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(54) Title: CARRIER IMMUNOGLOBULINS AND USES THEREOF

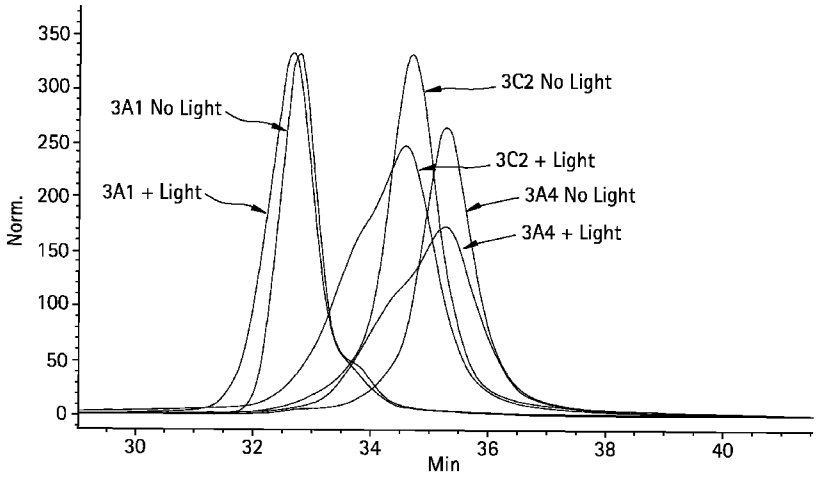

FIG. 16

(57) Abstract: Disclosed is an isolated antigen binding protein, such as but not limited to, an antibody or antibody fragment. Also disclosed are pharmaceutical compositions and medicaments comprising the antigen binding protein, isolated nucleic acid encoding it, vectors, host cells, and hybridomas useful in methods of making it. In some embodiments the antigen binding protein comprises one to twenty-four pharmacologically active chemical moieties conjugated thereto, such as a pharmacologically active polypeptide.

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CARRIER IMMUNOGLOBULINS AND USES THEREOF

[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-1537-WO-PCTSeqList031810-368_ST25.txt, and is 545 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] This invention relates to carrier antibodies to which one or more pharmacologically active chemical moieties can be conjugated for improved pharmacokinetic characteristics.

[0006] 2. Discussion of the Related Art.

[0007] A "carrier" moiety refers to a pharmacologically inactive molecule to which a pharmacologically active chemical moiety, such as a non-peptide organic moiety (i.e., "small molecule") or a polypeptide agent, can be covalently conjugated or fused. Effective carriers have been sought to prevent or mitigate in vivo degradation of pharmacologically active moieties by proteolysis or other in vivo activity-

diminishing chemical modifications of the pharmacologically active chemical moiety, or to reduce renal clearance, to enhance in vivo half-life or other pharmacokinetic properties of a therapeutic, such as increasing the rate of absorption, reducing toxicity or immunogenicity, improving solubility, and/or increasing manufacturability or storage stability, compared to an unconjugated form of the pharmacologically active moiety.

[0008] Examples of such carrier moieties that have been employed in the pharmaceutical industry include polyethylene glycol (see, e.g., Burg et al., Erythropoietin conjugates with polyethylene glycol, WO 01/02017), immunoglobulin Fc domain (see, e.g., Feige et al., Modified peptides as therapeutic agents, US Patent No. 6,660,843), human serum albumin (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), transthyretin (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 thyroxine-binding globulin, 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. Pharmacologically active moieties have also been conjugated to a 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).

[0009] Fischer et al. described a peptide-immunoglobulin-conjugate, in which the immunoglobulin consisted of two heavy chains or two heavy chains and two light chains, in which the immunoglobulin was not a functionable immunoglobulin (Fischer et al., A peptide-immunoglobulin conjugate, WO 2007/045463 A1).

[0010] The present invention provides carrier immunoglobulins yielding exceptional uniformity and efficiency of recombinant expression, in vitro stability and non-aggregation, resistance to photodegradation and oxidation, non-cross-reactivity with human antigens, and good pharmacokinetic properties.

SUMMARY OF THE INVENTION

[0011] The invention relates to antigen binding proteins. The inventive antigen binding proteins, including antibodies and antibody fragments, have reliable expression and purification characteristics, resulting in products that are stable and relatively uniform, and have outstanding pharmacokinetic (PK) properties in rats and cynomolgous monkeys. The inventive antigen binding proteins are found to specifically bind to dinitrophenol (DNP) or keyhole limpet hemocynanin (KLH), but have not been detected to bind to human proteins, cells or tissues. These antigen binding prioteins can be used for many purposes, including, but not limited to, quality control or analytical standards for antibody-based drugs and as controls for biologically relevant isotype-matched antibodies.

[0012] In some embodiments, the antigen binding protein of the present invention is used as a carrier for pharmacologically active chemical moieties, e.g., small molecules, peptides, and/or proteins to enhance their PK properties. The pharmacologically active moieties can be conjugated, i.e., covalently bound, to the inventive immunoglobulin by a chemical conjugation reaction, or through recombinant genetic expression, they can be fused to the antigen binding protein.

[0013] The invention also provides materials and methods for producing such inventive immunoglobulins, including isolated nucleic acids that encode them, vectors and isolated host cells, and hybridomas. Also provided are isolated nucleic acids encoding any of the immunoglobulin heavy and/or light chain sequences and/or VH and/or VL sequences and/or CDR sequences disclosed herein. In a related embodiment, an expression vector comprising any of the aforementioned nucleic acids is provided. In still another embodiment, a host cell is provided comprising any of the aforementioned nucleic acids or expression vectors.

[0014] The inventive immunoglobulin can be used in the manufacture of a pharmaceutical composition or medicament. The inventive pharmaceutical composition or medicament comprises the immunoglobulin conjugated with a

pharmacologically active agent, and a pharmaceutically acceptable diluent, carrier or excipient.

[0015] Numerous methods are contemplated in the present invention. For example, a method is provided involving culturing the aforementioned host cell comprising the expression vector of the invention such that the encoded antigen binding protein is expressed. A method is also provided involving culturing the aforementioned hybridoma in a culture medium under conditions permitting expression of the antigen binding protein by the hybridoma. Such methods can also comprise the step of recovering the antigen binding protein from the host cell culture. In a related embodiment, an isolated antigen binding protein produced by the aforementioned method is provided.

[0016] The foregoing summary is not intended to define every aspect of the invention, and additional aspects are described in other sections, such as the Detailed Description of Embodiments. The entire document is intended to be related as a unified disclosure, and it should be understood that all combinations of features described herein are contemplated, even if the combination of features are not found together in the same sentence, or paragraph, or section of this document.

[0017] In addition to the foregoing, the invention includes, as an additional aspect, all embodiments of the invention narrower in scope in any way than the variations defined by specific paragraphs above. For example, certain aspects of the invention that are described as a genus, and it should be understood that every member of a genus is, individually, an aspect of the invention. Also, aspects described as a genus or selecting a member of a genus, should be understood to embrace combinations of two or more members of the genus. Although the applicant(s) invented the full scope of the invention described herein, the applicants do not intend to claim subject matter described in the prior art work of others. Therefore, in the event that statutory prior art within the scope of a claim is brought to the attention of the applicants by a Patent Office or other entity or individual, the applicant(s) reserve the right to exercise amendment rights under applicable patent laws to redefine the subject

matter of such a claim to specifically exclude such statutory prior art or obvious variations of statutory prior art from the scope of such a claim. Variations of the invention defined by such amended claims also are intended as aspects of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] Figure 1A-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 1A 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 1B 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 1C 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 1D 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 1E 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 1F 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 1G 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 1H 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 1I 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 1J 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 1K 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 1L 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 1M 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 1N 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.

[0019] Figure 2A-B demonstrates by PatchXpress® electrophysiology that the monovalent aKLH HC-ShK(1-35 Q16K) Ab (SEQ ID NO:28, 29, 32), as described in Examples 4 and 5, is more potent in blocking human Kv1.3 current (Figure 2A) than human Kv1.1 current (Figure 2B).

[0020] Figure 3A 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, described in Example 4 herein. 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.

[0021] Figure 3B shows size exclusion chromatography on 50 μg of the final monovalent Fc-L10-ShK[1-35, Q16K]/anti-KLH Ab product, described in Example 4, 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.

[0022] Figure 3C shows an LC-MS analysis of the final sample of monovalent Fc-L10-ShK[1-35, Q16K]/anti-KLH Ab described in Example 4. 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 3000tt m/z and deconvoluted using the MaxEnt1 software provided by the instrument manufacturer.

[0023] Figure 4A shows a Coomassie brilliant blue stained Tris-glycine 4-20% SDS-PAGE of the final monovalent anti-KLH HC-L10-ShK[1-35, Q16K] Ab product described in Example 4. 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.

[0024] Figure 4B shows size exclusion chromatography on 25 μg of the final monovalent anti-KLH 120.6 HC-L10-ShK[1-35, Q16K] antibody product, described in Example 4, 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 detetcting the absorbance at 280 nm. The deflection observed at about 11 min is an injection-related artefact.

[0025] Figure 4C shows a MALDI mass spectral analysis of the final sample of monovalent anti-KLH HC-L10-ShK[1-35, Q16K] Ab, described in Example 4, 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.

[0026] Figure 5A 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, described in Example 4. 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.

[0027] Figure 5B shows size exclusion chromatography on 25 μg of the final bivalent anti-KLH HC-L10-ShK[1-35, Q16K] Ab product, described in Example 4, 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 detecting the absorbance at 280 nm. The deflection observed at about 11.5 min is an injection-related artefact.

[0028] Figure 5C shows a MALDI mass spectral analysis of the final sample of bivalent anti-KLH HC-L10-ShK[1-35, Q16K] Ab, described in Example 4, 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.

[0029] Figure 6A shows a Coomassie brilliant blue stained Tris-glycine 4-20% SDS-PAGE of the final monovalent KLH HC-L10-ShK[2-35, Q16K] Ab product,

described in Example 4. 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.

[0030] Figure 6B shows size exclusion chromatography on 20 μg of the final monovalent anti-KLH HC-L10-ShK[2-35, Q16K] Ab product, described in Example 4, 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 detecting the absorbance at 280 nm. The deflection observed at about 11 min is an injection-related artefact.

[0031] Figure 6C shows an LC-MS mass spectral analysis of the final sample of monovalent anti-KLH HC-L10-ShK[2-35, Q16K] Ab, described in Example 4. 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.

[0032] Figure 7 shows results of pharmacokinetic studies (single- subcutaneous dose = 6 mg/kg) performed in Sprague-Dawley rats. Open squares represent data for monovalent Fc/Fc-L10-ShK(1-35, Q16K) (heterodimer of SEQ ID NO: 1 and SEQ ID NO:26) closed circles represent data for monovalent anti-KLH antibody-ShK(1-35, Q16K) (tetramer of SEQ ID NO: 28, SEQ ID NO:29, SEQ ID NO:28, and SEQ ID NO:32); and closed triangles represent data for monovalent anti-KLH antibody (loop)-ShK(1-35, Q16K) (tetramer of SEQ ID NO: 28; SEQ ID NO:35; SEQ ID NO:28; and SEQ ID NO:34), described in Example 5 and Table 7H.

[0033] Figure 8 shows results of pharmacokinetic studies (single- subcutaneous dose = 6 mg/kg dose) performed in Sprague-Dawley rats for bivalent (open squares) and monovalent (closed circles) anti-KLH antibody-ShK(1-35, Q16K) (respectively, tetramers of [SEQ ID NO: 28, SEQ ID NO:32, SEQ ID NO:28, SEQ ID NO:32] and [SEQ ID NO: 28, SEQ ID NO:29, SEQ ID NO:28, SEQ ID NO:32]), as further described in Example 5, and Table 7J.

[0034] Figure 9 shows results of pharmacokinetic studies (single- subcutaneous dose = 6 mg/kg) performed in Sprague-Dawley rats for bivalent (open squares) and monovalent (closed circles) anti-KLH antibody (loop)-ShK(1-35, Q16K) (respectively, tetramers of [SEQ ID NO: 28, SEQ ID NO:35, SEQ ID NO:28, SEQ ID NO:35] and [SEQ ID NO: 28, SEQ ID NO:34, SEQ ID NO:28, SEQ ID NO:35]), as further described in Example 5, and Table 7L.

[0035] Figure 10 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/KLH 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 5.

[0036] Figure 11 shows analysis of antibodies on a 1.0 mm Tris-glycine 4-20% SDS-PAGE (Novex) developed at 220V using reducing loading buffer and staining with QuickBlue (Boston Biologicals). Lanes were loaded as follows (left to right): lane 1, Novex Mark 12 standards; lane 2, 2 μg aDNP 3B1 Ab from transient cell culture; lane 3, 2 μg aDNP-3B1 Ab from stable cell culture; lane 4, 2 μg aDNP 3H4 Ab from transient cell culture; lane 5, 2 μg aDNP 3H4 Ab from stable cell culture; lane 6, 2 μg aDNP 3A1 Ab from transient cell culture; lane 7, 2 μg aDNP 3C2 Ab from transient cell culture; and lane 8, 2 μg aDNP 3A4 Ab from transient cell culture.

[0037] Figure 12A-B shows analysis of antibodies on a 1.0 mm Tris-glycine 4-20% SDS-PAGE (Novex) developed at 220V using non-reducing loading buffer and

staining with QuickBlue (Boston Biologicals). Lanes were loaded as follows (left to right): (Figure 12A): lane 1, Novex Mark 12 standards; lane 2, 0.5 μg aDNP 3A1 Ab; lane 3, 0.5 μg aDNP 3A4 Ab; lane 4, 0.5 μg aDNP 3C2 Ab; lane 5, 0.5 μg aKLH 120.6 Ab; lane 6, Novex Mark 12 standards; lane 7, 5 μg aDNP 3A1 Ab; lane 8, 5 μg aDNP 3A4 Ab; lane 9, 5 μg aDNP 3C2 Ab; lane 10, 5 μg aKLH 120.6 Ab; (Figure 12B): lane 1, Novex Mark 12 standards; lane2, 0.5 μg aDNP 3B1 Ab; lane 3, blank; lane 4, Novex Mark 12 standards; lane 5, 5 μg aDNP 3B1 Ab.

[0038] Figure 13A shows analysis of antibodies on a 1.0 mm Tris-glycine 4-20% SDS-PAGE (Novex) developed at 220V using non-reducing loading buffer and staining with QuickBlue (Boston Biologicals). Lanes were loaded as follows (left to right): lane 1, Novex Mark 12 standards; lane 2, blank; lane 3, 0.2 μg aDNP 3B1 Ab; lane 4, 0.2 μg aDNP 3A1 Ab, lane 5, blank; lane 6, 0.6 μg aDNP 3B1 Ab; lane 7, 0.6 μg aDNP 3A1 Ab; lane 8, blank; lane 9, 1.8 μg aDNP 3B1 Ab; lane 10, 1.8 μg aDNP 3A1 Ab.

[0039] Figure 13B shows analysis of antibodies on a 1.0 mm Bis-Tris 4-12% NuPAGE (Novex) developed at 220V using non-reducing loading buffer and staining with QuickBlue (Boston Biologicals); Lanes were loaded as follows (left to right)::lane 1, Novex Mark 12 standards; lane2, blank; lane 3, 0.2 μg aDNP 3B1 Ab; lane 4, 0.2 μg aDNP 3A1 Ab; lane 5, blank; lane 6, 0.6 μg aDNP 3B1 Ab; lane 7, 0.6 μg aDNP 3A1 Ab; lane 8, blank; lane 9, 1.8 μg aDNP 3B1 Ab; lane 10, 1.8 μg aDNP 3A1 Ab.

[0040] Figure 14A-B shows analysis of antibodies on a 1.0 mm Tris-glycine 4-20% SDS-PAGE (Novex) developed at 220V using non-reducing loading buffer and staining with QuickBlue (Boston Biologicals). Lanes were loaded as follows (left to right): (Figure 14A: with 0.1% SDS in running buffer): lane 1, Novex Mark 12 standards; lane 2, 0.5 μg aDNP 3B1 Ab incubated at room temperature for 10 min; lane 3, 0.5 μg aDNP 3B1 Ab incubated at 85°C for 5 min; lane 4, 0.5 μg aDNP 3B1 Ab incubated at 100°C for 10 min; lane 5, blank; lane 6, 1 μg aDNP 3B1 Ab incubated at room temperature for 10 min; lane 7, 1 μg aDNP 3B1 Ab incubated at

85°C for 5 min; lane 8, 1 μg aDNP 3B1 Ab incubated at 100°C for 10 min; (Figure 14B: 0.4% SDS in running buffer; 85°C treatment for 5 min): lane 1, Novex Mark 12 standards, lane 2, blank; lane 3, 0.25 μg aDNP 3B1 Ab; lane 4, blank; lane 5, 0.5 μg aDNP 3B1 Ab; lane 6, blank; lane 7, 1.0 μg aDNP 3B1 Ab; lane 8, blank; lane 9, 2.0 μg aDNP 3B1 Ab.

[0041] Figure 15 shows analysis, using two size exclusion columns (TSK-GEL G3000SWXL, 5 mm particle size, 7.8 x 300 mm, TosohBioscience, 08541) in series with a 100 mM sodium phosphate, 250 mM NaCl at pH 6.8 mobile phase flowed at 0.5 mL/min., of antibodies: aDNP 3A1 ("3A1", darker trace with post shoulder); aDNP 3B1 ("3B1"); aKLH 120.6 ("KLH"); aDNP 3C2 ("3C2"), and aDNP 3A4 ("3A4").

[0042] Figure 16 shows analysis of antibodies aDNP 3A1 ("3A1"), aDNP 3C2 ("3C2") and DNP-3A4 before and after 3 weeks of light exposure, using two size exclusion columns (TSK-GEL G3000SWXL, 5 mm particle size, 7.8 x 300 mm, TosohBioscience, 08541) in series with a 100 mM sodium phosphate, 250 mM NaCl at pH 6.8 mobile phase flowed at 0.5 mL/min.

