                 235                 240

Leu Ser Pro Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser
                245                 250                 255

Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys Cys Lys
            260                 265                 270

His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys Gly Thr
        275                 280                 285

Cys
```

<210> 347
<211> 289
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Fusion polypeptide

<400> 347

```
Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu Ser Val Thr Thr Gly
1               5                  10                  15

Val His Ser Glu Arg Lys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro
            20                  25                  30

Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
        35                  40                  45

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
    50                  55                  60

Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
65                  70                  75                  80

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
                85                  90                  95

Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu
            100                 105                 110

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala
        115                 120                 125

Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro
    130                 135                 140

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
145                 150                 155                 160

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
                165                 170                 175

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
            180                 185                 190

Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
        195                 200                 205

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
    210                 215                 220

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
225                 230                 235                 240

Leu Ser Pro Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser
                245                 250                 255
```

```
Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys Cys Lys
            260                 265                 270

His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys Gly Thr
        275                 280                 285

Cys
```

<210> 348
<211> 290
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> IgG2 Fc-L10-ShK(1-35, Q16K) fusion protein

<400> 348

```
Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu Ser Val Thr Thr Gly
1               5                   10                  15

Val His Ser Glu Arg Lys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro
            20                  25                  30

Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
        35                  40                  45

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
    50                  55                  60

Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
65                  70                  75                  80

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
                85                  90                  95

Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu
            100                 105                 110

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala
        115                 120                 125

Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro
    130                 135                 140

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
145                 150                 155                 160

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
                165                 170                 175
```

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
180 185 190

Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
195 200 205

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
210 215 220

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
225 230 235 240

Leu Ser Pro Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Arg
245 250 255

Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys Cys
260 265 270

Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys Gly
275 280 285

Thr Cys
290

<210> 349
<211> 24
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Random primer with an extension adapter

<220>
<221> misc_feature
<222> (18)..(23)
<223> Any deoxriboneucleotide

<400> 349
ggccggatag gcctccannn nnnt 24

<210> 350
<211> 45
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Forward primer

<400> 350
gtggttgaga ggtgccagat gtgacattgt gatgactcag tctcc 45

<210> 351
<211> 41
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>

<223> Reverse primer

<400> 351
aaccgtttaa acgcggccgc tcaacactct cccctgttga a 41

<210> 352
<211> 43
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Optimized Kozak seqence

<400> 352
aagctcgagg tcgactagac caccatggac atgagggtcc ccg 43

<210> 353
<211> 45
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 353
aagctcgagg tcgactagac caccatggac atgagggtgc ccgct 45

<210> 354
<211> 25
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 354
tcatctggat gtcacatctg gcacc 25

<210> 355
<211> 25
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 355
ggtgccagat gtgacatcca gatga 25

<210> 356
<211> 1398
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> aKLH 120.6-VK1SP-IgG2 Heavy Chain coding sequence

<400> 356
atggacatga gggtgcccgc tcagctcctg gggctcctgc tgctgtggct gagaggtgcc 60

agatgtcagg tgcagctggt gcagtctggg gctgaggtga agaagcctgg ggcctcagtg 120

aaggtctcct gcaaggcttc tggatacacc ttcaccggct accacatgca ctgggtgcga 180

caggcccctg gacaagggct tgagtggatg ggatggatca accctaacag tggtggcaca 240

aactatgcac agaagtttca gggcagggtc accatgacca gggacacgtc catcagcaca 300

gcctacatgg agctgagcag gctgagatct gacgacacgg ccgtgtatta ctgtgcgaga 360

gatcgtggga gctactactg gttcgacccc tggggccagg gaaccctggt caccgtctcc 420

tcagcctcca ccaagggccc atcggtcttc cccctggcgc cctgctccag gagcacctcc 480

gagagcacag cggccctggg ctgcctggtc aaggactact tccccgaacc ggtgacggtg 540

tcgtggaact caggcgctct gaccagcggc gtgcacacct tcccagctgt cctacagtcc 600

tcaggactct actccctcag cagcgtggtg accgtgccct ccagcaactt cggcacccag 660

acctacacct gcaacgtaga tcacaagccc agcaacacca aggtggacaa gacagttgag 720

cgcaaatgtt gtgtcgagtg cccaccgtgc ccagcaccac ctgtggcagg accgtcagtc 780

ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcacg 840

tgcgtggtgg tggacgtgag ccacgaagac cccgaggtcc agttcaactg gtacgtggac 900

ggcgtggagg tgcataatgc caagacaaag ccacgggagg agcagttcaa cagcacgttc 960

cgtgtggtca gcgtcctcac cgttgtgcac caggactggc tgaacggcaa ggagtacaag 1020

tgcaaggtct ccaacaaagg cctcccagcc cccatcgaga aaaccatctc caaaaccaaa 1080

gggcagcccc gagaaccaca ggtgtacacc ctgcccccat cccgggagga gatgaccaag 1140

aaccaggtca gcctgacctg cctggtcaaa ggcttctacc ccagcgacat cgccgtggag 1200

tgggagagca atgggcagcc ggagaacaac tacaagacca cacctcccat gctggactcc 1260

gacggctcct tcttcctcta cagcaagctc accgtggaca agagcaggtg gcagcagggg 1320

aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc 1380

ctctccctgt ctccgggt 1398

<210> 357
<211> 466
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> aKLH 120.6-VK1SP-IgG2 Heavy Chain

<400> 357

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

Leu Arg Gly Ala Arg Cys Gln Val Gln Leu Val Gln Ser Gly Ala Glu
20 25 30

Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly
35 40 45

Tyr Thr Phe Thr Gly Tyr His Met His Trp Val Arg Gln Ala Pro Gly
50 55 60

```
Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr
65                  70                  75                  80

Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr
                85                  90                  95

Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp
            100                 105                 110

Thr Ala Val Tyr Tyr Cys Ala Arg Asp Arg Gly Ser Tyr Tyr Trp Phe
        115                 120                 125

Asp Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr
    130                 135                 140

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser
145                 150                 155                 160

Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
                165                 170                 175

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
            180                 185                 190

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
        195                 200                 205

Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys
    210                 215                 220

Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu
225                 230                 235                 240

Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala
                245                 250                 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
            260                 265                 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        275                 280                 285

Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    290                 295                 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe
305                 310                 315                 320

Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly
                325                 330                 335
```

```
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile
            340                 345                 350

Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val
        355                 360                 365

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
    370                 375                 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385                 390                 395                 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                405                 410                 415

Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            420                 425                 430

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        435                 440                 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    450                 455                 460

Pro Gly
465


<210>  358
<211>  34
<212>  DNA
<213>  ARTIFICIAL SEQUENCE

<220>
<223>  Primer sequence

<400>  358
ggatcctcct cctccacccg gagacaggga gagg                                  34


<210>  359
<211>  34
<212>  DNA
<213>  ARTIFICIAL SEQUENCE

<220>
<223>  Primer sequence

<400>  359
tccctgtctc cgggtggagg aggaggatcc ggag                                  34


<210>  360
<211>  39
<212>  PRT
<213>  ARTIFICIAL SEQUENCE

<220>
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<223> Gly-Gly-ShK-Gly-Gly sequence

<400> 360

Gly Gly Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala
1 5 10 15

Phe Gln Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys
20 25 30

Thr Cys Gly Thr Cys Gly Gly
35

<210> 361
<211> 127
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Forward primer

<220>
<221> misc_feature
<222> (6)..(11)
<223> HINDIII Site

<400> 361
tgcagaagct tctagaccac catggaatgg agctgggtct ttctcttctt cctgtcagta 60

acgactggtg tccactcccg cagctgcatc gacaccatcc ccaagagccg ctgcaccgcc 120

ttccagt 127

<210> 362
<211> 115
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Reverse primer