[0043] Figure 17A-B show analysis, using two size exclusion columns (TSK-GEL G3000SWXL, 5 mm particle size, 7.8 x 300 mm, TosohBioscience, 08541) in series with a 100 mM sodium phosphate, 250 mM NaCl at pH 6.8 mobile phase flowed at 0.5 mL/min, of antibodies aDNP 3A4, aDNP 3A4-Y ("W1010Y"), aDNP 3A4-F ("W101F"), aDNP 3A4 YSS ("W101Y/CCSS"), and aDNP-3A4-FSS ("W101F/CCSS") before (Figure 17A) and after (Figure 17B) 2 days of light exposure.

[0044] Figure 18 shows ion exchange analysis of aDNP antibodies (aDNP-3A4, aDNP-3A4-Y, aDNP-3A4-F, aDNP-3A4-YSS and aDNP-3A4-FSS). They were analyzed for homogeneity using a Tosohaas SP-5PW column (10-μm particle, 7.5 mm ID X 7.5 cm long) using Buffer A (10 mM sodium acetate, pH 5.0) and Buffer B (10 mM sodium acetate, 600 mM NaCl, pH 5.0) flowed at 1 ml/min with a

programmed linear gradient (1 min 0%B, 10 min 35% B, 30 min 70% B, 3 min 90%B and 3 min 0%B).

[0045] Figure 19 shows an analysis of aDNP 3B1 (Figure 19A), aDNP 3A4-F (Figure 19B), and aDNP 3A4-FSS (Figure 19C) antibodies by non-reducing CE-SDS with detection of absorbance at 220 nm. A bare-fused silica capillary 50 μm x 30.2 cm was used for the separation analysis.

[0046] Figure 20 shows an analysis of aDNP 3B1 (Figure 20A), aDNP 3A4-F (Figure 20B), and aDNP 3A4-FSS (Figure 20C) antibodies by reducing CE-SDS with detection of absorbance at 220 nm. A bare-fused silica capillary 50 μm x 30.2 cm was used for the separation analysis.

[0047] Figure 21 shows an analysis of aDNP-3A4-F (dotted curve), aDNP-3A4-FSS (solid curve) and aDNP-3B1 (dashed curve) antibodies were analyzed by DSC using a MicrCal VP-DSC where the samples were heated from 20°C to 95°C at a rate of 1°C per minute. The protein concentration was 0.5 mg/ml in 10 mM sodium acetate, 9% sucrose, pH 5.0.

[0048] Figure 22 shows serum concentrations of aDNP 3A4-F, aDNP 3A4-FSS, and aDNP 3B1 antibodies in rats receiving a single subcutaneous injection of 5 mg/kg, as determined by ELISA. Blood samples were collected at 0, 0.25, 1, 4, 24, 48, 72, 96, 168, 336, 504, 672, 840 and 1008 hours post-dose.

[0049] Figure 23 shows plasma concentrations of aDNP 3A4 or aKLH 120.6 in male cynomolgus monkeys receiving a bolus intravenous injection aDNP 3A4 (4 mg/kg) or aKLH 120.6 (3mg/kg) antibodies, respectively. Serum samples were taken periodically and plasma concentrations of the antibodies was determined by ELISA. The data for aDNP 3A4 was normalized to 3 mg/kg for comparison purposes.

[0050] Figure 24 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,

described in Example 4. 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.

[0051] Figure 25 shows size exclusion chromatography on 25 μg of the final monovalent aKLH 120.6 LC-ShK[1-35, Q16K] Ab product, described in Example 4, 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.

[0052] Figure 26A-B shows non-reducing (Figure 26A) and reducing (Figure 26B) MALDI-MS mass spectral analysis of the final sample of monovalent aKLH 120.6 LC-ShK[1-35, Q16K] product, described in Example 4, 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.

[0053] Figure 27 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, described in Example 4. 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.

[0054] Figure 28 shows size exclusion chromatography on 25 μg of the final bivalent aKLH 120.6 LC-ShK[1-35, Q16K] Ab product, described in Example 4,

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.

[0055] Figure 29A-B shows non-reducing (Figure 29A) and reducing (Figure 29B) MALDI-MS mass spectral analysis of the final sample of bivalent aKLH 120.6 LC-ShK[1-35, Q16K] Ab product, described in Example 4, 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.

[0056] Figure 30 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, described in Example 4. 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.

[0057] Figure 31 shows size exclusion chromatography on 25 μg of the final trivalent aKLH 120.6 LC-ShK[1-35, Q16K] Ab product, described in Example 4, 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.

[0058] Figure 32A-B shows non-reducing (Figure 32A) and reducing (Figure 32B) MALDI-MS mass spectral analysis of the final sample of trivalent aKLH 120.6 LC-ShK[1-35, Q16K] Ab product, described in Example 4, 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.

[0059] Figure 33 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, described in Example 4. 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.

[0060] Figure 34 shows size exclusion chromatography on 25 μg of the final monovalent aKLH 120.6 IgG2 HC-Shk[1-35, R1A, I4A, Q16K] Ab product, described in Example 4, 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.

[0061] Figure 35 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 product, described in Example 4. 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.

[0062] Figure 36 shows a Coomassie brilliant blue stained Tris-glycine 4-20% SDS-PAGE of the final aKLH 120.6 IgG2 HC-C681 Ab product, described in Example 11. 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.

[0063] Figure 37 shows size exclusion chromatography on 25 μg of the final aKLH 120.6 IgG2 HC-C681 Ab product, described in Example 11, 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.

[0064] Figure 38A-B shows non-reducing (Figure 38A) and reducing (Figure 38B) MALDI-MS mass spectral analysis of the final sample of aKLH 120.6 IgG2 HC-C681 product, described in Example 11, 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.

[0065] Figure 39 shows size exclusion chromatography on 50 μg each of aKLH IgG1(N297Q), AMP5-HC aKLH IgG2, HC-AMP5 aKLH IgG2, AMP5-LC aKLH IgG1 and LC-AMP5 aKLH IgG1) products, described in Example 9, 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.

[0066] Figure 40A-E shows analysis of antibodies (described in Example 9) aKLH IgG1 N297Q (Figure 40A), AMP5-HC aKLH IgG2 (Figure 40B), LC-AMP5 aKLH IgG2 (Figure 40C), HC-AMP5 aKLH IgG2 (Figure 40D), and AMP5-LC aKLH IgG1 (Figure 40E) on a 1.0 mm Tris-glycine 4-20% SDS-PAGE (Novex) developed at 220V using non-reducing loading buffer and staining with QuickBlue (Boston Biologicals). 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.

[0067] Figure 41A-D shows mass spectrographic analysis of reduced samples of LC-AMP5 aKLH IgG2 (Figure 41A), AMP5-HC aKLH IgG2 (Figure 41B), HC-AMP5 aKLH IgG2 (Figure 41C), and AMP5-LC aKLH IgG1 (Figure 41D), described in Example 9. Each sample was chromatographed through a Waters Massprep micro desalting column (2.1 x 5 mm) using an Acquity UPLC system then introduced into a Waters time-of-flight LCT premier mass spectrometer for mass measurement, and the mass spectrum was deconvoluted using the MaxEnt1 software.

[0068] Figure 42 is a schematic map of the Exendin-4 ("Ex4")-1kG-aKLH 120.6 LC fusion construct, described in Example 10.

[0069] Figure 43 shows size exclusion chromatography of 25 μg of the final Ex4-1kG-aKLH 120.6 LC antibody fusion, described in Example 10, 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.

[0070] Figure 44 shows analysis of on a 1.0 mm Tris-glycine 4-20% SDS-PAGE (Novex) developed at 220V using reducing and non-reducing loading buffers and staining with QuickBlue (Boston Biologicals). Lanes 1-10 were loaded as follows: lane 1: Novex Mark12 wide range protein standards (10 μl); lane 2: 0.5 μg other protein; lane 3: 0.5 μg Ex4-aKLH 120.6 Ab, non-reduced; lane 4: 2.0 μg other protein, lane 5: 2.0 μg Ex4-aKLH 120.6 Ab, non-reduced; lane 6: Novex Mark12 wide range protein standards (10 μl); lane 7: 0.5 μg other protein; lane 8: 0.5 μg Ex4-aKLH 120.6 Ab, reduced; lane 9: 2.0 μg other protein, lane 10: 2.0 μg Ex4-aKLH 120.6 Ab, reduced.

[0071] Figure 45 shows a schematic representation of N-terminal and C-terminal fusions of pharmacologically active chemical moieties with the HC and LC monomers of an antibody of the invention, as further exemplified in Example 9.

DETAILED DESCRIPTION OF EMBODIMENTS

[0072] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0073] Definitions

[0074] Unless otherwise defined herein, scientific and technical terms used in connection with the present application shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Thus, 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. For example, reference to “a protein” includes a plurality of proteins; reference to “a cell” includes populations of a plurality of cells.

[0075] "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, fusion proteins can be derivatized as described herein by well-known organic chemistry techniques.

[0076] The term “isolated protein” referred means that a subject protein (1) is free of at least some other proteins with which it would normally be found in nature, (2) is essentially free of other proteins from the same source, e.g., from the same species, (3) is expressed recombinantly by a cell of a heterologous species or kind, (4) has

been separated from at least about 50 percent of polynucleotides, lipids, carbohydrates, or other materials with which it is associated in nature, (5) is operably associated (by covalent or noncovalent interaction) with a polypeptide with which it is not associated in nature, and/or (6) does not occur in nature. Typically, an "isolated protein" constitutes at least about 5%, at least about 10%, at least about 25%, or at least about 50% of a given sample. Genomic DNA, cDNA, mRNA or other RNA, of synthetic origin, or any combination thereof may encode such an isolated protein. Preferably, the isolated protein is substantially free from proteins or polypeptides or other contaminants that are found in its natural environment that would interfere with its therapeutic, diagnostic, prophylactic, research or other use.

[0077] A "variant" of a polypeptide (e.g., an antigen binding protein, or an antibody) comprises an amino acid sequence wherein one or more amino acid residues are inserted into, deleted from and/or substituted into the amino acid sequence relative to another polypeptide sequence. Variants include fusion proteins.

[0078] 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.

[0079] 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.

[0080] 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.

[0081] 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. Examples of such molecular biological procedures are found in Maniatis et al., Molecular Cloning. A Laboratory Manual. Cold Spring Harbour Laboratory, Cold Spring Harbour, N.Y(1982). 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.

[0082] The term “polynucleotide” or “nucleic acid” includes both single-stranded and double-stranded nucleotide polymers containing two or more nucleotide residues. The nucleotide residues comprising the polynucleotide can be ribonucleotides or deoxyribonucleotides or a modified form of either type of nucleotide. Said modifications include base modifications such as bromouridine and inosine derivatives, ribose modifications such as 2’,3’-dideoxyribose, and internucleotide linkage modifications such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate and phosphoroamidate.

[0083] The term “oligonucleotide” means a polynucleotide comprising 200 or fewer nucleotide residues. In some embodiments, oligonucleotides are 10 to 60 bases in length. In other embodiments, oligonucleotides are 12, 13, 14, 15, 16, 17, 18, 19, or 20 to 40 nucleotides in length. Oligonucleotides may be single stranded or double stranded, e.g., for use in the construction of a mutant gene. Oligonucleotides may be sense or antisense oligonucleotides. An oligonucleotide can include a label, including a radiolabel, a fluorescent label, a hapten or an antigenic label, for detection assays. Oligonucleotides may be used, for example, as PCR primers, cloning primers or hybridization probes.

[0084] A “polynucleotide sequence” or “nucleotide sequence” or "nucleic acid sequence," as used interchangeably herein, is the primary sequence of nucleotide residues in a polynucleotide, including of an oligonucleotide, a DNA, and RNA, a nucleic acid, or a character string representing the primary sequence of nucleotide residues, 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. Unless specified otherwise, the left-hand end of any single-stranded polynucleotide sequence discussed herein is the 5’ end; the left-hand direction of double-stranded polynucleotide sequences is referred to as the 5’ direction. The direction of 5' to 3' addition of nascent RNA transcripts is referred to as the transcription direction;

sequence regions on the DNA strand having the same sequence as the RNA transcript that are 5' to the 5' end of the RNA transcript are referred to as "upstream sequences;" sequence regions on the DNA strand having the same sequence as the RNA transcript that are 3' to the 3' end of the RNA transcript are referred to as "downstream sequences."

[0085] As used herein, an "isolated nucleic acid molecule" or "isolated nucleic acid sequence" is a nucleic acid molecule that is either (1) identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the nucleic acid or (2) cloned, amplified, tagged, or otherwise distinguished from background nucleic acids such that the sequence of the nucleic acid of interest can be determined. An isolated nucleic acid molecule is other than in the form or setting in which it is found in nature. However, an isolated nucleic acid molecule includes a nucleic acid molecule contained in cells that ordinarily express the antigen binding protein (e.g., antibody) where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

[0086] 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.

[0087] 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.

[0088] "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.

[0089] 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).

[0090] The term "control sequence" or "control signal" refers to a polynucleotide sequence that can, in a particular host cell, affect the expression and processing of coding sequences to which it is ligated. The nature of such control sequences may depend upon the host organism. In particular embodiments, control sequences for prokaryotes may include a promoter, a ribosomal binding site, and a transcription termination sequence. Control sequences for eukaryotes may include promoters comprising one or a plurality of recognition sites for transcription factors, transcription enhancer sequences or elements, polyadenylation sites, and transcription termination sequences. Control sequences can include leader sequences and/or fusion partner sequences. Promoters and enhancers consist of short arrays of DNA 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)).

[0091] The term "vector" means any molecule or entity (e.g., nucleic acid, plasmid, bacteriophage or virus) used to transfer protein coding information into a host cell.

[0092] The term "expression vector" or "expression construct" as used herein refers to a recombinant DNA molecule containing a desired coding sequence and appropriate nucleic acid control sequences necessary for the expression of the operably linked coding sequence in a particular host cell. An expression vector can include, but is not limited to, sequences that affect or control transcription, translation, and, if introns are present, affect RNA splicing of a coding region operably linked thereto. 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 of interest, so that the expressed polypeptide can be secreted by the recombinant host cell, for more facile isolation of the polypeptide of interest 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).

[0093] 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. For example, a control sequence in a vector that is "operably linked" to a protein coding sequence is ligated thereto so that expression of the protein coding sequence is achieved under conditions compatible with the transcriptional activity of the control sequences.

[0094] The term “host cell” means a cell that has been transformed, or is capable of being transformed, with a nucleic acid and thereby expresses a gene of interest. The term includes the progeny of the parent cell, whether or not the progeny is identical in morphology or in genetic make-up to the original parent cell, so long as the gene of interest is present. 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 HEK-293 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.

[0095] The term "transfection" means the uptake of foreign or exogenous DNA by a cell, and a cell has been "transfected" when the exogenous DNA has been introduced inside the cell membrane. A number of transfection techniques are well known in the art and are disclosed herein. See, e.g., Graham et al., 1973, Virology 52:456; Sambrook et al., 2001, Molecular Cloning: A Laboratory Manual, supra; Davis et al., 1986, Basic Methods in Molecular Biology, Elsevier; Chu et al., 1981, Gene 13:197. Such techniques can be used to introduce one or more exogenous DNA moieties into suitable host cells.

[0096] The term "transformation" refers to a change in a cell's genetic characteristics, and a cell has been transformed when it has been modified to contain new DNA or RNA. For example, a cell is transformed where it is genetically modified from its native state by introducing new genetic material via transfection, transduction, or other techniques. Following transfection or transduction, the transforming DNA may recombine with that of the cell by physically integrating into a chromosome of the cell, or may be maintained transiently as an episomal element without being replicated, or may replicate independently as a plasmid. A cell is considered to have been "stably transformed" when the transforming DNA is replicated with the division of the cell.

[0097] By "physiologically acceptable salt" of a composition of matter, for example a salt of the antigen binding protein, such as an antibody, 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.

[0098] A "domain" or "region" (used interchangeably herein) 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).

[0099] "Treatment" or "treating" is an intervention performed with the intention of preventing the development or altering the pathology of a disorder. Accordingly, "treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented. "Treatment" includes any indicia of success in the amelioration of an injury, pathology or condition, including any objective or subjective parameter such as abatement; remission; diminishing of symptoms or making the injury, pathology or condition more tolerable to the patient; slowing in the rate of degeneration or decline; making the final point of degeneration less debilitating; improving a patient's physical or mental well-being. The treatment or amelioration of symptoms can be based on objective or subjective parameters; including the results of a physical examination, self-reporting by a patient, neuropsychiatric exams, and/or a psychiatric evaluation.

[00100] An "effective amount" is generally an amount sufficient to reduce the severity and/or frequency of symptoms, eliminate the symptoms and/or underlying cause, prevent the occurrence of symptoms and/or their underlying cause, and/or improve or remediate the damage that results from or is associated with migraine headache. In some embodiments, the effective amount is a therapeutically effective amount or a prophylactically effective amount. A "therapeutically effective amount" is an amount sufficient to remedy a disease state (e.g., transplant rejection or GVHD, inflammation, multiple sclerosis, cancer, diabetes, neuropathy, pain) or symptom(s), particularly a state or symptom(s) associated with the disease state, or otherwise prevent, hinder, retard or reverse the progression of the disease state or any other undesirable symptom associated with the disease in any way whatsoever (i.e. that provides "therapeutic efficacy"). A "prophylactically effective amount" is an amount of a pharmaceutical composition that, when administered to a subject, will

have the intended prophylactic effect, e.g., preventing or delaying the onset (or reoccurrence) of migraine headache or multiple sclerosis symptoms, or reducing the likelihood of the onset (or reoccurrence) of migraine headache, migraine headache symptoms, or multiple sclerosis symptoms. The full therapeutic or prophylactic effect does not necessarily occur by administration of one dose, and may occur only after administration of a series of doses. Thus, a therapeutically or prophylactically effective amount may be administered in one or more administrations.

[00101] "Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, rats, mice, monkeys, etc. Preferably, the mammal is human.

[00102] The term "naturally occurring" as used throughout the specification in connection with biological materials such as polypeptides, nucleic acids, host cells, and the like, refers to materials which are found in nature.