<220>
<221> misc_feature
<222> (5)..(10)
<223> BAMHI Site

<400> 362
ctccggatcc tcctcctccg caggtgccgc aggtcttgcg gcagaagctc aggcggtact 60

tcatgctgtg cttgcactgg aaggcggtgc agcggctctt ggggatggtg tcgat 115

<210> 363
<211> 38
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Forward primer

<220>

<221> misc_feature
<222> (4)..(9)
<223> BAMHI Site

<400> 363
gtaggatccg gaggaggagg aagcgacaaa actcacac 38

<210> 364
<211> 35
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Reverse primer

<220>
<221> misc_feature
<222> (4)..(11)
<223> NOTI Site

<400> 364
cgagcggccg cttactattt acccggagac aggga 35

<210> 365
<211> 879
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> VH21 SP-ShK(1-35)-L10-IgG1 Fc coding sequence

<400> 365
atggaatgga gctgggtctt tctcttcttc ctgtcagtaa cgactggtgt ccactcccgc 60

agctgcatcg acaccatccc caagagccgc tgcaccgcct tccagtgcaa gcacagcatg 120

aagtaccgcc tgagcttctg ccgcaagacc tgcggcacct gcggaggagg aggatccgga 180

ggaggaggaa gcgacaaaac tcacacatgc ccaccgtgcc cagcacctga actcctgggg 240

ggaccgtcag tcttcctctt cccccccaaaa cccaaggaca ccctcatgat ctcccggacc 300

cctgaggtca catgcgtggt ggtggacgtg agccacgaag accctgaggt caagttcaac 360

tggtacgtgg acggcgtgga ggtgcataat gccaagacaa agccgcggga ggagcagtac 420

aacagcacgt accgtgtggt cagcgtcctc accgtcctgc accaggactg gctgaatggc 480

aaggagtaca agtgcaaggt ctccaacaaa gccctcccag cccccatcga gaaaaccatc 540

tccaaagcca aagggcagcc ccgagaacca caggtgtaca ccctgccccc atcccgggat 600

gagctgacca agaaccaggt cagcctgacc tgcctggtca aaggcttcta tcccagcgac 660

atcgccgtgg agtgggagag caatgggcag ccggagaaca actacaagac cacgcctccc 720

gtgctggact ccgacggctc cttcttcctc tacagcaagc tcaccgtgga caagagcagg 780

tggcagcagg ggaacgtctt ctcatgctcc gtgatgcatg aggctctgca caaccactac 840

acgcagaaga gcctctccct gtctccgggt aaatagtaa 879

<210> 366
<211> 291

<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> VH21 SP-ShK(1-35)-L10-IgG1 Fc

<400> 366

Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu Ser Val Thr Thr Gly
1 5 10 15

Val His Ser Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr
20 25 30

Ala Phe Gln Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg
35 40 45

Lys Thr Cys Gly Thr Cys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
50 55 60

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
65 70 75 80

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
85 90 95

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
100 105 110

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
115 120 125

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
130 135 140

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
145 150 155 160

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
165 170 175

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
180 185 190

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
195 200 205

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
210 215 220

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
225 230 235 240

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
245 250 255

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
260 265 270

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
275 280 285

Pro Gly Lys
290

<210> 367
<211> 23
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 367
cattctagac caccatggaa tgg 23

<210> 368
<211> 29
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 368
cagctgcacc tggcttcctc ctcctccgg 29

<210> 369
<211> 192
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> VH21 SP-ShK(1-35, Q16K)-L10 coding sequence

<400> 369
atggaatgga gctgggtctt tctcttcttc ctgtcagtaa cgactggtgt ccactcccgc 60

agctgcatcg acaccatccc caagagccgc tgcaccgcct tcaagtgcaa gcacagcatg 120

aagtaccgcc tgagcttctg ccgcaagacc tgcggcacct gcggaggagg aggatccgga 180

ggaggaggaa gc 192

<210> 370
<211> 64
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> VH21 SP-ShK(1-35, Q16K)-L10

<400> 370

Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu Ser Val Thr Thr Gly
1 5 10 15

Val His Ser Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr
20 25 30

Ala Phe Lys Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg
35 40 45

Lys Thr Cys Gly Thr Cys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
50 55 60

<210> 371
<211> 30
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 371
ggaggaggaa gccaggtgca gctggtgcag 30

<210> 372
<211> 21
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 372
catgcggccg ctcatttacc c 21

<210> 373
<211> 1338
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> aKLH 120.6-HC coding sequence

<400> 373
caggtgcagc tggtgcagtc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc 60

tcctgcaagg cttctggata caccttcacc ggctaccaca tgcactgggt gcgacaggcc 120

cctggacaag ggcttgagtg gatgggatgg atcaacccta acagtggtgg cacaaactat 180

gcacagaagt ttcagggcag ggtcaccatg accagggaca cgtccatcag cacagcctac 240

atggagctga gcaggctgag atctgacgac acggccgtgt attactgtgc gagagatcgt 300

gggagctact actggttcga cccctggggc cagggaaccc tggtcaccgt ctcctcagcc 360

tccaccaagg gcccatcggt cttccccctg gcgccctgct ccaggagcac ctccgagagc 420

acagcggccc tgggctgcct ggtcaaggac tacttccccg aaccggtgac ggtgtcgtgg 480

aactcaggcg ctctgaccag cggcgtgcac accttcccag ctgtcctaca gtcctcagga 540

ctctactccc tcagcagcgt ggtgaccgtg ccctccagca acttcggcac ccagacctac 600

acctgcaacg tagatcacaa gcccagcaac accaaggtgg acaagacagt tgagcgcaaa 660

tgttgtgtcg agtgcccacc gtgcccagca ccacctgtgg caggaccgtc agtcttcctc 720

ttcccccaa aacccaagga caccctcatg atctcccgga cccctgaggt cacgtgcgtg 780

gtggtggacg tgagccacga agaccccgag gtccagttca actggtacgt ggacggcgtg 840

gaggtgcata atgccaagac aaagccacgg gaggagcagt tcaacagcac gttccgtgtg 900

gtcagcgtcc tcaccgttgt gcaccaggac tggctgaacg gcaaggagta caagtgcaag 960

gtctccaaca aaggcctccc agcccccatc gagaaaacca tctccaaaac caaagggcag 1020

ccccgagaac cacaggtgta caccctgccc ccatcccggg aggagatgac caagaaccag 1080

gtcagcctga cctgcctggt caaaggcttc taccccagcg acatcgccgt ggagtgggag 1140

agcaatgggc agccggagaa caactacaag accacacctc ccatgctgga ctccgacggc 1200

tccttcttcc tctacagcaa gctcaccgtg gacaagagca ggtggcagca ggggaacgtc 1260

ttctcatgct ccgtgatgca tgaggctctg cacaaccact acacgcagaa gagcctctcc 1320

ctgtctccgg gtaaatga 1338

<210> 374
<211> 445
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> aKLH 120.6-HC polypeptide sequence

<400> 374

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
20 25 30

His Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Arg Gly Ser Tyr Tyr Trp Phe Asp Pro Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

```
Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
    130                 135                 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145                 150                 155                 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
                165                 170                 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
            180                 185                 190

Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro
        195                 200                 205

Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu
    210                 215                 220

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
225                 230                 235                 240

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
                245                 250                 255

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln
            260                 265                 270

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
        275                 280                 285

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu
    290                 295                 300

Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305                 310                 315                 320

Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
                325                 330                 335

Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
            340                 345                 350

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
        355                 360                 365

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
    370                 375                 380

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly
385                 390                 395                 400
```

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
405 410 415

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
420 425 430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 375
<211> 29
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 375
catctggatg tcgcttcctc ctcctccgg 29

<210> 376

<400> 376
000

<210> 377

<400> 377
000

<210> 378
<211> 32
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 378
ggaggaggaa gcgacatcca gatgacccag tc 32

<210> 379
<211> 21
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 379
catctcgagc ggccgctcaa c 21

<210> 380
<211> 837
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> VH21 SP-ShK[1-35, Q16K]-L10-aKLH120.6 Light Chain coding sequence