[00103] The term "antibody", or interchangeably "Ab",is used in the broadest sense and includes fully assembled antibodies, monoclonal antibodies (including human, humanized or chimeric antibodies), polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments that can bind antigen (e.g., Fab, Fab', $F(ab')_2$, Fv, single chain antibodies, diabodies), comprising complementarity determining regions (CDRs) of the foregoing as long as they exhibit the desired biological activity. Multimers or aggregates of intact molecules and/or fragments, including chemically derivatized antibodies, are contemplated. Antibodies of any isotype class or subclass, including IgG, IgM, IgD, IgA, and IgE, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2, or any allotype, are contemplated. Different isotypes have different effector functions; for example, IgG1 and IgG3 isotypes have antibody-dependent cellular cytotoxicity (ADCC) activity.

[00104] The term "antigen binding protein" (ABP) includes antibodies or antibody fragments, as defined above, and recombinant peptides or other compounds that contain sequences derived from CDRs having the desired antigen-binding properties.

[00105] In general, an antigen binding protein, e.g., an antibody or antibody fragment, "specifically binds" to an antigen (e.g., keyhole limpet hemocynin (KLH) or dinitrophenol (DNP)) when it has a significantly higher binding affinity for, and consequently is capable of distinguishing, that antigen, compared to its affinity for other unrelated proteins, under similar binding assay conditions. Typically, an antigen binding protein is said to "specifically bind" its target antigen when the dissociation constant (K_D) is $\leq 10^{-8}$ M. The antibody specifically binds antigen with "high affinity" when the K_D is $\leq 5x\ 10^{-9}$ M, and with "very high affinity" when the K_D is $\leq 5x\ 10^{-10}$ M. In one embodiment, the antibodies will bind to KLH or DNP with a K_D of between about 10^{-8} M and 10^{-10} M, and in yet another embodiment the antibodies will bind with a $K_D \leq 5x\ 10^{-9}$.

[00106] "Antigen binding region" or "antigen binding site" means a portion of a protein, that specifically binds a specified antigen, e.g., keyhole limpet hemocynin (KLH) or dinitrophenol (DNP). For example, that portion of an antigen binding protein that contains the amino acid residues that interact with an antigen and confer on the antigen binding protein its specificity and affinity for the antigen is referred to as "antigen binding region." An antigen binding region typically includes one or more "complementary binding regions" ("CDRs"). Certain antigen binding regions also include one or more "framework" regions ("FRs"). A "CDR" is an amino acid sequence that contributes to antigen binding specificity and affinity. "Framework" regions can aid in maintaining the proper conformation of the CDRs to promote binding between the antigen binding region and an antigen.

[00107] An "isolated" antibody is one that has been identified and separated from one or more components of its natural environment or of a culture medium in which it has been secreted by a producing cell. "Contaminant" components of its natural environment or medium are materials that would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In some embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody, and most preferably more than 99% by weight, or (2) to homogeneity by SDS-PAGE under reducing or

nonreducing conditions, optionally using a stain, e.g., Coomassie blue or silver stain. Isolated naturally occurring antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Typically, however, isolated antibody will be prepared by at least one purification step.

[00108] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against an individual antigenic site or epitope, in contrast to polyclonal antibody preparations that typically include different antibodies directed against different epitopes. Nonlimiting examples of monoclonal antibodies include murine, rabbit, rat, chicken, chimeric, humanized, or human antibodies, fully assembled antibodies, multispecific antibodies (including bispecific antibodies), antibody fragments that can bind an antigen (including, Fab, Fab', $F(ab')_2$, Fv, single chain antibodies, diabodies), maxibodies, nanobodies, and recombinant peptides comprising CDRs of the foregoing as long as they exhibit the desired biological activity, or variants or derivatives thereof.

[00109] The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler et al., Nature, 256:495 [1975], or may be made by recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson et al., Nature,352:624-628[1991] and Marks et al., J. Mol. Biol., 222:581-597 (1991), for example.

[00110] A "multispecific" binding agent or antigen binding protein or antibody is one that targets more than one antigen or epitope.

[00111] A "bispecific," "dual-specific" or "bifunctional" binding agent or antigen binding protein or antibody is a hybrid having two different antigen binding sites. Biantigen binding proteins, antigen binding proteins and antibodies are a species of multiantigen binding protein, antigen binding protein or multispecific antibody and may be produced by a variety of methods including, but not limited to, fusion of hybridomas or linking of Fab' fragments. See, e.g., Songsivilai and Lachmann, 1990, Clin. Exp. Immunol. 79:315-321; Kostelny et al., 1992, J. Immunol. 148:1547-1553. The two binding sites of a bispecific antigen binding protein or antibody will bind to two different epitopes, which may reside on the same or different protein targets.

[00112] 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").

[00113] An "antibody" 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.

[00114] 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.

[00115] 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.

[00116] 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.

[00117] 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.

[00118] "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).

[00119] 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.

[00120] 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.

[00121] 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 .

[00122] "Framework" or "FR" residues are those variable region residues other than the hypervariable region residues.

[00123] "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.

[00124] 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.

[00125] 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).

[00126] 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.

[00127] 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.

[00128] 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.

[00129] "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.

[00130] "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.

[00131] "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.

[00132] 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).

[00133] 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.

[00134] The term “compete” when used in the context of antigen binding proteins (e.g., neutralizing antigen binding proteins or neutralizing antibodies) that compete for the same epitope means competition between antigen binding proteins is determined by an assay in which the antigen binding protein (e.g., antibody or immunologically functional fragment thereof) under test prevents or inhibits specific binding of a reference antigen binding protein (e.g., a ligand, or a reference antibody) to a common antigen (e.g., KLH or a fragment thereof, or DNP). Numerous types of competitive binding assays can be used, for example: solid phase direct or indirect radioimmunoassay (RIA), solid phase direct or indirect enzyme immunoassay (EIA), sandwich competition assay (see, e.g., Stahli et al., 1983, Methods in Enzymology 9:242-253); solid phase direct biotin-avidin EIA (see, e.g., Kirkland et al., 1986, J. Immunol. 137:3614-3619) solid phase direct labeled assay, solid phase direct labeled sandwich assay (see, e.g., Harlow and Lane, 1988, Antibodies, A Laboratory Manual, Cold Spring Harbor Press); solid phase direct label RIA using I-125 label (see, e.g., Morel et al., 1988, Molec. Immunol. 25:7-15); solid phase direct biotin-avidin EIA (see, e.g., Cheung, et al., 1990, Virology 176:546-552); and direct labeled RIA (Moldenhauer et al., 1990, Scand. J. Immunol. 32:77-82). Typically,

such an assay involves the use of purified antigen bound to a solid surface or cells bearing either of these, an unlabelled test antigen binding protein and a labeled reference antigen binding protein. Competitive inhibition is measured by determining the amount of label bound to the solid surface or cells in the presence of the test antigen binding protein. Usually the test antigen binding protein is present in excess. Antigen binding proteins identified by competition assay (competing antigen binding proteins) include antigen binding proteins binding to the same epitope as the reference antigen binding proteins and antigen binding proteins binding to an adjacent epitope sufficiently proximal to the epitope bound by the reference antigen binding protein for steric hindrance to occur. Additional details regarding methods for determining competitive binding are provided in the examples herein. Usually, when a competing antigen binding protein is present in excess, it will inhibit specific binding of a reference antigen binding protein to a common antigen by at least 40%, 45%, 50%, 55%, 60%, 65%, 70% or 75%. In some instance, binding is inhibited by at least 80%, 85%, 90%, 95%, or 97% or more.

[00135] The term "antigen" refers to a molecule or a portion of a molecule capable of being bound by a selective binding agent, such as an antigen binding protein (including, e.g., an antibody or immunological functional fragment thereof), and additionally capable of being used in an animal to produce antibodies capable of binding to that antigen. An antigen may possess one or more epitopes that are capable of interacting with different antigen binding proteins, e.g., antibodies.

[00136] 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.

[00137] 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.

[00138] 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.

[00139] 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.

[00140] 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.

[00141] Recommended parameters for determining percent identity for polypeptides or nucleotide sequences using the GAP program include the following:

[00142] Algorithm: Needleman et al., 1970, J. Mol. Biol. 48:443-453;

[00143] Comparison matrix: BLOSUM 62 from Henikoff et al., 1992, supra;

[00144] Gap Penalty: 12 (but with no penalty for end gaps)

[00145] Gap Length Penalty: 4

[00146] Threshold of Similarity: 0

[00147] 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.

[00148] The term "modification" when used in connection with antigen binding proteins, 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. Modified antigen binding proteins of the invention will retain the binding properties of unmodified molecules of the invention.

[00149] The term "derivative" when used in connection with antigen binding proteins (including antibodies and antibody fragments) of the invention refers to antigen binding 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.

[00150] Immunoglobulin Embodiments of Antigen Binding Proteins

[00151] In full-length immunoglobulin light and heavy chains, the variable and constant regions are joined by a "J" region of about twelve or more amino acids, with the heavy chain also including a "D" region of about ten more amino acids. *See, e.g.,* Fundamental Immunology, 2nd ed., Ch. 7 (Paul, W., ed.) 1989, New York: Raven Press (hereby incorporated by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair typically form the antigen binding site.

[00152] One example of a human IgG2 heavy chain (HC) constant domain has the amino acid sequence:

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVE
CPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWY

VDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGL
PAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL
HNHYTQKSLSLSPGK// SEQ. ID NO:86.

[00153] Constant region sequences of other IgG isotypes are known in the art for making recombinant versions of the inventive antigen binding protein having an IgG1, IgG2, IgG3, or IgG4 immunoglobulin isotype, if desired. In general, human IgG2 can be used for targets where effector functions are not desired, and human IgG1 in situations where such effector functions (e.g., antibody-dependent cytotoxicity (ADCC)) are desired. Human IgG3 has a relatively short half life and human IgG4 forms antibody "half-molecules." There are four known allotypes of human IgG1. The preferred allotype is referred to as "hIgG1z", also known as the "KEEM" allotype. Human IgG1 allotypes "hIgG1za" (KDEL), "hIgG1f" (REEM), and "hIgG1fa" are also useful; all appear to have ADCC effector function.

[00154] Human hIgG1z heavy chain (HC) constant domain has the amino acid sequence:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD
KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV
MHEALHNHYTQKSLSLSPGK// SEQ ID NO:87.

[00155] Human hIgG1za heavy chain (HC) constant domain has the amino acid sequence:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD
KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK

FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI
AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM
HEALHNHYTQKSLSLSPGK// SEQ ID NO:88.

[00156] Human hIgG1f heavy chain (HC) constant domain has the amino acid sequence:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCD
KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV
MHEALHNHYTQKSLSLSPGK// SEQ ID NO:89.

[00157] Human hIgG1fa heavy chain (HC) constant domain has the amino acid sequence:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCD
KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI
AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM
HEALHNHYTQKSLSLSPGK// SEQ ID NO:90.

[00158] One example of a human immunoglobulin light chain (LC) constant region sequence is the following (designated “CL-1”):

GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKA
GVETTKPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAP
TECS// SEQ ID NO:91.

[00159] CL-1 is useful to increase the pI of antibodies and is convenient. There are three other human immunoglobulin light chain constant regions, designated "CL-2", "CL-3" and "CL-7", which can also be used within the scope of the present invention. CL-2 and CL-3 are more common in the human population.

[00160] CL-2 human light chain (LC) constant domain has the amino acid sequence:

GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAG VETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTE CS// SEQ ID NO:92.

[00161] CL-3 human LC constant domain has the amino acid sequence:

GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAG VETTTPSKQSNNKYAASSYLSLTPEQWKSHKSYSCQVTHEGSTVEKTVAPTE CS// SEQ ID NO:93.

[00162] CL-7 human LC constant domain has the amino acid sequence:

GQPKAAPSVTLFPPSSEELQANKATLVCLVSDFYPGAVTVAWKADGSPVKV GVETTKPSKQSNNKYAASSYLSLTPEQWKSHRSYSCRVTHEGSTVEKTVAP AECS// SEQ ID NO:94.

[00163] Variable regions of immunoglobulin chains generally exhibit the same overall structure, comprising relatively conserved framework regions (FR) joined by three hypervariable regions, more often called "complementarity determining regions" or CDRs. The CDRs from the two chains of each heavy chain/light chain pair mentioned above typically are aligned by the framework regions to form a structure that binds specifically with a specific epitope or domain on the target (*e.g.*, KLH or DNP). From N-terminal to C-terminal, naturally-occurring light and heavy chain variable regions both typically conform with the following order of these elements: FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. A numbering system has been devised for assigning numbers to amino acids that occupy positions in each of

these domains. This numbering system is defined in Kabat Sequences of Proteins of Immunological Interest (1987 and 1991, NIH, Bethesda, MD), or Chothia & Lesk, 1987, *J. Mol. Biol.* 196:901-917; Chothia *et al.*, 1989, *Nature* 342:878-883.

[00164] Specific examples of some of the full length light and heavy chains of the antibodies that are provided and their corresponding amino acid sequences are summarized in Table 1A and Table 1B below. Table 1A shows exemplary light chain sequences, all of which have a common constant region lambda constant region 1 (CL-1; SEQ ID NO:91) for all lambda light chains. Table 1B shows exemplary heavy chain sequences, all of which include constant region human IgG2 (SEQ ID NO:86). However, encompassed within the present invention are immunoglobulins with sequence changes in the constant or framework regions of those listed in Table 1A and/or Table 1B (e.g. IgG4 vs IgG2, CL2 vs CL1). Also, the signal peptide (SP) sequences for all of the sequence in Table 1A and Table 1B are the same, i.e., the VK-1 SP signal peptide: MDMRVPAQLLGLLLLWLRGARC (SEQ ID NO:103; single underlined) that is used in the high throughput cloning process, but any other suitable signal peptide sequence may be employed within the scope of the invention. Another example of a useful signal peptide sequence is VH21 SP MEWSWVFLFFLSVTTGVHS (SEQ ID NO:95). Other exemplary signal peptide sequences are shown in Table 1A-B.

Table 1A. Immunoglobulin Light Chain Sequences. Signal peptide sequences are indicated by a double underline, CDR regions are indicated by single underline, and framework and constant regions are not underlined.

SEQ ID NO:	**Designation**	**Contained in Clone(s)**	**Sequence**
Anti-DNP			
105	L1	3A1	MDMRVPAQLLGLLLLWLRGARCDIQMTQ SPSSVSASVGDRVTITCRASQGISNWLAWY QRKPGKAPKLLIYAASSLQSGVPSRFSGSGS GTDFTLTISSLQPEDFAAYYCQQASSFPWTF GQGTRVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSG

			NSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC
109	L2	3A4	MDMRVPAQLLGLLLLWLRGARCDIQMTQ SPSSVSASVGDRVTITCRASQGISRRLAWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSG SGTDFTLTISSLQPEDFATYYCQQANSFPFT FGPGTKVDIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQS GNSQESVTEQDSKDSTYSLSSTLTLSKADY EKHKVYACEVTHQGLSSPVTKSFNRGEC
121	L3	3B1	MDMRVPAQLLGLLLLWLRGARCDIQMTQ SPSSLSASEGDRVTITCRASQGIRNDLGWY QQKPGKAPKRLIYAASSLQSGVPLRFSGSG SGTEFTLTISSLQPEDFATYYCLQYNSYPWT FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQS GNSQESVTEQDSKDSTYSLSSTLTLSKADY EKHKVYACEVTHQGLSSPVTKSFNRGEC
125	L4	3C2	MDMRVPAQLLGLLLLWLRGARCDIQMTQ SPSSLSASVGDRVTITCRASQGMSNYLAWY QQKPRKVPKLLIYAASTLQSGVPSRFSGSG SGTDFTLTISSLQPEDVATYYCQKFNSAPFT FGPGTKVDIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQS GNSQESVTEQDSKDSTYSLSSTLTLSKADY EKHKVYACEVTHQGLSSPVTKSFNRGEC
127	L5	3H4	MDMRVPAQLLGLLLLWLRGARCDIQMTLS PSSLSASVGDRVTITCRASQGIRNDLGWYQ QKPGKAPKRLIYAASSLQSGVPSRFSGSGS GTEFTLTISSLQPEDFATYYCLQYNSSPWTF GQGTEVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC
Anti-KLH			
131	L6	16.3.1	MDMRVPAQLLGLLLLWLSGARCDIQMTQS PSSLSVSVGDRVTITCQAGQDIRNYLNWYQ QKPGKAPKLLIYDASNLETGVPSRFSGSGS GTAFTFTISSLQPEDIATYYCQQYDNLTFGQ GTKLEIKRTVAAPSVFIFPPSDEQLKSGTAS

			VVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC
135	L7	108.1.2	METPAQLLFLLLLWLPDTTGEIVLTQSPGT LSLSPGERATLSCRASQNISTNYLAWYQQK PGQAPRFLIYGASSRATGIPDRFSGSGSGTD FTLTISRLEPEDFAVYYCQQFGRSPRCSFGQ GTKLEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC
137	L8	108.1.2 (N>Q, C>S)	METPAQLLFLLLLWLPDTTGEIVLTQSPGT LSLSPGERATLSCRASQQISTNYLAWYQQK PGQAPRFLIYGASSRATGIPDRFSGSGSGTD FTLTISRLEPEDFAVYYCQQFGRSPRSSFGQ GTKLEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC
141	L9	120.6	MDMRVPAQLLGLLLLWFPGARCDIQMTQS PSSLSASVGDRVTITCRASQGIRNDLGWYQ QKPGKAPKRLIYAASSLQSGVPSRFSGSGS GTEFTLTISSLQPEDFATYYCLQHNSYPLTF GGGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC
28	L10	120.6	MDMRVPAQLLGLLLLWLRGARCDIQMTQ SPSSLSASVGDRVTITCRASQGIRNDLGWY QQKPGKAPKRLIYAASSLQSGVPSRFSGSG SGTEFTLTISSLQPEDFATYYCLQHNSYPLT FGGGTKVEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDSTYSLSSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFNRGEC

Table 1B. Immunoglobulin Heavy Chain Sequences. Signal peptide sequences are indicated by a double underline, CDR regions are indicated by single underline, and framework and constant regions are not underlined.