<400> 380
atggaatgga gctgggtctt tctcttcttc ctgtcagtaa cgactggtgt ccactcccgc 60

agctgcatcg acaccatccc caagagccgc tgcaccgcct tcaagtgcaa gcacagcatg 120

aagtaccgcc tgagcttctg ccgcaagacc tgcggcacct gcggaggagg aggatccgga 180

ggaggaggaa gcgacatcca gatgacccag tctccatcct ccctgtctgc atctgtagga 240

gacagagtca ccatcacttg ccgggcaagt cagggcatta gaaatgattt aggctggtat 300

cagcagaaac cagggaaagc ccctaaacgc ctgatctatg ctgcatccag tttgcaaagt 360

ggggtcccat caaggttcag cggcagtgga tctgggacag aattcactct cacaatcagc 420

agcctgcagc ctgaagattt tgcaacttat tactgtctac agcataatag ttacccgctc 480

actttcggcg gagggaccaa ggtggagatc aaacgaactg tggctgcacc atctgtcttc 540

atcttcccgc catctgatga gcagttgaaa tctggaactg cctctgttgt gtgcctgctg 600

aataacttct atcccagaga ggccaaagta cagtggaagg tggataacgc cctccaatcg 660

ggtaactccc aggagagtgt cacagagcag gacagcaagg acagcaccta cagcctcagc 720

agcaccctga cgctgagcaa agcagactac gagaaacaca aagtctacgc ctgcgaagtc 780

acccatcagg gcctgagctc gcccgtcaca aagagcttca acaggggaga gtgttga 837

<210> 381
<211> 278
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> VH21 SP-ShK[1-35, Q16K]-L10-aKLH120.6 Light Chain

<400> 381

Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu Ser Val Thr Thr Gly
1 5 10 15

Val His Ser Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr
20 25 30

Ala Phe Lys Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg
35 40 45

Lys Thr Cys Gly Thr Cys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
50 55 60

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
65 70 75 80

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp
85 90 95

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
100 105 110

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
115 120 125

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
130 135 140

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln His Asn Ser Tyr Pro Leu
145 150 155 160

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
165 170 175

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
180 185 190

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
195 200 205

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
210 215 220

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
225 230 235 240

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
245 250 255

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
260 265 270

Phe Asn Arg Gly Glu Cys
275

<210> 382

<400> 382
000

<210> 383
<211> 1038
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Coding sequence - IgG2 fragment

<400> 383
atggacatga gggtgcccgc tcagctcctg gggctcctgc tgctgtggct gagaggtgcg 60

cgctgtcagg tgcagctggt ggagtctggg ggaggcgtgg tccagcctgg gaggtccctg 120

agactctcct gtgcagcgtc tggattcacc ttcagtagct atggcatgca ctgggtccgc 180

caggctccag gcaaggggct ggagtgggtg gcagttatat ggtatgatgg aagtaataaa 240

tactatgcag actccgtgaa gggccgattc actatctcca gagacaattc caagaacacg 300

ctgtatctgc aaatgaacag cctgagagcc gaggacacgg ctgtgtatta ctgtgcgagg 360

tataacttca actacggtat ggacgtctgg ggccaaggga ccacggtcac cgtctctagt 420

gcctccacca agggcccatc ggtcttcccc ctggcgccct gctccaggag cacctccgag 480

agcacagcgg ccctgggctg cctggtcaag gactacttcc ccgaaccggt gacggtgtcg 540

tggaactcag gcgctctgac cagcggcgtg cacaccttcc cagctgtcct acagtcctca 600

ggactctact ccctcagcag cgtggtgacc gtgccctcca gcaacttcgg cacccagacc 660

tacacctgca acgtagatca caagcccagc aacaccaagg tggacaagac agttgagcgc 720

aaatgttgtg tcgagtgccc accgtgccca gcaccacctg tggcaggacc gtcagtcttc 780

ctcttccccc caaaacccaa ggacaccctc atgatctccc ggaccctga ggtcacgtgc 840

gtggtggtgg acgtgagcca cgaagacccc gaggtccagt tcaactggta cgtggacggc 900

gtggaggtgc ataatgccaa gacaaagcca cgggaggagc agttcaacag cacgttccgt 960

gtggtcagcg tcctcaccgt tgtgcaccag gactggctga acggcaagga gtacaagtgc 1020

aaggtctcca acaaaggc 1038

<210> 384
<211> 346
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> IgG2 fragment

<400> 384

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

Leu Arg Gly Ala Arg Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly
20 25 30

Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
35 40 45

Phe Thr Phe Ser Ser Tyr Gly Met His Trp Val Arg Gln Ala Pro Gly
50 55 60

Lys Gly Leu Glu Trp Val Ala Val Ile Trp Tyr Asp Gly Ser Asn Lys
65 70 75 80

Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn
85 90 95

Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
100 105 110

Thr Ala Val Tyr Tyr Cys Ala Arg Tyr Asn Phe Asn Tyr Gly Met Asp
115 120 125

```
Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys
    130                 135                 140

Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu
145                 150                 155                 160

Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
                165                 170                 175

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
            180                 185                 190

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
        195                 200                 205

Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn
    210                 215                 220

Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg
225                 230                 235                 240

Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly
                245                 250                 255

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
            260                 265                 270

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
        275                 280                 285

Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
    290                 295                 300

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg
305                 310                 315                 320

Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys
                325                 330                 335

Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
            340                 345


<210>  385
<211>  37
<212>  DNA
<213>  ARTIFICIAL SEQUENCE

<220>
<223>  Forward primer

<400>  385
tttttttgc gcgctgtgac atccagatga cccagtc                                  37
```

<210> 386

<400> 386
000

<210> 387
<211> 510
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> aKLH 120.6 IgG2-ShK[2-35, Q16K] fusion

<400> 387

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1   5   10   15

Leu Arg Gly Ala Arg Cys Gln Val Gln Leu Val Gln Ser Gly Ala Glu
            20   25   30

Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly
        35   40   45

Tyr Thr Phe Thr Gly Tyr His Met His Trp Val Arg Gln Ala Pro Gly
    50   55   60

Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr
65   70   75   80

Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr
                85   90   95

Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp
            100   105   110

Thr Ala Val Tyr Tyr Cys Ala Arg Asp Arg Gly Ser Tyr Tyr Trp Phe
        115   120   125

Asp Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr
    130   135   140

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser
145   150   155   160

Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
                165   170   175

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
            180   185   190

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
        195   200   205

```
Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys
    210                 215                 220

Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu
225                 230                 235                 240

Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala
                245                 250                 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
            260                 265                 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        275                 280                 285

Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    290                 295                 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe
305                 310                 315                 320

Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly
                325                 330                 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile
            340                 345                 350

Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val
        355                 360                 365

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
    370                 375                 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385                 390                 395                 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                405                 410                 415

Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            420                 425                 430

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        435                 440                 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    450                 455                 460

Pro Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Cys Ile Asp
465                 470                 475                 480
```

```
Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys Cys Lys His Ser Met
                485                 490                 495


Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys Gly Thr Cys
            500                 505                 510


<210>  388
<211>  684
<212>  DNA
<213>  Homo sapiens


<220>
<221>  CDS
<222>  (1)..(684)

<400>  388
atg gac aaa act cac aca tgt cca cct tgt cca gct ccg gaa ctc ctg       48
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc       96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc      144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag      192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac agc acg      240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat      288
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc      336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag      384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc      432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg      480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct      528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc      576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg      624
```

```
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg      672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg ggt aaa                                                      684
Ser Pro Gly Lys
225
```

<210> 389
<211> 228
<212> PRT
<213> Homo sapiens

<400> 389

```
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
```

195 200 205

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
210 215 220

Ser Pro Gly Lys
225

<210> 390

<400> 390
000

<210> 391
<211> 744
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Coding sequence - IgG2 fragment

<400> 391
atggaatgga gctgggtctt tctcttcttc ctgtcagtaa cgactggtgt ccactccgag 60

cgcaaagtcg agtgcccacc gtgcccagca ccacctgtgg caggaccgtc agtcttcctc 120

ttcccccaa aacccaagga caccctcatg atctcccgga cccctgaggt cacgtgcgtg 180

gtggtggacg tgagccacga agaccccgag gtccagttca actggtacgt ggacggcgtg 240

gaggtgcata atgccaagac aaagccacgg gaggagcagt tcaacagcac gttccgtgtg 300

gtcagcgtcc tcaccgttgt gcaccaggac tggctgaacg gcaaggagta caagtgcaag 360

gtctccaaca aaggcctccc agcccccatc gagaaaacca tctccaaaac caaagggcag 420

ccccgagaac cacaggtgta caccctgccc ccatcccggg aggagatgac caagaaccag 480

gtcagcctga cctgcctggt caaaggcttc taccccagcg acatcgccgt ggagtgggag 540

agcaatgggc agccggagaa caactacaag accacacctc ccatgctgga ctccgacggc 600

tccttcttcc tctacagcaa gctcaccgtg gacaagagca ggtggcagca ggggaacgtc 660

ttctcatgct ccgtgatgca tgaggctctg cacaaccact acacgcagaa gagcctctcc 720

ctgtctccgg gtaaaggagg agga 744

<210> 392
<211> 248
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> IgG2 fragment

<400> 392

Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu Ser Val Thr Thr Gly
1 5 10 15

Val His Ser Glu Arg Lys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro
20 25 30

Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
35 40 45

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
50 55 60

Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
65 70 75 80

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser
85 90 95

Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu
100 105 110

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala
115 120 125

Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro
130 135 140

Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
145 150 155 160

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
165 170 175

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
180 185 190

Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
195 200 205

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
210 215 220

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
225 230 235 240

Leu Ser Pro Gly Lys Gly Gly Gly
245

<210> 393
<211> 30
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 393
cattctagac ccaccatgga catgagggtg 30

<210> 394
<211> 511
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> IgG2-HC-L10-ShK[1-35] fusion polypeptide

<400> 394

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

Leu Arg Gly Ala Arg Cys Gln Val Gln Leu Val Gln Ser Gly Ala Glu
20 25 30

Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly
35 40 45

Tyr Thr Phe Thr Gly Tyr His Met His Trp Val Arg Gln Ala Pro Gly
50 55 60

Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr
65 70 75 80

Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr
85 90 95

Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp
100 105 110

Thr Ala Val Tyr Tyr Cys Ala Arg Asp Arg Gly Ser Tyr Tyr Trp Phe
115 120 125

Asp Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr
130 135 140

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser
145 150 155 160

Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
165 170 175

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
180 185 190

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
195 200 205

Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys
210 215 220

```
Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu
225                 230                 235                 240

Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala
                245                 250                 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
            260                 265                 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        275                 280                 285

Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    290                 295                 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe
305                 310                 315                 320

Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly
                325                 330                 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile
            340                 345                 350

Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val
        355                 360                 365

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
    370                 375                 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385                 390                 395                 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                405                 410                 415

Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            420                 425                 430

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        435                 440                 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    450                 455                 460

Pro Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Arg Ser Cys Ile
465                 470                 475                 480

Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln Cys Lys His Ser
                485                 490                 495
```

Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys Gly Thr Cys
500 505 510

<210> 395
<211> 501
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> truncated IgG2 Fc-L10-ShK(1-35, Q16K) coding sequence

<400> 395
ctcccagccc ccatcgagaa aaccatctcc aaaaccaaag ggcagccccg agaaccacag 60

gtgtacaccc tgcccccatc ccgggaggag atgaccaaga accaggtcag cctgacctgc 120

ctggtcaaag gcttctaccc cagcgacatc gccgtggagt gggagagcaa tgggcagccg 180

gagaacaact acaagaccac acctcccatg ctggactccg acggctcctt cttcctctac 240

agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgctccgtg 300

atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccgggtaaa 360

ggaggaggag gatccggagg aggaggaagc cgcagctgca tcgacaccat ccccaagagc 420

cgctgcaccg ccttcaagtg caagcacagc atgaagtacc gcctgagctt ctgccgcaag 480

acctgcggca cctgctaatg a 501

<210> 396
<211> 165
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> truncated IgG2 Fc-L10-ShK(1-35, Q16K) amino acid sequence

<400> 396

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro
1 5 10 15

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr
20 25 30

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
35 40 45

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
50 55 60

Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
65 70 75 80

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
85 90 95

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
100 105 110

Ser Leu Ser Leu Ser Pro Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125

Gly Ser Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala
130 135 140

Phe Lys Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys
145 150 155 160

Thr Cys Gly Thr Cys
165

<210> 397
<211> 129
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Coding sequence

<400> 397
ggatccggag gaggaggaag cagctgcatc gacaccatcc ccaagagccg ctgcaccgcc 60

ttcaagtgca agcacagcat gaagtaccgc ctgagcttct gccgcaagac ctgcggcacc 120

tgctaatga 129

<210> 398
<211> 41
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Polypeptide sequence

<400> 398

Gly Ser Gly Gly Gly Gly Ser Ser Cys Ile Asp Thr Ile Pro Lys Ser
1 5 10 15

Arg Cys Thr Ala Phe Lys Cys Lys His Ser Met Lys Tyr Arg Leu Ser
20 25 30

Phe Cys Arg Lys Thr Cys Gly Thr Cys
35 40

<210> 399
<211> 1407
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Coding sequence

<400> 399
atggacatga gggtgcccgc tcagctcctg gggctcctgc tgctgtggct gagaggtgcc 60

agatgtcagg tgcagctggt gcagtctggg gctgaggtga agaagcctgg ggcctcagtg 120

aaggtctcct gcaaggcttc tggatacacc ttcaccggct accacatgca ctgggtgcga 180

caggcccctg gacaagggct tgagtggatg ggatggatca accctaacag tggtggcaca 240

aactatgcac agaagtttca gggcagggtc accatgacca gggacacgtc catcagcaca 300

gcctacatgg agctgagcag gctgagatct gacgacacgg ccgtgtatta ctgtgcgaga 360

gatcgtggga gctactactg gttcgacccc tggggccagg gaaccctggt caccgtctcc 420

tcagcctcca ccaagggccc atcggtcttc cccctggcgc cctgctccag gagcacctcc 480

gagagcacag cggccctggg ctgcctggtc aaggactact tccccgaacc ggtgacggtg 540

tcgtggaact caggcgctct gaccagcggc gtgcacacct tcccagctgt cctacagtcc 600

tcaggactct actccctcag cagcgtggtg accgtgccct ccagcaactt cggcacccag 660

acctacacct gcaacgtaga tcacaagccc agcaacacca aggtggacaa gacagttgag 720

cgcaaatgtt gtgtcgagtg cccaccgtgc ccagcaccac ctgtggcagg accgtcagtc 780

ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcacg 840

tgcgtggtgg tggacgtgag ccacgaagac cccgaggtcc agttcaactg gtacgtggac 900

ggcgtggagg tgcataatgc caagacaaag ccacgggagg agcagttcaa cagcacgttc 960

cgtgtggtca gcgtcctcac cgttgtgcac caggactggc tgaacggcaa ggagtacaag 1020

tgcaaggtct ccaacaaagg cctcccagcc cccatcgaga aaaccatctc caaaaccaaa 1080

gggcagcccc gagaaccaca ggtgtacacc ctgcccccat cccgggagga gatgaccaag 1140

aaccaggtca gcctgacctg cctggtcaaa ggcttctacc ccagcgacat cgccgtggag 1200

tgggagagca atgggcagcc ggagaacaac tacaagacca cacctcccat gctggactcc 1260

gacggctcct tcttcctcta cagcaagctc accgtggaca agagcaggtg gcagcagggg 1320

aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc 1380

ctctccctgt ctccgggtgg aggagga 1407

<210> 400
<211> 469
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Polypeptide sequence