SEQ ID NO:	Designation	Contained in Clone(s)	Sequence
Anti-DNP			
107	H1	3A1	MDMRVPAQLLGLLLLWLRGARCQVQLQE SGPGLVKPSETLSLTCTVSGGSISHYYWSW IRQPPGKGLGWIGYIYYSGSTNYNPSLKSR VTISVDTSKNQFSLKLTSVTAADTAVYYC ARARGDGYNYPDAFDIWGQGTMVTVSSA STKGPSVFPLAPCSRSTSESTAALGCLVKD YFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSNFGTQTYTCNVDHKPSN TKVDKTVERKCCVECPPCPAPPVAGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPE VQFNWYVDGVEVHNAKTKPREEQFNSTF RVVSVLTVVHQDWLNGKEYKCKVSNKGL PAPIEKTISKTKGQPREPQVYTLPPSREEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPMLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG K
111	H2	3A4 3C2	MDMRVPAQLLGLLLLWLRGARCQVQLVE SGGGVVQPGRSLRLSCAASGFTFSSYGMH WVRQAPGKGLEWVAVIWYDGSNKYYAD SVKGRFTISRDNSKNTLYLQMNSLRAEDT AVYYCARYNWNYGMDVWGQGTTVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSNFGTQTYTCNVDHKPS NTKVDKTVERKCCVECPPCPAPPVAGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVQFNWYVDGVEVHNAKTKPREEQFNS TFRVVSVLTVVHQDWLNGKEYKCKVSNK GLPAPIEKTISKTKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPMLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSP GK
113	H3	3A4-F	MDMRVPAQLLGLLLLWLRGARCQVQLVE SGGGVVQPGRSLRLSCAASGFTFSSYGMH

		(W101F)	WVRQAPGKGLEWVAVIWYDGSNKYYAD SVKGRFTISRDNSKNTLYLQMNSLRAEDT AVYYCARYNFNYGMDVWGQGTTVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSNFGTQTYTCNVDHKPS NTKVDKTVERKCCVECPPCPAPPVAGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVQFNWYVDGVEVHNAKTKPREEQFNS TFRVVSVLTVVHQDWLNGKEYKCKVSNK GLPAPIEKTISKTKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPMLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSP GK
115	H4	3A4-Y (W101Y)	MDMRVPAQLLGLLLLWLRGARCQVQLVE SGGGVVQPGRSLRLSCAASGFTFSSYGMH WVRQAPGKGLEWVAVIWYDGSNKYYAD SVKGRFTISRDNSKNTLYLQMNSLRAEDT AVYYCARYNYNYGMDVWGQGTTVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSNFGTQTYTCNVDHKPS NTKVDKTVERKCCVECPPCPAPPVAGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVQFNWYVDGVEVHNAKTKPREEQFNS TFRVVSVLTVVHQDWLNGKEYKCKVSNK GLPAPIEKTISKTKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPMLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSP GK
117	H5	3A4-FSS	MDMRVPAQLLGLLLLWLRGARCQVQLVE SGGGVVQPGRSLRLSCAASGFTFSSYGMH WVRQAPGKGLEWVAVIWYDGSNKYYAD SVKGRFTISRDNSKNTLYLQMNSLRAEDT AVYYCARYNFNYGMDVWGQGTTVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSNFGTQTYTCNVDHKPS NTKVDKTVERKSSVECPPCPAPPVAGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVQFNWYVDGVEVHNAKTKPREEQFNST FRVVSVLTVVHQDWLNGKEYKCKVSNKG

			LPAPIEKTISKTKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPMLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSP GK
119	H6	3A4-YSS	MDMRVPAQLLGLLLLWLRGARCQVQLVE SGGGVVQPGRSLRLSCAASGFTFSSYGMH WVRQAPGKGLEWVAVIWYDGSNKYYAD SVKGRFTISRDNSKNTLYLQMNSLRAEDT AVYYCARYNYNYGMDVWGQGTTVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSNFGTQTYTCNVDHKPS NTKVDKTVERKSSVECPPCPAPPVAGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVQFNWYVDGVEVHNAKTKPREEQFNST FRVVSVLTVVHQDWLNGKEYKCKVSNKG LPAPIEKTISKTKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPMLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSP GK
123	H7	3B1	MDMRVPAQLLGLLLLWLRGARCQVQLQE SGPGLVKPSETLSLTCTVSGGSISSYYWSWI RQPPGKGLEWIGYIYYSGNTNSNPSLKSRV TISVDTSKNQFSLKLSSVTAADTAVYYCAR TYYDSSGYYYRAFDIWGQGTMVTVSSAST KGPSVFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSNFGTQTYTCNVDHKPSNT KVDKTVERKCCVECPPCPAPPVAGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEV QFNWYVDGVEVHNAKTKPREEQFNSTFR VVSVLTVVHQDWLNGKEYKCKVSNKGLP APIEKTISKTKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPMLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGK
129	H8	3H4	MDMRVPAQLLGLLLLWLRGARCQVQLQE SGPGLVKPLQTLSLTCTVSGGSISSGGYYW SWIRQHPGKGLEWIGYIYYSRSTYYNPSLK SRVTISVDTSKNQFSLKLSSVTAADTAVYY CARTGYSSGWYPFDYWGQGTLVTVSSAST

			KGPSVFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSNFGTQTYTCNVDHKPSNT KVDKTVERKCCVECPPCPAPPVAGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEV QFNWYVDGVEVHNAKTKPREEQFNSTFR VVSVLTVVHQDWLNGKEYKCKVSNKGLP APIEKTISKTKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPMLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGK
144	H9	3A1	MDMRVPAQLLGLLLLWLRGARCQVQLQE SGPGLVKPSETLSLTCTVSGGSISHYYWSW IRQPPGKGLGWIGYIYYSGSTNYNPSLKSR VTISVDTSKNQFSLKLTSVTAADTAVYYC ARARGDGYNYPDAFDIWGQGTMVTVSSA STKGPSVFPLAPCSRSTSESTAALGCLVKD YFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSNFGTQTYTCNVDHKPSN TKVDKTVERKCCVECPPCPAPPVAGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPE VQFNWYVDGVEVHNAKTKPREEQFNSTF RVVSVLTVVHQDWLNGKEYKCKVSNKGL PAPIEKTISKTKGQPREPQVYTLPPSREEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPMLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG
145	H10	3A4 3C2	MDMRVPAQLLGLLLLWLRGARCQVQLVE SGGGVVQPGRSLRLSCAASGFTFSSYGMH WVRQAPGKGLEWVAVIWYDGSNKYYAD SVKGRFTISRDNSKNTLYLQMNSLRAEDT AVYYCARYNWNYGMDVWGQGTTVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSNFGTQTYTCNVDHKPS NTKVDKTVERKCCVECPPCPAPPVAGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVQFNWYVDGVEVHNAKTKPREEQFNS TFRVVSVLTVVHQDWLNGKEYKCKVSNK GLPAPIEKTISKTKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPMLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSP G

77	H11	3A4-F (W101F)	MDMRVPAQLLGLLLLWLRGARCQVQLVE SGGGVVQPGRSLRLSCAASGFTFSSYGMH WVRQAPGKGLEWVAVIWYDGSNKYYAD SVKGRFTISRDNSKNTLYLQMNSLRAEDT AVYYCARYNFNYGMDVWGQGTTVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSNFGTQTYTCNVDHKPS NTKVDKTVERKCCVECPPCPAPPVAGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVQFNWYVDGVEVHNAKTKPREEQFNS TFRVVSVLTVVHQDWLNGKEYKCKVSNK GLPAPIEKTISKTKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPMLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSP G
181	H12	3A4-Y (W101Y)	MDMRVPAQLLGLLLLWLRGARCQVQLVE SGGGVVQPGRSLRLSCAASGFTFSSYGMH WVRQAPGKGLEWVAVIWYDGSNKYYAD SVKGRFTISRDNSKNTLYLQMNSLRAEDT AVYYCARYNYNYGMDVWGQGTTVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSNFGTQTYTCNVDHKPS NTKVDKTVERKCCVECPPCPAPPVAGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVQFNWYVDGVEVHNAKTKPREEQFNS TFRVVSVLTVVHQDWLNGKEYKCKVSNK GLPAPIEKTISKTKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPMLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSP G
182	H13	3A4-FSS	MDMRVPAQLLGLLLLWLRGARCQVQLVE SGGGVVQPGRSLRLSCAASGFTFSSYGMH WVRQAPGKGLEWVAVIWYDGSNKYYAD SVKGRFTISRDNSKNTLYLQMNSLRAEDT AVYYCARYNFNYGMDVWGQGTTVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSNFGTQTYTCNVDHKPS NTKVDKTVERKSSVECPPCPAPPVAGPSVF

			LFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVQFNWYVDGVEVHNAKTKPREEQFNST FRVVSVLTVVHQDWLNGKEYKCKVSNKG LPAPIEKTISKTKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPMLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSP G
183	H14	3A4-YSS	MDMRVPAQLLGLLLLWLRGARCQVQLVE SGGGVVQPGRSLRLSCAASGFTFSSYGMH WVRQAPGKGLEWVAVIWYDGSNKYYAD SVKGRFTISRDNSKNTLYLQMNSLRAEDT AVYYCARYNYNYGMDVWGQGTTVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSNFGTQTYTCNVDHKPS NTKVDKTVERKSSVECPPCPAPPVAGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVQFNWYVDGVEVHNAKTKPREEQFNST FRVVSVLTVVHQDWLNGKEYKCKVSNKG LPAPIEKTISKTKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPMLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSP G
184	H15	3B1	MDMRVPAQLLGLLLLWLRGARCQVQLQE SGPGLVKPSETLSLTCTVSGGSISSYYWSWI RQPPGKGLEWIGYIYYSGNTNSNPSLKSRV TISVDTSKNQFSLKLSSVTAADTAVYYCAR TYYDSSGYYYRAFDIWGQGTMVTVSSAST KGPSVFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSNFGTQTYTCNVDHKPSNT KVDKTVERKCCVECPPCPAPPVAGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEV QFNWYVDGVEVHNAKTKPREEQFNSTFR VVSVLTVVHQDWLNGKEYKCKVSNKGLP APIEKTISKTKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPMLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPG
185	H16	3H4	MDMRVPAQLLGLLLLWLRGARCQVQLQE SGPGLVKPLQTLSLTCTVSGGSISSGGYYW

			SWIRQHPGKGLEWIGYIYYSRSTYYNPSLK SRVTISVDTSKNQFSLKLSSVTAADTAVYY CARTGYSSGWYPFDYWGQGTLVTVSSAST KGPSVFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSNFGTQTYTCNVDHKPSNT KVDKTVERKCCVECPPCPAPPVAGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEV QFNWYVDGVEVHNAKTKPREEQFNSTFR VVSVLTVVHQDWLNGKEYKCKVSNKGLP APIEKTISKTKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPMLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPG
Anti-KLH			
133	H17	16.3.1	MELGLSWVFLFAILEGVQCEVQLVESGGG LVQPGGSLRLSCAASGFTFSNYDMYWVRQ TTGKGLEWVSAIGTAGDTYYPGSVKGRFT ISRENAKNSLYLQMNSLRAGDTAVYYCAR EKSSTSAFDYWGQGTLVTVSSASTKGPSVF PLAPCSRSTSESTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSNFGTQTYTCNVDHKPSNTKVDKTV ERKCCVECPPCPAPPVAGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTFRVVSVLT VVHQDWLNGKEYKCKVSNKGLPAPIEKTI SKTKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTP PMLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
139	H18	108.1.2	MKHLWFFLLLVAAPRWVLSQLQLQESGP GLMKPSETLSLTCTVSGGSISSSSYFWGWI RQPPGKGLEWIGSIYYSGNTFYNPSLKSRV TISVDTSKNQFSLKLNSMTAADTAVYFCA RQGGIAARTGYWYFDLWGRGTTVTVSSA STKGPSVFPLAPCSRSTSESTAALGCLVKD YFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSNFGTQTYTCNVDHKPSN TKVDKTVERKCCVECPPCPAPPVAGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPE VQFNWYVDGVEVHNAKTKPREEQFNSTF RVVSVLTVVHQDWLNGKEYKCKVSNKGL

			PAPIEKTISKTKGQPREPQVYTLPPSREEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPMLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG K
143	H19	120.6	MDWTWRILFLVAAATGAHSQVQLVQSGA EVKKPGASVKVSCKASGYTFTGYHMHWV RQAPGQGLEWMGWINPNSGGTNYAQKFQ GRVTMTRDTSISTAYMELSRLRSDDTAVY YCARDRGSYYWFDPWGQGTLVTVSSAST KGPSVFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSNFGTQTYTCNVDHKPSNT KVDKTVERKCCVECPPCPAPPVAGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEV QFNWYVDGVEVHNAKTKPREEQFNSTFR VVSVLTVVHQDWLNGKEYKCKVSNKGLP APIEKTISKTKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPMLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGK
46	H20	120.6	MDMRVPAQLLGLLLLWLRGARCQVQLVQ SGAEVKKPGASVKVSCKASGYTFTGYHM HWVRQAPGQGLEWMGWINPNSGGTNYA QKFQGRVTMTRDTSISTAYMELSRLRSDD TAVYYCARDRGSYYWFDPWGQGTLVTVS SASTKGPSVFPLAPCSRSTSESTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVVTVPSSNFGTQTYTCNVDHK PSNTKVDKTVERKCCVECPPCPAPPVAGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVQFNWYVDGVEVHNAKTKPREEQFN STFRVVSVLTVVHQDWLNGKEYKCKVSN KGLPAPIEKTISKTKGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPMLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSL SPG
186	H21	16.3.1	MELGLSWVFLFAILEGVQCEVQLVESGGG LVQPGGSLRLSCAASGFTFSNYDMYWVRQ TTGKGLEWVSAIGTAGDTYYPGSVKGRFT ISRENAKNSLYLQMNSLRAGDTAVYYCAR

			EKSSTSAFDYWGQGTLVTVSSASTKGPSVF PLAPCSRSTSESTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSNFGTQTYTCNVDHKPSNTKVDKTV ERKCCVECPPCPAPPVAGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTFRVVSVLT VVHQDWLNGKEYKCKVSNKGLPAPIEKTI SKTKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTP PMLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPG
187	H22	108.1.2	MKHLWFFLLLVAAPRWVLSQLQLQESGP GLMKPSETLSLTCTVSGGSISSSSYFWGWI RQPPGKGLEWIGSIYYSGNTFYNPSLKSRV TISVDTSKNQFSLKLNSMTAADTAVYFCA RQGGIAARTGYWYFDLWGRGTTVTVSSA STKGPSVFPLAPCSRSTSESTAALGCLVKD YFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSNFGTQTYTCNVDHKPSN TKVDKTVERKCCVECPPCPAPPVAGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPE VQFNWYVDGVEVHNAKTKPREEQFNSTF RVVSVLTVVHQDWLNGKEYKCKVSNKGL PAPIEKTISKTKGQPREPQVYTLPPSREEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPMLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG
366	H23	120.6	MDWTWRILFLVAAATGAHSQVQLVQSGA EVKKPGASVKVSCKASGYTFTGYHMHWV RQAPGQGLEWMGWINPNSGGTNYAQKFQ GRVTMTRDTSISTAYMELSRLRSDDTAVY YCARDRGSYYWFDPWGQGTLVTVSSAST KGPSVFPLAPCSRSTSESTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSNFGTQTYTCNVDHKPSNT KVDKTVERKCCVECPPCPAPPVAGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEV QFNWYVDGVEVHNAKTKPREEQFNSTFR VVSVLTVVHQDWLNGKEYKCKVSNKGLP APIEKTISKTKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPMLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPG

367	H24	120.6	MDMRVPAQLLGLLLLWLRGARCQVQLVQ SGAEVKKPGASVKVSCKASGYTFTGYHM HWVRQAPGQGLEWMGWINPNSGGTNYA QKFQGRVTMTRDTSISTAYMELSRLRSDD TAVYYCARDRGSYYWFDPWGQGTLVTVS SASTKGPSVFPLAPCSRSTSESTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVVTVPSSNFGTQTYTCNVDHK PSNTKVDKTVERKCCVECPPCPAPPVAGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVQFNWYVDGVEVHNAKTKPREEQFN STFRVVSVLTVVHQDWLNGKEYKCKVSN KGLPAPIEKTISKTKGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPMLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSL SPGK

[00165] Some embodiments of the isolated anti-DNP antigen binding protein comprising an antibody or antibody fragment, comprise:

[00166] (a) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO:77, SEQ ID NO:107, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:123, SEQ ID NO:129, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:181, SEQ ID NO:182, SEQ ID NO:183, SEQ ID NO:184, or SEQ ID NO:185, or comprising any one of the foregoing sequences from which one, two, three, four or five amino acid residues are lacking from the N-terminal or C-terminal, or both;

[00167] (b) an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO:105, SEQ ID NO:109, SEQ ID NO:121; SEQ ID NO:125, or SEQ ID NO:127, or comprising any one of the foregoing sequences from which one, two, three, four or five amino acid residues are lacking from the N-terminal or C-terminal, or both; or

[00168] (c) the immunoglobulin heavy chain of (a) and the immunoglobulin light chain of (b).

[00169] Some embodiments of the isolated anti-KLH antigen binding protein comprising an antibody or antibody fragment, comprise:

[00170] (a) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO:46, SEQ ID NO:133, SEQ ID NO:139, SEQ ID NO:143, SEQ ID NO:186, or SEQ ID NO:187, SEQ ID NO:366, or SEQ ID NO:367, or comprising any one of the foregoing sequences from which one, two, three, four or five amino acid residues are lacking from the N-terminal or C-terminal, or both;

[00171] (b) an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO:28, SEQ ID NO:131, SEQ ID NO:135, SEQ ID NO:137; or SEQ ID NO:141, or comprising any one of the foregoing sequences from which one, two, three, four or five amino acid residues are lacking from the N-terminal or C-terminal, or both; or

[00172] (c) the immunoglobulin heavy chain of (a) and the immunoglobulin light chain of (b).