<400> 400

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

Leu Arg Gly Ala Arg Cys Gln Val Gln Leu Val Gln Ser Gly Ala Glu
20 25 30

Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly
35 40 45

```
Tyr Thr Phe Thr Gly Tyr His Met His Trp Val Arg Gln Ala Pro Gly
    50                  55                  60

Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr
65                  70                  75                  80

Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr
                85                  90                  95

Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp
            100                 105                 110

Thr Ala Val Tyr Tyr Cys Ala Arg Asp Arg Gly Ser Tyr Tyr Trp Phe
        115                 120                 125

Asp Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr
    130                 135                 140

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser
145                 150                 155                 160

Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
                165                 170                 175

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
            180                 185                 190

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
        195                 200                 205

Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys
    210                 215                 220

Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu
225                 230                 235                 240

Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala
                245                 250                 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
            260                 265                 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        275                 280                 285

Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    290                 295                 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe
305                 310                 315                 320
```

Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly
325 330 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile
340 345 350

Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val
355 360 365

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
370 375 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385 390 395 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
405 410 415

Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
420 425 430

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
435 440 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
450 455 460

Pro Gly Gly Gly Gly
465

<210> 401
<211> 510
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> IgG2-HC-L10-ShK[2-35] fusion polypeptide

<400> 401

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

Leu Arg Gly Ala Arg Cys Gln Val Gln Leu Val Gln Ser Gly Ala Glu
20 25 30

Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly
35 40 45

Tyr Thr Phe Thr Gly Tyr His Met His Trp Val Arg Gln Ala Pro Gly
50 55 60

Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr
65 70 75 80

```
Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr
                85                  90                  95

Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp
            100                 105                 110

Thr Ala Val Tyr Tyr Cys Ala Arg Asp Arg Gly Ser Tyr Tyr Trp Phe
        115                 120                 125

Asp Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr
    130                 135                 140

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser
145                 150                 155                 160

Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
                165                 170                 175

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
            180                 185                 190

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
        195                 200                 205

Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys
    210                 215                 220

Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu
225                 230                 235                 240

Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala
                245                 250                 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
            260                 265                 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        275                 280                 285

Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    290                 295                 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe
305                 310                 315                 320

Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly
                325                 330                 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile
            340                 345                 350
```

```
Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val
        355                 360                 365

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
    370                 375                 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385                 390                 395                 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                405                 410                 415

Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            420                 425                 430

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        435                 440                 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    450                 455                 460

Pro Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Cys Ile Asp
465                 470                 475                 480

Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys Cys Lys His Ser Met
                485                 490                 495

Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys Gly Thr Cys
            500                 505                 510


<210>  402
<211>  291
<212>  PRT
<213>  ARTIFICIAL SEQUENCE

<220>
<223>  VH21 SP-ShK(1-35, Q16K)-L10-IgG1 Fc

<400>  402

Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu Ser Val Thr Thr Gly
1               5                   10                  15

Val His Ser Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr
            20                  25                  30

Ala Phe Lys Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg
        35                  40                  45

Lys Thr Cys Gly Thr Cys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
    50                  55                  60
```

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
65 70 75 80

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
85 90 95

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
100 105 110

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
115 120 125

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
130 135 140

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
145 150 155 160

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
165 170 175

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
180 185 190

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
195 200 205

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
210 215 220

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
225 230 235 240

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
245 250 255

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
260 265 270

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
275 280 285

Pro Gly Lys
290

<210> 403
<211> 509
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> VH21 SP-ShK[1-35, Q16K]-L10-aKLH120.6-HC fusion polypeptide

<400> 403

```
Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu Ser Val Thr Thr Gly
1               5                  10                  15

Val His Ser Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr
            20                  25                  30

Ala Phe Lys Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg
        35                  40                  45

Lys Thr Cys Gly Thr Cys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
    50                  55                  60

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
65                  70                  75                  80

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
                85                  90                  95

His Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
            100                 105                 110

Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
        115                 120                 125

Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
    130                 135                 140

Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
145                 150                 155                 160

Ala Arg Asp Arg Gly Ser Tyr Tyr Trp Phe Asp Pro Trp Gly Gln Gly
                165                 170                 175

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
            180                 185                 190

Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
        195                 200                 205

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
    210                 215                 220

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
225                 230                 235                 240

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
                245                 250                 255

Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro
            260                 265                 270
```

Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu
        275   280   285

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
    290   295   300

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
305   310   315   320

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln
                325   330   335

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
            340   345   350

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu
        355   360   365

Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
    370   375   380

Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
385   390   395   400

Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
                405   410   415

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
            420   425   430

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
        435   440   445

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly
    450   455   460

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
465   470   475   480

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
                485   490   495

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
            500   505

<210> 404
<211> 278
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>

<223> Fusion polypeptide

<400> 404

```
Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu Ser Val Thr Thr Gly
1               5                  10                  15

Val His Ser Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr
            20                  25                  30

Ala Phe Lys Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg
        35                  40                  45

Lys Thr Cys Gly Thr Cys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
    50                  55                  60

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
65                  70                  75                  80

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp
                85                  90                  95

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
            100                 105                 110

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
        115                 120                 125

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
    130                 135                 140

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln His Asn Ser Tyr Pro Leu
145                 150                 155                 160

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
                165                 170                 175

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
            180                 185                 190

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
        195                 200                 205

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
    210                 215                 220

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
225                 230                 235                 240

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
                245                 250                 255
```

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
260 265 270

Phe Asn Arg Gly Glu Cys
275

<210> 405
<211> 465
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> anti-DNP 3A4 (W101F) IgG2 Heavy Chain

<400> 405

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

Leu Arg Gly Ala Arg Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly
20 25 30

Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
35 40 45

Phe Thr Phe Ser Ser Tyr Gly Met His Trp Val Arg Gln Ala Pro Gly
50 55 60

Lys Gly Leu Glu Trp Val Ala Val Ile Trp Tyr Asp Gly Ser Asn Lys
65 70 75 80

Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn
85 90 95

Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
100 105 110

Thr Ala Val Tyr Tyr Cys Ala Arg Tyr Asn Phe Asn Tyr Gly Met Asp
115 120 125

Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys
130 135 140

Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu
145 150 155 160

Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
165 170 175

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
180 185 190

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
195 200 205

```
Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn
    210                 215                 220

Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg
225                 230                 235                 240

Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly
                245                 250                 255

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
            260                 265                 270

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
        275                 280                 285

Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
    290                 295                 300

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg
305                 310                 315                 320

Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys
                325                 330                 335

Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu
            340                 345                 350

Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
        355                 360                 365

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
    370                 375                 380

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
385                 390                 395                 400

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met
                405                 410                 415

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
            420                 425                 430

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
        435                 440                 445

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
    450                 455                 460

Gly
465
```

<210> 406
<211> 510
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> aDNP 3A4 (W101F) IgG2 HC-L10-ShK[1-35, Q16K] fusion protein

<400> 406

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

Leu Arg Gly Ala Arg Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly
20 25 30

Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
35 40 45

Phe Thr Phe Ser Ser Tyr Gly Met His Trp Val Arg Gln Ala Pro Gly
50 55 60

Lys Gly Leu Glu Trp Val Ala Val Ile Trp Tyr Asp Gly Ser Asn Lys
65 70 75 80

Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn
85 90 95

Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
100 105 110

Thr Ala Val Tyr Tyr Cys Ala Arg Tyr Asn Phe Asn Tyr Gly Met Asp
115 120 125

Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys
130 135 140

Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu
145 150 155 160

Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
165 170 175

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
180 185 190

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
195 200 205

Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn
210 215 220

```
Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg
225                 230                 235                 240

Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly
                245                 250                 255

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
            260                 265                 270

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
        275                 280                 285

Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
    290                 295                 300

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg
305                 310                 315                 320

Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys
                325                 330                 335

Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu
            340                 345                 350

Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
        355                 360                 365

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
    370                 375                 380

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
385                 390                 395                 400

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met
                405                 410                 415

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
            420                 425                 430

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
        435                 440                 445

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
    450                 455                 460

Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Arg Ser Cys Ile Asp
465                 470                 475                 480

Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys Cys Lys His Ser Met
                485                 490                 495
```

Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys Gly Thr Cys
500 505 510

<210> 407
<211> 236
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> anti-DNP 3A4 Antibody Light Chain

<400> 407

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

Leu Arg Gly Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
20 25 30

Val Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
35 40 45

Gln Gly Ile Ser Arg Arg Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys
50 55 60

Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val
65 70 75 80

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
85 90 95

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
100 105 110

Ala Asn Ser Phe Pro Phe Thr Phe Gly Pro Gly Thr Lys Val Asp Ile
115 120 125

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
130 135 140

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
145 150 155 160

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
165 170 175

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
180 185 190

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
195 200 205

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
210 215 220

```
Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225                 230                 235


<210>  408
<211>  475
<212>  PRT
<213>  ARTIFICIAL SEQUENCE

<220>
<223>  Polypeptide

<400>  408

Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15


Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30


Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45


His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60


Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80


Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95


Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110


Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125


Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140


Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160


Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175


Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190


Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205
```

```
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

Ser Pro Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

Gly Gly Ser Gly Gly Gly Gly Ser Asp Lys Thr His Thr Cys Pro Pro
                245                 250                 255

Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
            260                 265                 270

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
        275                 280                 285

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
    290                 295                 300

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
305                 310                 315                 320

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
                325                 330                 335

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
            340                 345                 350

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
        355                 360                 365

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
    370                 375                 380

Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
385                 390                 395                 400

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
                405                 410                 415

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
            420                 425                 430

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
        435                 440                 445

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
    450                 455                 460

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
465                 470                 475
```

<210> 409
<211> 148
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Polypeptide

<400> 409

Met Lys Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
1 5 10 15

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
20 25 30

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
35 40 45

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
50 55 60

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
65 70 75 80

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
85 90 95

Ile Glu Lys Thr Ile Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
100 105 110

Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys
115 120 125

Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys
130 135 140

Gly Thr Cys Ala
145

<210> 410
<211> 521
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> scFc-Shk construct

<400> 410

Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1 5 10 15

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
20 25 30

```
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

Ser Pro Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

Gly Gly Ser Gly Gly Gly Gly Ser Asp Lys Thr His Thr Cys Pro Pro
                245                 250                 255

Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
            260                 265                 270

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
        275                 280                 285

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
    290                 295                 300
```

```
Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
305                 310                 315                 320

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
                325                 330                 335

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
            340                 345                 350

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
        355                 360                 365

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
    370                 375                 380

Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
385                 390                 395                 400

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
                405                 410                 415

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
            420                 425                 430

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
        435                 440                 445

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
    450                 455                 460

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Gly Gly Gly Gly Ser
465                 470                 475                 480

Gly Gly Gly Gly Ser Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg
                485                 490                 495

Cys Thr Ala Phe Lys Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe
            500                 505                 510

Cys Arg Lys Thr Cys Gly Thr Cys Ala
        515                 520


<210>  411
<211>  514
<212>  PRT
<213>  ARTIFICIAL SEQUENCE

<220>
<223>  scFc-Shk construct

<400>  411

Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                   10                  15
```

```
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130                 135                 140

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

Ser Pro Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
225                 230                 235                 240

Gly Gly Ser Gly Gly Gly Gly Ser Asp Lys Thr His Thr Cys Pro Pro
                245                 250                 255

Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
            260                 265                 270

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
        275                 280                 285
```

```
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
    290                 295                 300

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
305                 310                 315                 320

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
                325                 330                 335

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
            340                 345                 350

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
        355                 360                 365

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
    370                 375                 380

Glu Leu Gly Gly Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys
385                 390                 395                 400

Thr Ala Phe Gln Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe Cys
                405                 410                 415

Arg Lys Thr Cys Gly Thr Cys Gly Gly Thr Lys Asn Gln Val Ser Leu
            420                 425                 430

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
        435                 440                 445

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
    450                 455                 460

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
465                 470                 475                 480

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
                485                 490                 495

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
            500                 505                 510

Gly Lys
```

<210> 412
<211> 519
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>

<223> scFc-Shk construct

<400> 412

```
Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1               5                  10                  15

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50                  55                  60

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65                  70                  75                  80

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85                  90                  95

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100                 105                 110

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Gly Gly Arg Ser Cys
    130                 135                 140

Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Gln Cys Lys His
145                 150                 155                 160

Ser Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys Gly Thr Cys
                165                 170                 175

Gly Gly Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
            180                 185                 190

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
        195                 200                 205

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
    210                 215                 220

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
225                 230                 235                 240

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
                245                 250                 255
```

```
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Gly Gly Gly Gly Ser
            260                 265                 270

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
        275                 280                 285

Gly Gly Gly Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
    290                 295                 300

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
305                 310                 315                 320

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
                325                 330                 335

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
            340                 345                 350

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
        355                 360                 365

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
    370                 375                 380

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
385                 390                 395                 400

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
                405                 410                 415

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
            420                 425                 430

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
        435                 440                 445

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
    450                 455                 460

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
465                 470                 475                 480

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
                485                 490                 495

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
            500                 505                 510

Leu Ser Leu Ser Pro Gly Lys
        515
```

<210> 413
<211> 480
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> Polypeptide

<400> 413

Met Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
1   5   10   15

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20   25   30

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35   40   45

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    50   55   60

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
65   70   75   80

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                85   90   95

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
            100   105   110

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        115   120   125

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    130   135   140

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145   150   155   160

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165   170   175

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180   185   190

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195   200   205

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210   215   220

Ser Pro Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
225   230   235   240

```
Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Lys Thr
                245                 250                 255

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
            260                 265                 270

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
        275                 280                 285

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
    290                 295                 300

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
305                 310                 315                 320

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
                325                 330                 335

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
            340                 345                 350

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
        355                 360                 365

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
    370                 375                 380

Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys
385                 390                 395                 400

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
                405                 410                 415

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
            420                 425                 430

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
        435                 440                 445

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
    450                 455                 460

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
465                 470                 475                 480


<210>  414
<211>  45
<212>  DNA
<213>  ARTIFICIAL SEQUENCE

<220>
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<223> Primer sequence

<400> 414
aaacctgtgg cacctgtggc ggtaccaaaa accaggtgtc cctga 45

<210> 415
<211> 45
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 415
tatcgatgca agaacgaccg cccagttcgt cacgagacgg cggca 45

<210> 416
<211> 45
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 416
aaataccgtc tcagtttctg tcgtaaaacc tgtggcacct gtggc 45

<210> 417
<211> 45
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 417
cagtacagcg ggacttaggg attgtatcga tgcaagaacg accgc 45

<210> 418
<211> 45
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 418
caatgcaaac actcaatgaa ataccgtctc agtttctgtc gtaaa 45

<210> 419
<211> 43
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 419
tgagtgtttg cattgaaagg cagtacagcg ggacttaggg att 43

<210> 420
<211> 28

<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 420
taatgaattc gagctccgtc gacaagct 28

<210> 421
<211> 44
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 421
ccaccggatc caccaccacc tttacccgga gacagggaga ggct 44

<210> 422
<211> 28
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 422
tggtggatcc ggtggtggtg gctccggt 28

<210> 423
<211> 40
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 423
gagctcgaat tcattattta cccggagaca gagacaggga 40

<210> 424
<211> 36
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 424
ggtggcggtg gctctggtgg tggtggatcc ggtggt 36

<210> 425
<211> 39
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 425
agagccaccg ccacctttac ccggagacag ggagaggct 39

<210> 426
<211> 28
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 426
ggcggtacca agaaccaggt cagcctga 28

<210> 427
<211> 29
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 427
accgcccagc tcatcacggg atgggggca 29

<210> 428
<211> 42
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 428
cgtgatgagc tgggcggtcg ttcttgcatc gatacaatcc ct 42

<210> 429
<211> 42
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 429
acctggttct tggtaccgcc acaggtgcca caggttttac ga 42

<210> 430
<211> 33
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 430
cactcaatga ataccgtct cagtttctgt cgt 33

<210> 431
<211> 31
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>

<223> Primer sequence

<400> 431
gtatttcatt gagtgtttgc attgaaaggc a 31

<210> 432
<211> 28
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 432
taatgaattc gagctccgtc gacaagct 28

<210> 433
<211> 20
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 433
cccggagaca gagacaggga 20

<210> 434
<211> 32
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 434
gtctctgtct ccgggtaaag gcggcggcgg ca 32

<210> 435
<211> 34
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 435
agctcgaatt cattaagcac aggtgccaca ggtt 34

<210> 436
<211> 163
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Coding sequence ShK{1-35, Q16K) with an N-terminal linker

<400> 436
ggatccggag gaggaggaag ccgcagctgc atcgacacca tccccaagag ccgctgcacc 60

gccttcaagt gcaagcacag catgaagtac cgcctgagct tctgccgcaa gacctgcggc 120

acctgctaat gagcggccgc tcgaggccgg caaggccgga tcc 163

<210> 437
<211> 42
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK{1-35, Q16K) with an N-terminal linker

<400> 437

Gly Ser Gly Gly Gly Gly Ser Arg Ser Cys Ile Asp Thr Ile Pro Lys
1 5 10 15

Ser Arg Cys Thr Ala Phe Lys Cys Lys His Ser Met Lys Tyr Arg Leu
20 25 30

Ser Phe Cys Arg Lys Thr Cys Gly Thr Cys
35 40

<210> 438
<211> 36
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> ShK-192

<220>
<221> MISC_FEATURE
<222> (1)..(1)
<223> L-phosphonophenylalanine

<220>
<221> MISC_FEATURE
<222> (1)..(2)
<223> Between residues 1 and 2 is: {2-[2-Aminoethoxy]ethoxy}acetic acid

<220>
<221> MISC_FEATURE
<222> (22)..(22)
<223> Norleucine

<400> 438

Xaa Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe
1 5 10 15

Gln Cys Lys His Ser Xaa Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr
20 25 30

Cys Gly Thr Cys
35

<210> 439
<211> 281
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>

<223> aKLH 120.6 kappa LC-ShK[1-35, Q16K] fusion protein

<400> 439

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

Leu Arg Gly Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
20 25 30

Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
35 40 45

Gln Gly Ile Arg Asn Asp Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys
50 55 60

Ala Pro Lys Arg Leu Ile Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val
65 70 75 80

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr
85 90 95

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln
100 105 110

His Asn Ser Tyr Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile
115 120 125

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
130 135 140

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
145 150 155 160

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
165 170 175

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
180 185 190

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
195 200 205

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
210 215 220

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys Gly Gly Gly Gly
225 230 235 240

Ser Gly Gly Gly Gly Ser Arg Ser Cys Ile Asp Thr Ile Pro Lys Ser
245 250 255

Arg Cys Thr Ala Phe Lys Cys Lys His Ser Met Lys Tyr Arg Leu Ser
260 265 270

Phe Cys Arg Lys Thr Cys Gly Thr Cys
275 280

<210> 440
<211> 280
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> aKLH 120.6 kappa LC-ShK[2-35, Q16K] fusion protein

<400> 440

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

Leu Arg Gly Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
20 25 30

Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
35 40 45

Gln Gly Ile Arg Asn Asp Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys
50 55 60

Ala Pro Lys Arg Leu Ile Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val
65 70 75 80

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr
85 90 95

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln
100 105 110

His Asn Ser Tyr Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile
115 120 125

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
130 135 140

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
145 150 155 160

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
165 170 175

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
180 185 190

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
195 200 205

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
210 215 220

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys Gly Gly Gly Gly
225 230 235 240

Ser Gly Gly Gly Gly Ser Ser Cys Ile Asp Thr Ile Pro Lys Ser Arg
245 250 255

Cys Thr Ala Phe Lys Cys Lys His Ser Met Lys Tyr Arg Leu Ser Phe
260 265 270

Cys Arg Lys Thr Cys Gly Thr Cys
275 280

<210> 441
<211> 34
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 441
aacaggggag agtgtggagg aggaggatcc ggag 34

<210> 442
<211> 22
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 442
catgcggccg ctcattagca gg 22

<210> 443
<211> 26
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Forward primer sequence

<400> 443
ggacactgac atggactgaa ggagta 26

<210> 444
<211> 21
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Reverse primer sequence

<400> 444
ctcctgggag ttacccgatt g 21

<210> 445
<211> 40
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Reverse primer sequence

<400> 445
gatgggccct tggtggaggc tgaggagacg gtgaccgtgg 40

<210> 446
<211> 36
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 446
aagctcgagg tcgactagac caccatggac atgagg 36

<210> 447
<211> 41
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 447
aaccgtttaa acgcggccgc tcaacactct cccctgttga a 41

<210> 448
<211> 41
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 448
aagctcgagg tcgactagac caccatggaa ttgggactga g 41

<210> 449
<211> 41
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 449
aaccgtttaa acgcggccgc tcatttaccc ggagacaggg a 41

<210> 450
<211> 37
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>

<223> Primer sequence

<400> 450
tttttttgc gcgctgtgac atccagatga cccagtc 37

<210> 451
<211> 32
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 451
aaaaaacgta cgtttgatat ccactttggt cc 32

<210> 452
<211> 50
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 452
ctgtgtatta ctgtgcgagg tataacttca actacggtat ggacgtctgg 50

<210> 453
<211> 50
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 453
ccagacgtcc ataccgtagt tgaagttata cctcgcacag taatacacag 50

<210> 454
<211> 50
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 454
ctgtgtatta ctgtgcgagg tataactaca actacggtat ggacgtctgg 50

<210> 455
<211> 50
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 455
ccagacgtcc ataccgtagt tgtagttata cctcgcacag taatacacag 50

<210> 456
<211> 59

<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 456
aagctcgagg tcgactagac caccatggac atgagggtgc ccgctcagct cctggggct 59

<210> 457
<211> 41
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 457
aaccgtttaa acgcggccgc tcatttaccc ggagacaggg a 41

<210> 458
<211> 50
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 458
ggacaagaca gttgagcgca aatcttctgt cgagtgccca ccgtgcccag 50

<210> 459
<211> 50
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 459
ctgggcacgg tgggcactcg acagaagatt tgcgctcaac tgtcttgtcc 50

<210> 460
<211> 59
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 460
aagctcgagg tcgactagac caccatggac atgagggtgc ccgctcagct cctggggct 59

<210> 461
<211> 41
<212> DNA
<213> ARTIFICIAL SEQUENCE

<220>
<223> Primer sequence

<400> 461
aaccgtttaa acgcggccgc tcatttaccc ggagacaggg a 41

<210> 462
<211> 511
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> aKLH 120.6 IgG2-ShK[1-35, R1A, I4A, Q16K] fusion protein

<400> 462

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

Leu Arg Gly Ala Arg Cys Gln Val Gln Leu Val Gln Ser Gly Ala Glu
20 25 30

Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly
35 40 45

Tyr Thr Phe Thr Gly Tyr His Met His Trp Val Arg Gln Ala Pro Gly
50 55 60

Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr
65 70 75 80

Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr
85 90 95

Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp
100 105 110

Thr Ala Val Tyr Tyr Cys Ala Arg Asp Arg Gly Ser Tyr Tyr Trp Phe
115 120 125

Asp Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr
130 135 140

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser
145 150 155 160

Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
165 170 175

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
180 185 190

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
195 200 205

Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys
210 215 220

```
Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu
225                 230                 235                 240

Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala
                245                 250                 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
            260                 265                 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        275                 280                 285

Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    290                 295                 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe
305                 310                 315                 320

Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly
                325                 330                 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile
            340                 345                 350

Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val
        355                 360                 365