[00173] Again, each of the exemplary anti-DNP heavy chains (H1, H2, H3, . . . etc.) listed in Table 1B can be combined with any of the exemplary anti-DNP light chains shown in Table 1A to form an antibody. Examples of such combinations include H1 combined with any of L1 through L5; H2 combined with any of L1 through L5; H3 combined with any of L1 through L5, H4 combined with any of L1 through L5, and so on. In some instances, the antibodies include at least one anti-DNP heavy chain and one anti-DNP light chain from those listed in Table 1A and 1B. In some instances, the antibodies comprise two different anti-DNP heavy chains and two different anti-DNP light chains listed in Table 1A and Table 1B. In other instances, the antibodies contain two identical light chains and two identical heavy chains. As an example, an antibody or immunologically functional fragment may include two H1 heavy chains and two L1 light chains, or two H2 heavy chains and two L2 light chains, or two H3 heavy chains and two L3 light chains and other similar combinations of pairs of anti-DNP light chains and pairs of anti-DNP heavy chains as listed in Table 1A and Table 1B.

[00174] Again, each of the exemplary anti-KLH heavy chains (H1, H2, H3, . . . etc.) listed in Table 1B can be combined with any of the exemplary anti-KLH light chains shown in Table 1A to form an antibody. Examples of such combinations include H1 combined with any of L1 through L5; H2 combined with any of L1 through L5; H3 combined with any of L1 through L5, H4 combined with any of L1 through L5, and so on. In some instances, the antibodies include at least one anti-KLH heavy chain and one anti-KLH light chain from those listed in Table 1A and 1B. In some instances, the antibodies comprise two different anti-KLH heavy chains and two different anti-KLH light chains listed in Table 1A and Table 1B. In other instances, the antibodies contain two identical light chains and two identical heavy chains. As an example, an antibody or immunologically functional fragment may include two H1 heavy chains and two L1 light chains, or two H2 heavy chains and two L2 light chains, or two H3 heavy chains and two L3 light chains and other similar combinations of pairs of anti-KLH light chains and pairs of anti-KLH heavy chains as listed in Table 1A and Table 1B.

[00175] Other antigen binding proteins that are provided are variants of antibodies formed by combination of the heavy and light chains shown in Tables 1A and Table 1B and comprise light and/or heavy chains that each have at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or at least 99% identity to the amino acid sequences of these chains. In some instances, such antibodies include at least one heavy chain and one light chain, whereas in other instances the variant forms contain two identical light chains and two identical heavy chains. It is within the scope of the invention that the heavy chain(s) and/or light chain(s) may have one, two, three, four or five amino acid residues lacking from the N-terminal or C-terminal, or both, in relation to any one of the heavy and light chains set forth in Tables 1A and Table 1B, e.g., due to post-translational modifications. For example, CHO cells typically cleave off a C-terminal lysine.

[00176] Variable Domains of Antibodies

[00177] The various heavy chain and light chain variable regions provided herein are depicted in Table 2A-B. Each of these variable regions may be attached to the above heavy and light chain constant regions to form a complete antibody heavy and light chain, respectively. Further, each of the so generated heavy and light chain sequences may be combined to form a complete antibody structure. It should be understood that the heavy chain and light chain variable regions provided herein can also be attached to other constant domains having different sequences than the exemplary sequences listed above.

[00178] Also provided are antigen binding proteins, including antibodies or antibody fragments, that contain or include at least one immunoglobulin anti-DNP heavy chain variable region selected from V_H1, V_H2, V_H3, V_H4, V_H5, and V_H6 and/or at least one immunoglobulin anti-DNP light chain variable region selected from V_L1, V_L2, V_L3, V_L4, and V_L5, as shown in Table 2A below, and immunologically functional fragments, derivatives, muteins and variants of these light chain and heavy chain variable regions.

[00179] Also provided are antigen binding proteins, including antibodies or antibody fragments, that contain or include at least one immunoglobulin anti-KLH heavy chain variable region selected from V_H7, V_H8, and V_H9 and/or at least one immunoglobulin anti-KLH light chain variable region selected from V_L6, V_L7, V_L8, and V_L9, as shown in Table 2B below, and immunologically functional fragments, derivatives, muteins and variants of these light chain and heavy chain variable regions.

[00180] Antigen binding proteins of this type can generally be designated by the formula " V_Hx/ V_Ly," where "x" corresponds to the number of heavy chain variable regions included in the antigen binding protein and "y" corresponds to the number of the light chain variable regions included in the antigen binding protein (in general, x and y are each 1 or 2).

Table 2A. Exemplary anti-DNP V_H and V_L Chains: CDR regions are indicated by underline, and framework regions are not underlined. Optional N-terminal signal sequences are not shown (See, Table 1A-B).

Contained in Reference in Table 1A-B	Designation	SEQ ID NO	Amino Acid Sequence
L1	**VL1**	**232**	DIQMTQSPSSVSASVGDRVTITCRASQGISNWLAWYQRKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFAAYYCQQASSFPWTFGQGTRVEIK
L2	**VL2**	**234**	DIQMTQSPSSVSASVGDRVTITCRASQGISRRLAWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQANSFPFTFGPGTKVDIK
L3	**VL3**	**236**	DIQMTQSPSSLSASEGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPLRFSGSGSGTEFTLTISSLQPEDFATYYCLQYNSYPWTFGQGTKVEIK
L4	**VL4**	**238**	DIQMTQSPSSLSASVGDRVTITCRASQGMSNYLAWYQQKPRKVPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQKFNSAPFTFGPGTKVDIK
L5	**VL5**	**240**	DIQMTLSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCLQYNSSPWTFGQGTEVEIK
H1, H9	**VH1**	**250**	QVQLQESGPGLVKPSETLSLTCTVSGGSISHYYWSWIRQPPGKGLGWIGYIYYSGSTNYNPSLKSRVTISVDTSKNQFSLKLTSVTAADTAVYYCARARGDGYNYPDAFDIWGQGTMVTVSS
H2, H10	**VH2**	**252**	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYNWNYGMDVWGQGTTVTVSS

Contained in Reference in Table 1A-B	Designation	SEQ ID NO	Amino Acid Sequence
H3, H5, H11, H13	**VH3**	**254**	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYNFNYGMDVWGQGTTVTVSS
H4, H6, H12, H14	**VH4**	**256**	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYNYNYGMDVWGQGTTVTVSS
H7, H15	**VH5**	**258**	QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPPGKGLEWIGYIYYSGNTNSNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARTYYDSSGYYYRAFDIWGQGTMVTVSS
H8, H16	**VH6**	**260**	QVQLQESGPGLVKPLQTLSLTCTVSGGSISSGGYYWSWIRQHPGKGLEWIGYIYYSRSTYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARTGYSSGWYPFDYWGQGTLVTVSS

Table 2B. Exemplary anti-KLH V_H and V_L Chains: CDR regions are indicated by underline, and framework regions are not underlined. Optional N-terminal signal sequences are not shown (See, Table 1A-B; see, e.g., SEQ ID NO:95 and SEQ ID NO:103).

Contained in Reference in Table 1A-B	Designation	SEQ ID NO	Amino Acid Sequence
L6	**VL6**	**242**	DIQMTQSPSSLSVSVGDRVTITCQAGQDIRNYLN WYQQKPGKAPKLLIYDASNLETGVPSRFSGSGS GTAFTFTISSLQPEDIATYYCQQYDNLTFGQGTK LEIK
L7	**VL7**	**244**	EIVLTQSPGTLSLSPGERATLSCRASQNISTNYLA WYQQKPGQAPRFLIYGASSRATGIPDRFSGSGSG TDFTLTISRLEPEDFAVYYCQQFGRSPRCSFGQGT KLEIK
L8	**VL8**	**246**	EIVLTQSPGTLSLSPGERATLSCRASQQISTNYLA WYQQKPGQAPRFLIYGASSRATGIPDRFSGSGSG TDFTLTISRLEPEDFAVYYCQQFGRSPRSSFGQGT KLEIK
L9, L10	**VL9**	**248**	DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLG WYQQKPGKAPKRLIYAASSLQSGVPSRFSGSGSG TEFTLTISSLQPEDFATYYCLQHNSYPLTFGGGTK VEIK
H17, H21	**VH7**	**262**	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYD MYWVRQTTGKGLEWVSAIGTAGDTYYPGSVKG RFTISRENAKNSLYLQMNSLRAGDTAVYYCARE KSSTSAFDYWGQGTLVTVSS
H18, H22	**VH8**	**264**	QLQLQESGPGLMKPSETLSLTCTVSGGSISSSSYF WGWIRQPPGKGLEWIGSIYYSGNTFYNPSLKSRV TISVDTSKNQFSLKLNSMTAADTAVYFCARQGGI AARTGYWYFDLWGRGTTVTVSS
H19, H20, H23, H24	**VH9**	**266**	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGY HMHWVRQAPGQGLEWMGWINPNSGGTNYAQK FQGRVTMTRDTSISTAYMELSRLRSDDTAVYYC ARDRGSYYWFDPWGQGTLVTVSS

[00181] Some embodiments of the isolated antigen binding protein that comprises an anti-DNP antibody or antibody fragment, comprising an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region:

[00182] (a) the heavy chain variable region comprises an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of SEQ ID NO:250, SEQ ID NO:252, SEQ ID NO:254, SEQ ID NO:256, SEQ ID NO:258, or SEQ ID NO:260; or

[00183] (b) the light chain variable region comprises an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of SEQ ID NO:232, SEQ ID NO:234, SEQ ID NO:236, SEQ ID NO:238, or SEQ ID NO:240; or

[00184] (c) the heavy chain variable region of (a) and the light chain variable region of (b).

[00185] Some embodiments of the isolated antigen binding protein that comprises an anti-DNP antibody or antibody fragment, comprising an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region:

[00186] (a) the heavy chain variable region comprises an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of SEQ ID NO:262, SEQ ID NO:264, or SEQ ID NO:266; or

[00187] (b) the light chain variable region comprises an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of SEQ ID NO:242, SEQ ID NO:244, SEQ ID NO:246, or SEQ ID NO:248; or

[00188] (c) the heavy chain variable region of (a) and the light chain variable region of (b).

[00189] Each of the heavy chain variable regions listed in Table 2A, whether or not it is included in a larger heavy chain, may be combined with any of the light chain variable regions shown in Table 2A to form an antigen binding protein. Examples of such combinations include V_H1 combined with any of V_L1, V_L2, V_L3, V_L4, or V_L5; V_H2 combined with any of V_L1, V_L2, V_L3, V_L4, or V_L5; V_H3 combined with any of V_L1, V_L2, V_L3, V_L4, or V_L5; V_H4 combined with any of V_L1, V_L2, V_L3, V_L4, or V_L5, and so on.

[00190] Each of the heavy chain variable regions listed in Table 2B, whether or not it is included in a larger heavy chain, may be combined with any of the light chain variable regions shown in Table 2B to form an antigen binding protein. Examples of such combinations include V_H7 combined with any of V_L6, V_L7, V_L8 or V_L9; V_H8 combined with any of V_L6, V_L7, V_L8 or V_L9; V_H8 combined with any of V_L6, V_L7, V_L8 or V_L9; V_H9 combined with any of V_L6, V_L7, V_L8 or V_L9.

[00191] In some instances, the antigen binding protein includes at least one heavy chain variable region and/or one light chain variable region from those listed in Table 2A. In some instances, the antigen binding protein includes at least two different heavy chain variable regions and/or light chain variable regions from those listed in Table 2A. An example of such an antigen binding protein comprises (a) one V_H1, and (b) one of V_H2, V_H3, or V_H4, etc. Another example comprises (a) one V_H2, and (b) one of V_H1, V_H3, or V_H4, etc. Again another example comprises (a) one V_H3, and (b) one of V_H1, V_H2, or V_H4, etc. Again another example comprises (a) one V_H4, and (b) one of V_H1, V_H2, or V_H3, etc. Again another example comprises (a) one V_H5, and (b) one of V_H1, V_H2, or V_H3, etc. Again another example comprises (a) one V_H6, and (b) one of V_H1, V_H2, or V_H3, etc.

[00192] Again another example of such an antigen binding protein comprises (a) one V_L1, and (b) one of V_L2 or V_L3, etc. Again another example of such an antigen binding protein comprises (a) one V_L2, and (b) one of V_L1 or V_L3, etc. Again another example of such an antigen binding protein comprises (a) one V_L3, and (b) one of V_L1 or V_L2, etc., and so on.

[00193] The various combinations of heavy chain variable regions set forth in Table 2A may be combined with any of the various combinations of light chain variable regions set forth in Table 2A.

[00194] In other instances, the antigen binding protein contains two identical light chain variable regions and/or two identical heavy chain variable regions. As an example, the antigen binding protein may be an antibody or immunologically functional fragment that includes two light chain variable regions and two heavy chain variable regions in combinations of pairs of light chain variable regions and pairs of heavy chain variable regions as listed in Table 2A.

[00195] In some instances, the antigen binding protein includes at least one heavy chain variable region and/or one light chain variable region from those listed in Table 2B. In some instances, the antigen binding protein includes at least two different heavy chain variable regions and/or light chain variable regions from those listed in Table 2B. An example of such an antigen binding protein comprises (a) one V_H7, and (b) one of V_H7, V_H8, or V_H9. Another example comprises (a) one V_H8, and (b) one of V_H7, V_H8, or V_H9. Again another example comprises (a) one V_H9, and (b) one of V_H7, V_H8, or V_H9.

[00196] Again another example of such an antigen binding protein comprises (a) one V_L6, and (b) one of V_L6, V_L7, V_L8 or V_L9. Again another example of such an antigen binding protein comprises (a) one V_L7, and (b) one of V_L6, V_L7, V_L8 or V_L9. Again another example of such an antigen binding protein comprises (a) one V_L8, and (b) one of V_L6, V_L7, V_L8 or V_L9. Again another example of such an antigen binding protein comprises (a) one V_L9, and (b) one of V_L6, V_L7, V_L8 or V_L9.

[00197] The various combinations of heavy chain variable regions set forth in Table 2B may be combined with any of the various combinations of light chain variable regions set forth in Table 2B.

[00198] In other instances, the antigen binding protein contains two identical light chain variable regions and/or two identical heavy chain variable regions. As an

example, the antigen binding protein may be an antibody or immunologically functional fragment that includes two light chain variable regions and two heavy chain variable regions in combinations of pairs of light chain variable regions and pairs of heavy chain variable regions as listed in Table 2B.

[00199] Some antigen binding proteins that are provided comprise a heavy chain variable domain comprising a sequence of amino acids that differs from the sequence of a heavy chain variable domain selected from V_H1, V_H2, V_H3, V_H4, V_H5, V_H6, V_H7, V_H8, and V_H9, at only 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acid residues, wherein each such sequence difference is independently either a deletion, insertion or substitution of one amino acid, with the deletions, insertions and/or substitutions resulting in no more than 15 amino acid changes relative to the foregoing variable domain sequences. The heavy chain variable region in some antigen binding proteins comprises a sequence of amino acids that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or at least 99% sequence identity to the amino acid sequences of the heavy chain variable region of V_H1, V_H2, V_H3, V_H4, V_H5, V_H6, V_H7, V_H8, or V_H9.

[00200] Certain antigen binding proteins comprise a light chain variable domain comprising a sequence of amino acids that differs from the sequence of a light chain variable domain selected from V_L1, V_L2, V_L3, V_L4, V_L5, V_L6, V_L7, V_L8, and V_L9 at only 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acid residues, wherein each such sequence difference is independently either a deletion, insertion or substitution of one amino acid, with the deletions, insertions and/or substitutions resulting in no more than 15 amino acid changes relative to the foregoing variable domain sequences. The light chain variable region in some antigen binding proteins comprises a sequence of amino acids that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97% or at least 99% sequence identity to the amino acid sequences of the light chain variable region of V_L1, V_L2, V_L3, V_L4, V_L5, V_L6, V_L7, V_L8, or V_L9.

[00201] Still other antigen binding proteins, *e.g.*, antibodies or immunologically functional fragments, include variant forms of a variant heavy chain and a variant light chain as described herein.

[00202] CDRs

[00203] The antigen binding proteins disclosed herein are polypeptides into which one or more CDRs are grafted, inserted and/or joined. An antigen binding protein can have 1, 2, 3, 4, 5 or 6 CDRs. An antigen binding protein thus can have, for example, one heavy chain CDR1 ("CDRH1"), and/or one heavy chain CDR2 ("CDRH2"), and/or one heavy chain CDR3 ("CDRH3"), and/or one light chain CDR1 ("CDRL1"), and/or one light chain CDR2 ("CDRL2"), and/or one light chain CDR3 ("CDRL3"). Some antigen binding proteins include both a CDRH3 and a CDRL3. Specific heavy and light chain CDRs are identified in Table 3A-B (anti-DNP) and Table 3C-D (anti-KLH), respectively.

[00204] Complementarity determining regions (CDRs) and framework regions (FR) of a given antibody may be identified using the system described by Kabat et al. in Sequences of Proteins of Immunological Interest, 5th Ed., US Dept. of Health and Human Services, PHS, NIH, NIH Publication no. 91-3242, 1991. Certain antibodies that are disclosed herein comprise one or more amino acid sequences that are identical or have substantial sequence identity to the amino acid sequences of one or more of the CDRs presented in Table 3A (anti-DNP CDRHs), Table 3B (anti-DNP CDRLs), Table 3C (anti-KLH CDRHs), and Table 3D (anti-KLH CDRLs).

Table 3A: Exemplary Anti-DNP CDRH Sequences

Contained in HC	Designation	Sequence	SEQ ID NO:
H1, H9	CDRH 1-1	HYYWS	**188**
H2, H3, H4, H5, H6, H10, H11, H12, H13, H14	CDRH 1-2	SYGMH	**189**
H7, H15,	CDRH 1-3	SYYWS	**190**

Contained in HC	Designation	Sequence	SEQ ID NO:
H8, H16	CDRH 1-4	SGGYYWS	**191**
H1, H9	CDRH 2-1	YIYYSGSTNYNPSLKS	**192**
H2, H3, H4, H5, H6, H10, H11, H12, H13, H14	CDRH 2-2	VIWYDGSNKYYADSVKG	**193**
H7, H15	CDRH 2-3	VIYYSGNTNSNPSLKS	**194**
H8, H16	CDRH 2-4	YIYYSRSTYYNPSLKS	**195**
H1, H9	CDRH 3-1	ARGDGYNYPDAFDI	**196**
H2, H10	CDRH 3-2	YNWNYGMDV	**197**
H3, H5, H11, H13	CDRH 3-3	YNFNYGMDV	**198**
H4, H6, H12, H14	CDRH 3-4	YNYNYGMDV	**199**
H7, H15	CDRH 3-5	TYYDSSGYYYRAFDI	**200**
H8, H16	CDRH 3-6	TGYSSGWYPFDY	**201**

Table 3B: Exemplary Anti-DNP CDRL Sequences

Contained in LC	Designation	Sequence	SEQ ID NO:
L1	CDRL 1-1	RASQGISNWLA	**202**
L2	CDRL 1-2	RASQGISRRLA	**203**
L3, L5	CDRL 1-3	RASQGIRNDLG	**204**
L4	CDRL 1-4	RASQGMSNYLA	**205**
L1, L2, L3, L5	CDRL 2-1	AASSLQS	**206**
L4	CDRL 2-2	AASTLQS	**207**
L1	CDRL 3-1	QQASSFPWT	**208**
L2	CDRL 3-2	QQANSFPFT	**209**
L3	CDRL 3-3	LQYNSYPWT	**210**
L4	CDRL 3-4	QKFNSAPFT	**211**
L5	CDRL 3-5	LQYNSSPWT	**212**

Table 3C: Exemplary Anti-KLH CDRH Sequences

Contained in HC	Designation	Sequence	SEQ ID NO:
H17, H21	CDRH 1-5	NYDMY	**213**
H18, H22	CDRH 1-6	SSSYFWG	**214**
H19, H20, H23, H24	CDRH 1-7	GYHMH	**215**
H17, H21	CDRH 2-5	AIGTAGDTYYPGSVKG	**216**
H18, H22	CDRH 2-6	SIYYSGNTFYNPSLKS	**217**
H19, H20, H23, H24	CDRH 2-7	WINPNSGGTNYAQKFQG	**218**
H17, H21	CDRH 3-7	EKSSTSAFDY	**219**
H18, H22	CDRH 3-8	QGGIAARTGYWYFDL	**220**
H19, H20, H23, H24	CDRH 3-9	DRGSYYWFDP	**221**

Table 3D: Exemplary Anti-KLH CDRL Sequences

Contained in LC	Designation	Sequence	SEQ ID NO:
L6	CDRL 1-5	QAGQDIRNYLN	**222**
L7	CDRL 1-6	RASQNISTNYLA	**223**
L8	CDRL 1-7	RASQQISTNYLA	**224**
L9, L10	CDRL 1-8	RASQGIRNDLG	**204**
L6	CDRL 2-3	DASNLET	**225**
L7, L8	CDRL 2-4	GASSRAT	**226**
L9, L10	CDRL 2-5	AASSLQS	**206**
L6	CDRL 3-6	QQYDNLT	**227**
L7	CDRL 3-7	QQFGRSPRCS	**228**
L8	CDRL 3-8	QQFGRSPRSS	**229**
L9, L10	CDRL 3-9	LQHNSYPLT	**230**

[00205] The structure and properties of CDRs within a naturally occurring antibody have been described, *supra*. Briefly, in a traditional antibody, the CDRs are embedded within a framework in the heavy and light chain variable region where they constitute the regions responsible for antigen binding and recognition. A variable region comprises at least three heavy or light chain CDRs, *see, supra* (Kabat *et al.*, 1991, *Sequences of Proteins of Immunological Interest*, Public Health Service N.I.H., Bethesda, MD; *see* also Chothia and Lesk, 1987, *J. Mol. Biol.* 196:901-917; Chothia *et al.*, 1989, *Nature* 342: 877-883), within a framework region (designated framework regions 1-4, FR1, FR2, FR3, and FR4, by Kabat *et al.*, 1991, *supra*; *see* also Chothia and Lesk, 1987, *supra*). The CDRs provided herein, however, may not only be used to define the antigen binding domain of a traditional antibody structure, but may be embedded in a variety of other polypeptide structures, as described herein.

[00206] Some embodiments of the isolated antigen binding protein comprise an anti-DNP antibody or antibody fragment, comprising an immunoglobulin heavy

chain variable region and an immunoglobulin light chain variable region. The heavy chain variable region comprise three complementarity determining regions designated CDRHl, CDRH2 and CDRH3, and/or the light chain variable region comprises three CDRs designated CDRLl, CDRL2 and CDRL3, wherein:

[00207] (a) CDRHl has the amino acid sequence of SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, or SEQ ID NO:191; and/or

[00208] (b) CDRH2 has the amino acid sequence of SEQ ID NO:192, SEQ ID NO:193, SEQ ID NO:194, or SEQ ID NO:195; and/or

[00209] (c) CDRH3 has the amino acid sequence of SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, or SEQ ID NO:201; and/or

[00210] (d) CDRL1 has the amino acid sequence of SEQ ID NO:202, SEQ ID NO:203, SEQ ID NO:204, or SEQ ID NO:205; and/or

[00211] (e) CDRL2 has the amino acid sequence of SEQ ID NO:206 or SEQ ID NO:207; and/or

[00212] (f) CDRL3 has the amino acid sequence of SEQ ID NO:208, SEQ ID NO:209, SEQ ID NO:210, SEQ ID NO:211, or SEQ ID NO:212.

[00213] In other aspects, the CDRs provided are (A) a CDRH selected from (i) a CDRH1 selected from SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, and SEQ ID NO:191; (ii) a CDRH2 selected from SEQ ID NO:192, SEQ ID NO:193, SEQ ID NO:194, and SEQ ID NO:195; (iii) a CDRH3 selected from SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, and SEQ ID NO:201; and (iv) a CDRH of (i), (ii) and (iii) that contains one or more amino acid substitutions, deletions or insertions of no more than five, four, three, two, or one amino acids; (B) a CDRL selected from (i) a CDRL1 selected from SEQ ID NO:202, SEQ ID NO:203, SEQ ID NO:204, and SEQ ID NO:205; (ii) a CDRL2 selected from SEQ ID NO:206 and SEQ ID NO:207; (iii) a CDRL3 selected from

SEQ ID NO:208, SEQ ID NO:209, SEQ ID NO:210, SEQ ID NO:211, and SEQ ID NO:212; and (iv) a CDRL of (i), (ii) and (iii) that contains one or more amino acid substitutions, deletions or insertions of no more than five, four, three, two, or one amino acids amino acids.

[00214] Some embodiments of the isolated antigen binding protein comprise an anti-KLH antibody or antibody fragment, comprising an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region. The heavy chain variable region comprise three complementarity determining regions designated CDRHl, CDRH2 and CDRH3, and/or the light chain variable region comprises three CDRs designated CDRLl, CDRL2 and CDRL3, wherein:

[00215] (a) CDRHl has the amino acid sequence of SEQ ID NO:213, SEQ ID NO:214, or SEQ ID NO:215; and/or

[00216] (b) CDRH2 has the amino acid sequence of SEQ ID NO:216, SEQ ID NO:217, or SEQ ID NO:218; and/or

[00217] (c) CDRH3 has the amino acid sequence of SEQ ID NO:219, SEQ ID NO:220, or SEQ ID NO:221; and/or

[00218] (d) CDRL1 has the amino acid sequence of SEQ ID NO:204, SEQ ID NO:222, SEQ ID NO:223, or SEQ ID NO:224; and/or

[00219] (e) CDRL2 has the amino acid sequence of SEQ ID NO:206, SEQ ID NO:225, or SEQ ID NO:226; and/or

[00220] (f) CDRL3 has the amino acid sequence of SEQ ID NO:227, SEQ ID NO:228, SEQ ID NO:229, or SEQ ID NO:230.

[00221] In other aspects, the CDRs provided are (A) a CDRH selected from (i) a CDRH1 selected from SEQ ID NO:213, SEQ ID NO:214, and SEQ ID NO:215; (ii) a CDRH2 selected from SEQ ID NO:216, SEQ ID NO:217, and SEQ ID NO:218; (iii) a CDRH3 selected from SEQ ID NO:219, SEQ ID NO:220, and SEQ ID

NO:221; and (iv) a CDRH of (i), (ii) and (iii) that contains one or more amino acid substitutions, deletions or insertions of no more than five, four, three, two, or one amino acids; (B) a CDRL selected from (i) a CDRL1 selected from SEQ ID NO:204, SEQ ID NO:222, SEQ ID NO:223, and SEQ ID NO:224; (ii) a CDRL2 selected from SEQ ID NO:206, SEQ ID NO:225, and SEQ ID NO:226; (iii) a CDRL3 selected from SEQ ID NO:227, SEQ ID NO:228, SEQ ID NO:229, and SEQ ID NO:230; and (iv) a CDRL of (i), (ii) and (iii) that contains one or more amino acid substitutions, deletions or insertions of no more than five, four, three, two, or one amino acids amino acids.

[00222] In another aspect, an antigen binding protein includes 1, 2, 3, 4, 5, or 6 variant forms of the CDRs listed in Table 3A and Table 3B, each having at least 80%, at least 85%, at least 90% or at least 95% sequence identity to a CDR sequence listed in Table 3A and Table 3B. Some antigen binding proteins include 1, 2, 3, 4, 5, or 6 of the CDRs listed in Table 3A and Table 3B, each differing by no more than 1, 2, 3, 4 or 5 amino acids from the CDRs listed in these tables.

[00223] In another aspect, an antigen binding protein includes 1, 2, 3, 4, 5, or 6 variant forms of the CDRs listed in Table 3C and Table 3D, each having at least 80%, at least 85%, at least 90% or at least 95% sequence identity to a CDR sequence listed in Table 3C and Table 3D. Some antigen binding proteins include 1, 2, 3, 4, 5, or 6 of the CDRs listed in Table 3C and Table 3D, each differing by no more than 1, 2, 3, 4 or 5 amino acids from the CDRs listed in these tables.

[00224] In yet another aspect, the CDRs disclosed herein include consensus sequences derived from groups of related monoclonal antibodies. As described herein, a "consensus sequence" refers to amino acid sequences having conserved amino acids common among a number of sequences and variable amino acids that vary within a given amino acid sequences. The CDR consensus sequences provided include CDRs corresponding to each of CDRH1, CDRH2, CDRH3, CDRL1, CDRL2 and CDRL3.

[00225] Antibody-antigen interactions can be characterized by the association rate constant in $M^{-1}s^{-1}$ (k_a), or the dissociation rate constant in s^{-1} (k_d), or alternatively the dissociation equilibrium constant in M (K_D).

[00226] The present invention provides a variety of antigen binding proteins, including but not limited to antibodies that specifically bind DNP or KLH, respectively, that exhibit desirable characteristics such as binding affinity as measured by K_D (dissociation equilibrium constant) for DNP or KLH, respectively, in the range of 10^{-9} M or lower, ranging down to 10^{-12} M or lower, or avidity as measured by k_d (dissociation rate constant) for DNP or KLH, respectively, in the range of 10^{-4} s^{-1} or lower, or ranging down to 10^{-10} s^{-1} or lower. (See, Example 12 herein).

[00227] In some embodiments, the antigen binding proteins (e.g., antibodies or antibody fragments) exhibit desirable characteristics such as binding avidity as measured by k_d (dissociation rate constant) for DNP or KLH, respectively, of about 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10} s^{-1} or lower (lower values indicating higher binding avidity), and/or binding affinity as measured by K_D (dissociation equilibrium constant) for DNP or KLH, respectively, of about 10^{-9}, 10^{-10}, 10^{-11}, 10^{-12}, 10^{-13}, 10^{-14}, 10^{-15}, 10^{-16} M or lower (lower values indicating higher binding affinity). Association rate constants, dissociation rate constants, or dissociation equilibrium constants may be readily determined using kinetic analysis techniques such as surface plasmon resonance (BIAcore®; e.g., Fischer et al., A peptide-immunoglobulin-conjugate, WO 2007/045463 A1, Example 10, which is incorporated herein by reference in its entirety), or KinExA using general procedures outlined by the manufacturer or other methods known in the art. The kinetic data obtained by BIAcore® or KinExA may be analyzed by methods described by the manufacturer.

[00228] In some embodiments, the antibody comprises all three light chain CDRs, all three heavy chain CDRs, or all six CDRs. In some exemplary embodiments, two light chain CDRs from an antibody may be combined with a third light chain CDR

from a different antibody. Alternatively, a CDRL1 from one antibody can be combined with a CDRL2 from a different antibody and a CDRL3 from yet another antibody, particularly where the CDRs are highly homologous. Similarly, two heavy chain CDRs from an antibody may be combined with a third heavy chain CDR from a different antibody; or a CDRH1 from one antibody can be combined with a CDRH2 from a different antibody and a CDRH3 from yet another antibody, particularly where the CDRs are highly homologous.

[00229] Thus, the invention provides a variety of compositions comprising one, two, and/or three CDRs of a heavy chain variable region and/or a light chain variable region of an antibody including modifications or derivatives thereof. Such compositions may be generated by techniques described herein or known in the art.

[00230] In some embodiments, the antigen binding protein (including antibodies and antibody fragments) can be useful as a therapeutic molecule which can be used singularly or in combination with other therapeutics to achieve the desired effects. In such embodiments, the inventive antigen binding protein (including antibodies and antibody fragments) further comprises one to twenty-four, one to sixteen, one to eight, or one to four, pharmacologically active chemical moieties conjugated thereto, whether a small molecule or a polypeptide. The pharmacologically active small molecule or polypeptide chemical moieties can be conjugated at or via the N-terminal or C-terminal residue of the antigen binding protein immunoglobulin monomers (e.g., LC or HC monomers), chemical reactions known in the art and further described herein. Alternatively encompassed by the invention, is conjugation of the pharmacologically active chemical moiety, or moieties, at or via functional groups on one or more side chains of the amino acid residue(s) within the primary chain of the inventive antigen binding protein. Useful methods and internal conjugation sites (e.g., particular cysteine residues) within immunoglobulin chains are known in the art (e.g., Gegg et al., Modified Fc Molecules, published in WO 2007/022070 and US 20070269369, which are incorporated herein by reference in their entireties).

[00231] In other embodiments of the invention, in which the pharmacologically active chemical moiety is a polypeptide, a recombinant fusion protein can be produced with the pharmacologically active polypeptide being inserted in the primary amino acid sequence of the of the immunoglobulin heavy chain within an internal loop of the Fc domain of the immunoglobulin heavy chain, instead of at the N- and/or C-terminus, as further described in the Examples herein and in the art (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).

[00232] "Conjugated" means that the pharmacologically active chemical moieties are covalently linked, or bound, directly to an amino acid residue of the antigen binding protein, or optionally, to a peptidyl or non-peptidyl linker moiety that is covalently linked to the amino acid residue of the antigen binding protein.

[00233] As stated above, some embodiments of the inventive compositions involve at least one pharmacologically active polypeptide moiety conjugated to the pharmacologically inactive antigen binding protein of the invention, for example constituting a recombinant fusion protein of the pharmacologically active polypeptide moiety conjugated to the pharmacologically inactive antigen binding protein of the invention. The term "pharmacologically active" means that a substance so described is determined to have activity that affects a medical parameter (e.g., blood pressure, blood cell count, cholesterol level, pain perception) or disease state (e.g., cancer, autoimmune disorders, chronic pain). Conversely, the term "pharmacologically inactive" means that no activity affecting a medical parameter or disease state can be determined for that substance. Thus, pharmacologically active peptides or proteins comprise agonistic or mimetic and antagonistic peptides as defined below. The present invention encompasses the use of any pharmacologically active protein, which has an amino acid sequence ranging from about 5 to about 80 amino acid residues in length, and which is amenable to recombinant expression. In some useful embodiments of the invention, the

pharmacologically active protein is modified in one or more ways relative to a native sequence of interest, , including amino acid additions or insertions, amino acid deletions, peptide truncations, amino acid substitutions, or chemical derivatization of amino acid residues (accomplished by known chemical techniques), so long as the requisite bioactivity is maintained.

[00234] 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., ShK or OSK1 toxin peptides, or peptide analogs thereof. 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.

[00235] 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 or a G-Protein Coupled Receptor (GPCR)).

[00236] Examples of pharmacologically active proteins that can be used within the present invention include, but are not limited to, a toxin peptide (e.g., OSK1 or an OSK1 peptide analog; ShK or an ShK peptide analog), an IL-6 binding peptide, a CGRP peptide antagonist, a bradykinin B1 receptor peptide antagonist, a parathyroid hormone (PTH) agonist peptide, a parathyroid hormone (PTH) antagonist peptide, an ang-1 binding peptide, an ang-2 binding peptide, a myostatin binding peptide, an erythropoietin-mimetic (EPO-mimetic) peptide, a thrombopoietin-mimetic (TPO-mimetic) peptide (e.g., AMP2 or AMP5), a nerve growth factor (NGF) binding peptide, a B cell activating factor (BAFF) binding peptide, and a glucagon-like peptide (GLP)-1 or a peptide mimetic therof or GLP-2 or a peptide mimetic thereof.

Glucagon-like peptide 1 (GLP-1) and the related peptide glucagon are produced via differential processing of proglucagon and have opposing biological activities.