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
    370                 375                 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385                 390                 395                 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                405                 410                 415

Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            420                 425                 430

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        435                 440                 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    450                 455                 460

Pro Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Ser Cys Ala
465                 470                 475                 480

Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys Cys Lys His Ser
                485                 490                 495
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Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys Gly Thr Cys
500 505 510

<210> 463
<211> 511
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> aKLH 120.6 IgG2-ShK[1-35, R1A, Q16K, K30E] fusion protein

<400> 463

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

Leu Arg Gly Ala Arg Cys Gln Val Gln Leu Val Gln Ser Gly Ala Glu
20 25 30

Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly
35 40 45

Tyr Thr Phe Thr Gly Tyr His Met His Trp Val Arg Gln Ala Pro Gly
50 55 60

Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr
65 70 75 80

Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr
85 90 95

Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp
100 105 110

Thr Ala Val Tyr Tyr Cys Ala Arg Asp Arg Gly Ser Tyr Tyr Trp Phe
115 120 125

Asp Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr
130 135 140

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser
145 150 155 160

Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
165 170 175

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
180 185 190

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
195 200 205

Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys
210 215 220

```
Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu
225                 230                 235                 240

Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala
                245                 250                 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
            260                 265                 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        275                 280                 285

Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    290                 295                 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe
305                 310                 315                 320

Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly
                325                 330                 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile
            340                 345                 350

Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val
        355                 360                 365

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
    370                 375                 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385                 390                 395                 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                405                 410                 415

Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            420                 425                 430

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        435                 440                 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    450                 455                 460

Pro Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Ser Cys Ile
465                 470                 475                 480

Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys Cys Lys His Ser
                485                 490                 495
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Met Lys Tyr Arg Leu Ser Phe Cys Arg Glu Thr Cys Gly Thr Cys
500 505 510

<210> 464
<211> 511
<212> PRT
<213> ARTIFICIAL SEQUENCE

<220>
<223> aKLH 120.6 IgG2-ShK[1-35, R1H, I4A, Q16K] fusion protein

<400> 464

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

Leu Arg Gly Ala Arg Cys Gln Val Gln Leu Val Gln Ser Gly Ala Glu
20 25 30

Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly
35 40 45

Tyr Thr Phe Thr Gly Tyr His Met His Trp Val Arg Gln Ala Pro Gly
50 55 60

Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr
65 70 75 80

Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr
85 90 95

Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp
100 105 110

Thr Ala Val Tyr Tyr Cys Ala Arg Asp Arg Gly Ser Tyr Tyr Trp Phe
115 120 125

Asp Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr
130 135 140

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser
145 150 155 160

Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
165 170 175

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
180 185 190

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
195 200 205

```
Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys
    210                 215                 220

Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu
225                 230                 235                 240

Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala
                245                 250                 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
            260                 265                 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        275                 280                 285

Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    290                 295                 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe
305                 310                 315                 320

Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly
                325                 330                 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile
            340                 345                 350

Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val
        355                 360                 365

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
    370                 375                 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385                 390                 395                 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                405                 410                 415

Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            420                 425                 430

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        435                 440                 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    450                 455                 460

Pro Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His Ser Cys Ala
465                 470                 475                 480
```

```
Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys Cys Lys His Ser
                485                 490                 495

Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys Gly Thr Cys
            500                 505                 510


<210>  465
<211>  511
<212>  PRT
<213>  ARTIFICIAL SEQUENCE

<220>
<223>  aKLH 120.6 IgG2-ShK[1-35, R1H, Q16K, K30E] fusion protein

<400>  465

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1               5                   10                  15

Leu Arg Gly Ala Arg Cys Gln Val Gln Leu Val Gln Ser Gly Ala Glu
            20                  25                  30

Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly
        35                  40                  45

Tyr Thr Phe Thr Gly Tyr His Met His Trp Val Arg Gln Ala Pro Gly
    50                  55                  60

Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr
65                  70                  75                  80

Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr
                85                  90                  95

Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp
            100                 105                 110

Thr Ala Val Tyr Tyr Cys Ala Arg Asp Arg Gly Ser Tyr Tyr Trp Phe
        115                 120                 125

Asp Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr
    130                 135                 140

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser
145                 150                 155                 160

Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
                165                 170                 175

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
            180                 185                 190

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
        195                 200                 205
```

```
Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys
    210                 215                 220

Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu
225                 230                 235                 240

Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala
                245                 250                 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
            260                 265                 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        275                 280                 285

Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    290                 295                 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe
305                 310                 315                 320

Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly
                325                 330                 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile
            340                 345                 350

Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val
        355                 360                 365

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
    370                 375                 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385                 390                 395                 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                405                 410                 415

Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            420                 425                 430

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        435                 440                 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    450                 455                 460

Pro Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser His Ser Cys Ile
465                 470                 475                 480
```

```
Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys Cys Lys His Ser
                485                 490                 495

Met Lys Tyr Arg Leu Ser Phe Cys Arg Glu Thr Cys Gly Thr Cys
            500                 505                 510


<210>  466
<211>  511
<212>  PRT
<213>  ARTIFICIAL SEQUENCE

<220>
<223>  aKLH 120.6 HC (IgG2)-ShK[1-35, R1K, 14A,  Q16K] fusion protein

<400>  466

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1               5                   10                  15

Leu Arg Gly Ala Arg Cys Gln Val Gln Leu Val Gln Ser Gly Ala Glu
            20                  25                  30

Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly
        35                  40                  45

Tyr Thr Phe Thr Gly Tyr His Met His Trp Val Arg Gln Ala Pro Gly
    50                  55                  60

Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr
65                  70                  75                  80

Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr
                85                  90                  95

Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp
            100                 105                 110

Thr Ala Val Tyr Tyr Cys Ala Arg Asp Arg Gly Ser Tyr Tyr Trp Phe
        115                 120                 125

Asp Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr
    130                 135                 140

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser
145                 150                 155                 160

Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
                165                 170                 175

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
            180                 185                 190
```

```
Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
        195                 200                 205

Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys
    210                 215                 220

Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu
225                 230                 235                 240

Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala
                245                 250                 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
            260                 265                 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        275                 280                 285

Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    290                 295                 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe
305                 310                 315                 320

Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly
                325                 330                 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile
            340                 345                 350

Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val
        355                 360                 365

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
    370                 375                 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385                 390                 395                 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                405                 410                 415

Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            420                 425                 430

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        435                 440                 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    450                 455                 460
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Pro Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Lys Ser Cys Ala
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Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys Cys Lys His Ser
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Met Lys Tyr Arg Leu Ser Phe Cys Arg Lys Thr Cys Gly Thr Cys
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Leu Arg Gly Ala Arg Cys Gln Val Gln Leu Val Gln Ser Gly Ala Glu
20 25 30

Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly
35 40 45

Tyr Thr Phe Thr Gly Tyr His Met His Trp Val Arg Gln Ala Pro Gly
50 55 60

Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr
65 70 75 80

Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr
85 90 95

Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp
100 105 110

Thr Ala Val Tyr Tyr Cys Ala Arg Asp Arg Gly Ser Tyr Tyr Trp Phe
115 120 125

Asp Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr
130 135 140

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser
145 150 155 160

Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
165 170 175

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
180 185 190

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Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
        195                 200                 205

Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys
    210                 215                 220

Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu
225                 230                 235                 240

Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala
                245                 250                 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
            260                 265                 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        275                 280                 285

Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    290                 295                 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe
305                 310                 315                 320

Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly
                325                 330                 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile
            340                 345                 350

Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val
        355                 360                 365

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
    370                 375                 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385                 390                 395                 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                405                 410                 415

Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            420                 425                 430

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        435                 440                 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    450                 455                 460
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Pro Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Lys Ser Cys Ile
465 470 475 480

Asp Thr Ile Pro Lys Ser Arg Cys Thr Ala Phe Lys Cys Lys His Ser
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Met Lys Tyr Arg Leu Ser Phe Cys Arg Glu Thr Cys Gly Thr Cys
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