Proglucagon itself is produced in α-cells of the pancreas and in the enteroendocrine L-cells, which are located primarily in the distal small intestine and colon. In the pancreas, glucagon is selectively cleaved from proglucagon. In the intestine, in contrast, proglucagon is processed to form GLP-1 and glucagon-like peptide 2 (GLP-2), which correspond to amino acid residues 78-107 and 126-158 of proglucagon, respectively (see, e.g., Irwin and Wong, 1995, *Mol. Endocrinol.* 9:267-277 and Bell *et al.*, 1983, *Nature* 304:368-371). By convention, the numbering of the amino acids of GLP-1 is based on the GLP-1 (1-37) formed from cleavage of proglucagon. The biologically active forms are generated from further processing of this peptide, which, in one numbering convention, yields GLP-1 (7-37)-OH and GLP-1 (7-36)-NH_2. Both GLP-1 (7-37)-OH (or simply GLP-1 (7-37)) and GLP-1 (7-36)-NH_2 have the same activities. For convenience, the term "GLP-1", is used to refer to both of these forms. The first amino acid of these processed peptides is His7 in this numbering convention. Another numbering convention recognized in the art, however, assumes that the numbering of the processed peptide begins with His as position 1 rather than position 7. Thus, in this numbering scheme, GLP-1 (1-31) is the same as GLP-1(7-37), and GLP-1(1-30) is the same as GLP-1 (7-36). Examples of GLP-1 mimetic polypeptide sequences include:

HGEGTFTSDQSSYLEGQAAKEFIAWLVKGRG// (SEQ ID NO:290);
HGEGTFTSDQSSYLEGQAAKEFIAWLQKGRG// (SEQ ID NO:291);
HGEGTFTSDVSSYQEGQAAKEFIAWLVKGRG// (SEQ ID NO:292);
HGEGTFTSDVSSYLEGQAAKEFIAQLVKGRG// (SEQ ID NO:293);
HGEGTFTSDVSSYLEGQAAKEFIAQLQKGRG// (SEQ ID NO:294);
HGEGTFTSDVSSYLEGQAAKEFIAWLQKGRG// (SEQ ID NO:295);
HNETTFTSDVSSYLEGQAAKEFIAWLVKGRG// (SEQ ID NO:296)
HGEGTFTSDVSSYLENQTAKEFIAWLVKGRG// (SEQ ID NO:297);
HGEGTFTSDVSSYLEGNATKEFIAWLVKGRG// (SEQ ID NO:298);
HGEGTFTSDVSSYLEGQAAKEFIAWLVNGTG// (SEQ ID NO:299);
HGEGTFTSDVSSYLEGQAAKEFIAWLVKNRT// (SEQ ID NO:300);
HGEGTFTSDVSSYLEGQAAKEFIAWLVKGRNGT// (SEQ ID NO:301);
HGEGTFTSDVSSYLEGQAAKEFIAWLVKGRGGTGNGT// (SEQ ID NO:302);

and
HGEGTFTSDVSSYLEGQAAKEFIAWLVKGRGGSGNGT// (SEQ ID NO:303).

[00237] Human GLP-2 and GLP-2-mimetic analogs are also known in the art. (See, e.g., Prasad et al., Glucagonlike peptide-2 analogue enhances intestinal mucosal mass after ischemia and reperfusion, J. Pediatr. Surg. 2000 Feb;35(2):357-59 (2000); Yusta et al., Glucagon-like peptide-2 receptor activation engages bad and glycogen synthase kinase-3 in a protein kinase A-dependent manner and prevents apoptosis following inhibition of phosphatidylinositol 3-kinase, J. Biol. Chem. 277(28):24896-906 (2002)).

[00238] "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)).

[00239] 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 of these has been determined by NMR spectroscopy, illustrating their compact structure and verifying conservation of their family fold. (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)). Examples of pharmacologically active toxin peptides for which the practice of the present invention can be useful include, but are not limited to ShK, OSK1, charybdotoxin (ChTx), kaliotoxin1 KTX1), or maurotoxin, or toxin peptide analogs of any of these, modified from the native sequences at one or more amino acid residues. 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, and published as US20090291885 on November 26, 2009), Sullivan et al., WO 2008/088422; Lebrun et al., U.S. Patent No. 6,689,749, and Sullivan et al., Selective and Potent Peptide Inhibitors of Kv1.3, U.S. Provisional Application No. 61/210,594, filed March 20, 2009, which are each incorporated by reference in their entireties.

[00240] 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). 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. "Toxin peptide analogs", such as, but not limited to, an OSK1 peptide analog, ShK peptide analog, or ChTx 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.

[00241] A "CGRP peptide antagonist" is a peptide that preferentially binds the $CGRP_1$ receptor, such as, but not limited to, a CGRP peptide analog, and that

antagonizes, blocks, decreases, reduces, impedes, or inhibits $CGRP_1$ receptor activation by full length native human αCGRP or βCGRP under physiological conditions of temperature, pH, and ionic strength. CGRP peptide antagonists include full and partial antagonists. Such antagonist activity can be detected by known *in vitro* methods or *in vivo* functional assay methods. (See, e.g., Smith et al., Modifications to the N-terminus but not the C-terminus of calcitonin gene-related peptide(8-37) produce antagonists with increased affinity, J. Med. Chem., 46:2427-2435 (2003)). Examples of useful CGRP peptide antagonists are disclosed in Gegg et al., CGRP peptide antagonists and conjugates, WO 2007/048026 A2 and U.S. Serial No. 11/584,177, filed on October 19, 2006, published as US 2008/0020978 A1, which is incorporated herein by reference in its entirety.

[00242] The terms "parathyroid hormone (PTH) agonist" and "PTH agonist"refer to a molecule that binds to PTH-1 or PTH-2 receptor and increases or decreases one or more PTH activity assay parameters as does full-length native human parathyroid hormone. Examples of useful PTH agonist peptides are disclosed in Table 1 of U.S. Patent No. 6,756,480, titled Modulators of receptors for parathyroid hormone and parathyroid hormone-related protein, which is incorporated herein by reference in its entirety. An exemplary PTH activity assay is disclosed in Example 1 of U.S. Patent No. 6,756,480.

[00243] The term "parathyroid hormone (PTH) antagonist" refers to a molecule that binds to PTH-1 or PTH-2 receptor and blocks or prevents the normal effect on those parameters by full length native human parathyroid hormone. Examples of useful PTH antagonist peptides are disclosed in Table 2 of U.S. Patent No. 6,756,480, which is incorporated herein by reference in its entirety. An exemplary PTH activity assay is disclosed in Example 2 of U.S. Patent No. 6,756,480.

[00244] The terms "bradykinin B1 receptor antagonist peptide" and "bradykinin B1 receptor peptide antagonist" mean a peptide with antagonist activity with respect to human bradykinin B1 receptor (hB1). Useful bradykinin B1 receptor antagonist peptides can be identified or derived as described in Ng et al., Antagonist of the

bradykinin B1 receptor, US 2005/0215470 A1, published September 29, 2005, which issued as U.S. Patent No. 7,605,120; U.S. Patent Nos. 5,834,431 or 5,849,863. An exemplary B1 receptor activity assays are disclosed in Examples 6-8 of US 2005/0215470 A1.

[00245] The terms "thrombopoietin (TPO)-mimetic peptide" and "TPO-mimetic peptide" refer to peptides that can be identified or derived as described in Cwirla et al. (1997), Science 276: 1696-9 , U.S. Pat. Nos. 5,869,451 and 5,932,946, which are incorporated by reference in their entireties; U.S. Pat. App. No. 2003/0176352, published Sept. 18, 2003, which is incorporated by reference in its entirety; WO 03/031589, published April 17, 2003; WO 00/24770, published May 4, 2000; and any peptides appearing in Table 5 of published application US 2006/0140934 (U.S. Serial No. 11/234,731, filed September 23, 2005, titled Modified Fc Molecules, which is incorporated herein by reference in its entirety). Those of ordinary skill in the art appreciate that each of these references enables one to select different peptides than actually disclosed therein by following the disclosed procedures with different peptide libraries.

[00246] The terms "EPO-mimetic peptide" and "erythropoietin-mimetic peptide" refers to peptides that can be identified or derived as described in Wrighton et al. (1996), Science 273: 458-63, and Naranda et al. (1999), Proc. Natl. Acad. Sci. USA 96: 7569-74, both of which are incorporated herein by reference in their entireties. Useful EPO-mimetic peptides include EPO-mimetic peptides listed in Table 5 of published U.S. patent application US 2007/0269369 A1and in U.S. Pat. No. 6,660,843, which are both hereby incorporated by reference in their entireties.

[00247] The term "ang-2-binding peptide" comprises peptides that can be identified or derived as described in U.S. Pat. App. No. 2003/0229023, published Dec. 11, 2003; WO 03/057134, published July, 17, 2003; U.S. 2003/0236193, published Dec. 25, 2003 (each of which is incorporated herein by reference in its entirety); and any peptides appearing in Table 6 of published application US 2006/0140934 (U.S. Serial No. 11/234,731, filed September 23, 2005, titled

Modified Fc Molecules, which is incorporated herein by reference in its entirety). Those of ordinary skill in the art appreciate that each of these references enables one to select different peptides than actually disclosed therein by following the disclosed procedures with different peptide libraries.

[00248] The terms "nerve growth factor (NGF) binding peptide" and "NGF-binding peptide" comprise peptides that can be identified or derived as described in WO 04/026329, published April 1, 2004 and any peptides identified in Table 7 of published application US 2006/0140934 (U.S. Serial No. 11/234,731, filed September 23, 2005, titled Modified Fc Molecules, which is incorporated herein by reference in its entirety). Those of ordinary skill in the art appreciate that this reference enables one to select different peptides than actually disclosed therein by following the disclosed procedures with different peptide libraries.

[00249] The term "myostatin-binding peptide" comprises peptides that can be identified or derived as described in U.S. Ser. No. 10/742,379, filed December 19, 2003, which is incorporated herein by reference in its entirety, and peptides appearing in Table 8 of published application US 2006/0140934 (U.S. Serial No. 11/234,731, filed September 23, 2005, titled Modified Fc Molecules, which is incorporated herein by reference in its entirety). Those of ordinary skill in the art appreciate that each of these references enables one to select different peptides than actually disclosed therein by following the disclosed procedures with different peptide libraries.

[00250] The terms "BAFF-antagonist peptide" and "BAFF binding peptide" comprise peptides that can be identified or derived as described in U.S. Pat. Appln. No. 2003/0195156 A1, which is incorporated herein by reference in its entirety and those peptides appearing in Table 9 of published application US 2006/0140934 (U.S. Serial No. 11/234,731, filed September 23, 2005, titled Modified Fc Molecules, which is incorporated herein by reference in its entirety). Those of ordinary skill in the art appreciate that the foregoing references enable one to select different peptides

than actually disclosed therein by following the disclosed procedures with different peptide libraries.

[00251] The foregoing are intended merely as non-limiting examples of the pharmacologically active polypeptides that can be usefully conjugated or fused to the inventive antigen binding proteins (including antibodies and antibody fragements). Any include pharmacologically active polypeptide moiety can be used within the scope of the invention, including a polypeptide having a so-called avimer structure (see, e.g., Kolkman et al., Novel Proteins with Targeted Binding, US 2005/0089932; Baker et al., IL-6 Binding Proteins, US 2008/0281076; Stemmer et al., Protein Scaffolds and Uses Thereof, US 2006/0223114 and US 2006/0234299).

[00252] Useful preclinical animal models are known in the art for use in validating a drug in a therapeutic indication of interest (e.g., an adoptive-transfer model of periodontal disease by Valverde et al., J. Bone Mineral Res. 19:155 (2004); an ultrasonic perivascular Doppler flow meter-based animal model of arterial thrombosis in Gruner et al., Blood 105:1492-99 (2005); pulmonary thromboembolism model, aorta occlusion model, and murine stroke model in Braun et al., WO 2009/115609 A1). For example, an adoptive transfer experimental autoimmune encephalomyelitis (AT-EAE) model of multiple sclerosis has been described for investigations concerning immune diseases, such as multiple sclerosis (Beeton et al., J. Immunol. 166:936 (2001); Beeton et al., PNAS 98:13942 (2001); Sullivan et al., Example 45 of WO 2008/088422 A2, incorporated herein by reference in its entirety). In the AT-EAE model, significantly reduced disease severity and increased survival are expected for animals treated with an effective amount of the inventive pharmaceutical composition, while untreated animals are expected to develop severe disease and/or mortality. For running the AT-EAE model, the encephalomyelogenic CD4+ rat T cell line, PAS, specific for myelin-basic protein (MBP) originated from Dr. Evelyne Beraud. The maintenance of these cells in vitro and their use in the AT-EAE model has been described earlier [Beeton et al. (2001) PNAS 98, 13942]. PAS T cells are 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) involves incubating the cells for 2 days with 10 μg/ml MBP and 15 $\times 10^6$/ml syngeneic irradiated (3500 rad) thymocytes. On day 2 after in vitro activation, 10-15 $\times 10^6$ viable PAS T cells are 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) or test pharmaceutical composition are given from days –1 to 3, where day –1 represent 1 day prior to injection of PAS T cells (day 0). In vehicle treated rats, acute EAE is expected to develop 4 to 5 days after injection of PAS T cells. Typically, serum is collected by tail vein bleeding at day 4 and by cardiac puncture at day 8 (end of the study) for analysis of levels of inhibitor. Rats are typically weighed on days –1, 4, 6, and 8. Animals may be 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 are evaluated as the total score of the degree of paresis of each limb and tail. Clinical scoring: 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 5.0 are typically euthanized.

[00253] Production of Antibody Embodiments of the Antigen Binding Proteins

[00254] Polyclonal antibodies. Polyclonal antibodies are preferably raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. Alternatively, antigen may be injected directly into the animal's lymph node (see Kilpatrick et al., Hybridoma, 16:381-389, 1997). An improved antibody response may be obtained by conjugating the relevant antigen to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride or other agents known in the art.

[00255] Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining, e.g., 100 μg of the protein or conjugate (for mice) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later, the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. At 7-14 days post-booster injection, the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

[00256] Monoclonal Antibodies. The inventive antigen binding proteins or antigen binding proteins that are provided include monoclonal antibodies that bind to DNP or KLH, respectively. Monoclonal antibodies may be produced using any technique known in the art, e.g., by immortalizing spleen cells harvested from the transgenic animal after completion of the immunization schedule. The spleen cells can be immortalized using any technique known in the art, e.g., by fusing them with myeloma cells to produce hybridomas. For example, monoclonal antibodies may be made using the hybridoma method first described by Kohler et al., Nature, 256:495 (1975), or may be made by recombinant DNA methods (e.g., Cabilly et al., Methods of producing immunoglobulins, vectors and transformed host cells for use therein, US Patent No. 6,331,415), including methods, such as the "split DHFR" method, that facilitate the generally equimolar production of light and heavy chains, optionally using mammalian cell lines (e.g., CHO cells) that can glycosylate the antibody (See, e.g., Page, Antibody production, EP0481790 A2 and US Patent No. 5,545,403).

[00257] In the hybridoma method, a mouse or other appropriate host mammal, such as rats, hamster or macaque monkey, is immunized as herein described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized in vitro. Lymphocytes then are fused with myeloma cells using a

suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, pp.59-103 (Academic Press, 1986)).

[00258] In some instances, a hybridoma cell line is produced by immunizing a transgenic animal having human immunoglobulin sequences with a DNP or KLH immunogen; harvesting spleen cells from the immunized animal; fusing the harvested spleen cells to a myeloma cell line, thereby generating hybridoma cells; establishing hybridoma cell lines from the hybridoma cells, and identifying a hybridoma cell line that produces an antibody that binds DNP or KLH, respectively. Such hybridoma cell lines, and monoclonal antibodies produced by them, are aspects of the present invention.

[00259] The present invention also encompasses a hybridoma that produces the inventive antigen binding protein that is a monoclonal antibody. Accordingly, the present invention is also directed to a method, comprising:

[00260] (a) culturing the hybridoma in a culture medium under conditions permitting expression of the antigen binding protein by thehybridoma; and

[00261] (b) recovering the antigen binding protein from the culture medium, which can be accomplished by known antibody purification techniques, such as but not limited to, monoclonal antibody purification techniques disclosed in Example 1 herein.

[00262] The hybridoma cells, once prepared, are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

[00263] Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, J. Immunol., 133: 3001 (1984) ;Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)). Myeloma cells for use in hybridoma-producing fusion procedures preferably are non-antibody-producing, have high fusion efficiency, and enzyme deficiencies that render them incapable of growing in certain selective media which support the growth of only the desired fused cells (hybridomas). Examples of suitable cell lines for use in mouse fusions include Sp-20, P3-X63/Ag8, P3-X63-Ag8.653, NS1/1.Ag 4 1, Sp210-Ag14, FO, NSO/U, MPC-11, MPC11-X45-GTG 1.7 and S194/5XXO Bul; examples of cell lines used in rat fusions include R210.RCY3, Y3-Ag 1.2.3, IR983F and 4B210. Other cell lines useful for cell fusions are U-266, GM1500-GRG2, LICR-LON-HMy2 and UC729-6.

[00264] Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). The binding affinity of the monoclonal antibody can, for example, be determined by BIAcore® or Scatchard analysis (Munson et al., Anal. Biochem., 107:220 (1980); Fischer et al., A peptide-immunoglobulin-conjugate, WO 2007/045463 A1, Example 10, which is incorporated herein by reference in its entirety).

[00265] After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, Monoclonal Antibodies: Principles and Practice, pp.59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown in vivo as ascites tumors in an animal.

[00266] Hybridomas or mAbs may be further screened to identify mAbs with particular properties, such as the ability to inhibit K^{1+} flux though Kv1.x channels. Examples of such screens are provided in the examples below. The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, affinity chromatography, or any other suitable purification technique known in the art.

[00267] Recombinant Production of Antibodies. The invention provides isolated nucleic acids encoding any of the antibodies (polyclonal and monoclonal), including antibody fragments, of the invention described herein, optionally operably linked to control sequences recognized by a host cell, vectors and host cells comprising the nucleic acids, and recombinant techniques for the production of the antibodies, which may comprise culturing the host cell so that the nucleic acid is expressed and, optionally, recovering the antibody from the host cell culture or culture medium. Similar materials and methods apply to production of polypeptide-based antigen binding proteins.

[00268] Relevant amino acid sequences from an immunoglobulin or polypeptide of interest may be determined by direct protein sequencing, and suitable encoding nucleotide sequences can be designed according to a universal codon table. Alternatively, genomic or cDNA encoding the monoclonal antibodies may be isolated and sequenced from cells producing such antibodies using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies).

[00269] Cloning of DNA is carried out using standard techniques (see, e.g., Sambrook et al. (1989) Molecular Cloning: A Laboratory Guide, Vols 1-3, Cold Spring Harbor Press, which is incorporated herein by reference). For example, a cDNA library may be constructed by reverse transcription of polyA+ mRNA,

preferably membrane-associated mRNA, and the library screened using probes specific for human immunoglobulin polypeptide gene sequences. In one embodiment, however, the polymerase chain reaction (PCR) is used to amplify cDNAs (or portions of full-length cDNAs) encoding an immunoglobulin gene segment of interest (e.g., a light or heavy chain variable segment). The amplified sequences can be readily cloned into any suitable vector, e.g., expression vectors, minigene vectors, or phage display vectors. It will be appreciated that the particular method of cloning used is not critical, so long as it is possible to determine the sequence of some portion of the immunoglobulin polypeptide of interest.

[00270] One source for antibody nucleic acids is a hybridoma produced by obtaining a B cell from an animal immunized with the antigen of interest and fusing it to an immortal cell. Alternatively, nucleic acid can be isolated from B cells (or whole spleen) of the immunized animal. Yet another source of nucleic acids encoding antibodies is a library of such nucleic acids generated, for example, through phage display technology. Polynucleotides encoding peptides of interest, e.g., variable region peptides with desired binding characteristics, can be identified by standard techniques such as panning.

[00271] The sequence encoding an entire variable region of the immunoglobulin polypeptide may be determined; however, it will sometimes be adequate to sequence only a portion of a variable region, for example, the CDR-encoding portion. Sequencing is carried out using standard techniques (see, e.g., Sambrook et al. (1989) Molecular Cloning: A Laboratory Guide, Vols 1-3, Cold Spring Harbor Press, and Sanger, F. et al. (1977) Proc. Natl. Acad. Sci. USA 74: 5463-5467, which is incorporated herein by reference). By comparing the sequence of the cloned nucleic acid with published sequences of human immunoglobulin genes and cDNAs, one of skill will readily be able to determine, depending on the region sequenced, (i) the germline segment usage of the hybridoma immunoglobulin polypeptide (including the isotype of the heavy chain) and (ii) the sequence of the heavy and light chain variable regions, including sequences resulting from N-region addition and the process of somatic mutation. One source of immunoglobulin gene sequence

information is the National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, Md.

[00272] Isolated DNA can be operably linked to control sequences or placed into expression vectors, which are then transfected into host cells that do not otherwise produce immunoglobulin protein, to direct the synthesis of monoclonal antibodies in the recombinant host cells. Recombinant production of antibodies is well known in the art.

[00273] Nucleic acid is operably linked when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, operably linked means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

[00274] Many vectors are known in the art. Vector components may include one or more of the following: a signal sequence (that may, for example, direct secretion of the antibody; e.g., ATGGACATGAGGGTGCCCGCTCAGCTCCTGGGGCTCCTGCTGCTGTGGCT GAGAGGTGCGCGCTGT// SEQ ID NO:102, which encodes the VK-1 signal peptide sequence MDMRVPAQLLGLLLLWLRGARC// SEQ ID NO:103), an origin of replication, one or more selective marker genes (that may, for example, confer antibiotic or other drug resistance, complement auxotrophic deficiencies, or supply critical nutrients not available in the media), an enhancer element, a promoter, and a transcription termination sequence, all of which are well known in the art.

[00275] Cell, cell line, and cell culture are often used interchangeably and all such designations herein include progeny. Transformants and transformed cells include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included.

[00276] Exemplary host cells include prokaryote, yeast, or higher eukaryote cells. Prokaryotic host cells include eubacteria, such as Gram-negative or Gram-positive organisms, for example, *Enterobacteriaceae* such as Escherichia, *e.g.*, E. coli, Enterobacter, Erwinia, Klebsiella, Proteus, Salmonella, *e.g.*, Salmonella typhimurium, Serratia, *e.g.*, Serratia marcescans, and Shigella, as well as Bacillus such as B. subtilis and B. licheniformis, Pseudomonas, and Streptomyces. Eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for recombinant polypeptides or antibodies. Saccharomyces cerevisiae, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as Pichia, e.g. P. pastoris, Schizosaccharomyces pombe; Kluyveromyces, Yarrowia; Candida; Trichoderma reesia; Neurospora crassa; Schwanniomyces such as Schwanniomyces occidentalis; and filamentous fungi such as, *e.g.*, Neurospora, Penicillium, Tolypocladium, and Aspergillus hosts such as A. nidulans and A. niger.

[00277] Host cells for the expression of glycosylated antigen binding protein, including antibody, can be derived from multicellular organisms. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as Spodoptera frugiperda (caterpillar), Aedes aegypti (mosquito), Aedes albopictus (mosquito), Drosophila melanogaster (fruitfly), and Bombyx mori have been identified. A variety of viral strains for transfection of such cells are publicly

available, *e.g.*, the L-1 variant of Autographa californica NPV and the Bm-5 strain of Bombyx mori NPV.

[00278] Vertebrate host cells are also suitable hosts, and recombinant production of antigen binding protein (including antibody) from such cells has become routine procedure. Examples of useful mammalian host cell lines are Chinese hamster ovary cells, including CHOK1 cells (ATCC CCL61), DXB-11, DG-44, and Chinese hamster ovary cells/-DHFR (CHO, Urlaub et al., Proc. Natl. Acad. Sci. USA 77: 4216 (1980)); monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, [Graham et al., *J. Gen Virol. 36*: 59 (1977)]; baby hamster kidney cells (BHK, ATCC CCL 10); mouse sertoli cells (TM4, Mather, Biol. Reprod. 23: 243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human hepatoma cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., Annals N.Y Acad. Sci. 383: 44-68 (1982)); MRC 5 cells or FS4 cells; or mammalian myeloma cells.

[00279] Host cells are transformed or transfected with the above-described nucleic acids or vectors for production antigen binding proteins and are cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. In addition, novel vectors and transfected cell lines with multiple copies of transcription units separated by a selective marker are particularly useful for the expression of antigen binding proteins.

[00280] The host cells used to produce the antigen binding proteins of the invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable

for culturing the host cells. In addition, any of the media described in Ham et al., Meth. Enz. 58: 44 (1979), Barnes et al., Anal. Biochem. 102: 255 (1980), U.S. Patent Nos. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO90103430; WO 87/00195; or U.S. Patent Re. No. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as Gentamycin™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

[00281] Upon culturing the host cells, the antigen binding protein can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the antigen binding protein is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, is removed, for example, by centrifugation or ultrafiltration.

[00282] The antigen binding protein (e.g., an antibody or antibody fragment) can be purified using, for example, hydroxylapatite chromatography, cation or anion exchange chromatography, or preferably affinity chromatography, using the antigen of interest or protein A or protein G as an affinity ligand. Protein A can be used to purify proteins that include polypeptides are based on human γ1, γ2, or γ4 heavy chains (Lindmark et al., J. Immunol. Meth. *62*: 1-13 (1983)). Protein G is recommended for all mouse isotypes and for human γ3 (Guss et al., EMBO J. 5: 15671575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the protein

comprises a C_H 3 domain, the Bakerbond ABX™resin (J. T. Baker, Phillipsburg, N.J.) is useful for purification. Other techniques for protein purification such as ethanol precipitation, Reverse Phase HPLC, chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also possible depending on the antibody to be recovered.

[00283] Chimeric, Humanized and Human Engineered™ monoclonal antibodies. Chimeric monoclonal antibodies, in which the variable Ig domains of a rodent monoclonal antibody are fused to human constant Ig domains, can be generated using standard procedures known in the art (See Morrison, S. L., et al. (1984) Chimeric Human Antibody Molecules; Mouse Antigen Binding Domains with Human Constant Region Domains, Proc. Natl. Acad. Sci. USA 81, 6841-6855; and, Boulianne, G. L., et al, Nature 312, 643-646 . (1984)). A number of techniques have been described for humanizing or modifying antibody sequence to be more human-like, for example, by (1) grafting the non-human complementarity determining regions (CDRs) onto a human framework and constant region (a process referred to in the art as humanizing through "CDR grafting") or (2) transplanting the entire non-human variable domains, but "cloaking" them with a human-like surface by replacement of surface residues (a process referred to in the art as "veneering") or (3) modifying selected non-human amino acid residues to be more human, based on each residue's likelihood of participating in antigen-binding or antibody structure and its likelihood for immunogenicity. See, e.g., Jones et al., Nature 321:522 525 (1986); Morrison et al., Proc. Natl. Acad. Sci., U.S.A., 81:6851 6855 (1984); Morrison and Oi, Adv. Immunol., 44:65 92 (1988); Verhoeyer et al., Science 239:1534 1536 (1988); Padlan, Molec. Immun. 28:489 498 (1991); Padlan, Molec. Immunol. 31(3):169 217 (1994); and Kettleborough, C.A. et al., Protein Eng. 4(7):773 83 (1991); Co, M. S., et al. (1994), J. Immunol. 152, 2968-2976); Studnicka et al. Protein Engineering 7: 805-814 (1994); each of which is incorporated herein by reference in its entirety.

[00284] A number of techniques have been described for humanizing or modifying antibody sequence to be more human-like, for example, by (1) grafting the non-

human complementarity determining regions (CDRs) onto a human framework and constant region (a process referred to in the art as humanizing through "CDR grafting") or (2) transplanting the entire non-human variable domains, but "cloaking" them with a human-like surface by replacement of surface residues (a process referred to in the art as "veneering") or (3) modifying selected non-human amino acid residues to be more human, based on each residue's likelihood of participating in antigen-binding or antibody structure and its likelihood for immunogenicity. See, e.g., Jones et al., Nature 321:522 525 (1986); Morrison et al., Proc. Natl. Acad. Sci., U.S.A., 81:6851 6855 (1984); Morrison and Oi, Adv. Immunol., 44:65 92 (1988); Verhoeyer et al., Science 239:1534 1536 (1988); Padlan, Molec. Immun. 28:489 498 (1991); Padlan, Molec. Immunol. 31(3):169 217 (1994); and Kettleborough, C.A. et al., Protein Eng. 4(7):773 83 (1991); Co, M. S., et al. (1994), J. Immunol. 152, 2968-2976); Studnicka et al. Protein Engineering 7: 805-814 (1994); each of which is incorporated herein by reference in its entirety.

[00285] In one aspect, the CDRs of the light and heavy chain variable regions of the antibodies provided herein (see, Table 2A-B) are grafted to framework regions (FRs) from antibodies from the same, or a different, phylogenetic species. For example, the CDRs of the heavy chain variable regions (e.g., V_H1, V_H2, V_H3, V_H4, V_H5, V_H6, V_H7, V_H8, or V_H9) and/or light chain variable regions (e.g., V_L1, V_L2, V_L3, V_L4, V_L5, V_L6, V_L7, V_L8, or V_L9) can be grafted to consensus human FRs. To create consensus human FRs, FRs from several human heavy chain or light chain amino acid sequences may be aligned to identify a consensus amino acid sequence. In other embodiments, the FRs of a heavy chain or light chain disclosed herein are replaced with the FRs from a different heavy chain or light chain. In one aspect, rare amino acids in the FRs of the heavy and light chains of the antibody are not replaced, while the rest of the FR amino acids are replaced. A "rare amino acid" is a specific amino acid that is in a position in which this particular amino acid is not usually found in an FR. Alternatively, the grafted variable regions from the one heavy or light chain may be used with a constant region that is different from the constant

region of that particular heavy or light chain as disclosed herein. In other embodiments, the grafted variable regions are part of a single chain Fv antibody.

[00286] Antibodies can also be produced using transgenic animals that have no endogenous immunoglobulin production and are engineered to contain human immunoglobulin loci. For example, WO 98/24893 discloses transgenic animals having a human Ig locus wherein the animals do not produce functional endogenous immunoglobulins due to the inactivation of endogenous heavy and light chain loci. WO 91/10741 also discloses transgenic non-primate mammalian hosts capable of mounting an immune response to an immunogen, wherein the antibodies have primate constant and/or variable regions, and wherein the endogenous immunoglobulin encoding loci are substituted or inactivated. WO 96/30498 discloses the use of the Cre/Lox system to modify the immunoglobulin locus in a mammal, such as to replace all or a portion of the constant or variable region to form a modified antibody molecule. WO 94/02602 discloses non-human mammalian hosts having inactivated endogenous Ig loci and functional human Ig loci. U.S. Patent No. 5,939,598 discloses methods of making transgenic mice in which the mice lack endogenous heavy chains, and express an exogenous immunoglobulin locus comprising one or more xenogeneic constant regions.

[00287] Using a transgenic animal described above, an immune response can be produced to a selected antigenic molecule, and antibody producing cells can be removed from the animal and used to produce hybridomas that secrete human-derived monoclonal antibodies. Immunization protocols, adjuvants, and the like are known in the art, and are used in immunization of, for example, a transgenic mouse as described in WO 96/33735. The monoclonal antibodies can be tested for the ability to inhibit or neutralize the biological activity or physiological effect of the corresponding protein. See also Jakobovits et al., Proc. Natl. Acad. Sci. USA, 90:2551 (1993); Jakobovits et al., Nature, 362:255-258 (1993); Bruggermann et al., Year in Immuno., 7:33 (1993); Mendez et al., *Nat. Genet.* 15:146-156 (1997); and U.S. Pat. No. 5,591,669, U.S. Patent No. 5,589,369, U.S. Patent No. 5,545,807; and U.S Patent Application No. 20020199213. U.S. Patent Application No. and

20030092125 describes methods for biasing the immune response of an animal to the desired epitope. Human antibodies may also be generated by *in vitro* activated B cells (see U.S. Pat. Nos. 5,567,610 and 5,229,275).

[00288] Antibody production by phage display techniques

[00289] The development of technologies for making repertoires of recombinant human antibody genes, and the display of the encoded antibody fragments on the surface of filamentous bacteriophage, has provided another means for generating human-derived antibodies. Phage display is described in e.g., Dower et al., WO 91/17271, McCafferty et al., WO 92/01047, and Caton and Koprowski, Proc. Natl. Acad. Sci. USA, 87:6450-6454 (1990), each of which is incorporated herein by reference in its entirety. The antibodies produced by phage technology are usually produced as antigen binding fragments, e.g. Fv or Fab fragments, in bacteria and thus lack effector functions. Effector functions can be introduced by one of two strategies: The fragments can be engineered either into complete antibodies for expression in mammalian cells, or into bispecific antibody fragments with a second binding site capable of triggering an effector function.

[00290] Typically, the Fd fragment (V_H-C_H1) and light chain (V_L-C_L) of antibodies are separately cloned by PCR and recombined randomly in combinatorial phage display libraries, which can then be selected for binding to a particular antigen. The antibody fragments are expressed on the phage surface, and selection of Fv or Fab (and therefore the phage containing the DNA encoding the antibody fragment) by antigen binding is accomplished through several rounds of antigen binding and re-amplification, a procedure termed panning. Antibody fragments specific for the antigen are enriched and finally isolated.

[00291] Phage display techniques can also be used in an approach for the humanization of rodent monoclonal antibodies, called "guided selection" (see Jespers, L. S., et al., Bio/Technology 12, 899-903 (1994)). For this, the Fd fragment of the mouse monoclonal antibody can be displayed in combination with a human light chain library, and the resulting hybrid Fab library may then be selected with

antigen. The mouse Fd fragment thereby provides a template to guide the selection. Subsequently, the selected human light chains are combined with a human Fd fragment library. Selection of the resulting library yields entirely human Fab.

[00292] A variety of procedures have been described for deriving human antibodies from phage-display libraries (See, for example, Hoogenboom et al., J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol, 222:581-597 (1991); U.S. Pat. Nos. 5,565,332 and 5,573,905; Clackson, T., and Wells, J. A., TIBTECH 12, 173-184 (1994)). In particular, in vitro selection and evolution of antibodies derived from phage display libraries has become a powerful tool (See Burton, D. R., and Barbas III, C. F., Adv. Immunol. 57, 191-280 (1994); and, Winter, G., et al., Annu. Rev. Immunol. 12, 433-455 (1994); U.S. patent application no. 20020004215 and WO92/01047; U.S. patent application no. 20030190317 published October 9, 2003 and U.S. Patent No. 6,054,287; U.S. Patent No. 5,877,293.

[00293] Watkins, "Screening of Phage-Expressed Antibody Libraries by Capture Lift," Methods in Molecular Biology, Antibody Phage Display: Methods and Protocols 178: 187-193, and U.S. Patent Application Publication No. 20030044772 published March 6, 2003 describes methods for screening phage-expressed antibody libraries or other binding molecules by capture lift, a method involving immobilization of the candidate binding molecules on a solid support.

[00294] Other Embodiments of Antigen binding proteins: Antibody Fragments

[00295] As noted above, antibody fragments comprise a portion of an intact full length antibody, preferably an antigen binding or variable region of the intact antibody, and include linear antibodies and multispecific antibodies formed from antibody fragments. Nonlimiting examples of antibody fragments include Fab, Fab', F(ab')2, Fv, Fd, domain antibody (dAb), complementarity determining region (CDR) fragments, single-chain antibodies (scFv), single chain antibody fragments, maxibodies, diabodies, triabodies, tetrabodies, minibodies, linear antibodies, chelating recombinant antibodies, tribodies or bibodies, intrabodies, nanobodies, small modular immunopharmaceuticals (SMIPs), an antigen-binding-domain

immunoglobulin fusion protein, a camelized antibody, a VHH containing antibody, or muteins or derivatives thereof, and polypeptides that contain at least a portion of an immunoglobulin that is sufficient to confer specific antigen binding to the polypeptide, such as a CDR sequence, as long as the antibody retains the desired biological activity. Such antigen fragments may be produced by the modification of whole antibodies or synthesized *de novo* using recombinant DNA technologies or peptide synthesis.

[00296] Additional antibody fragments include a domain antibody (dAb) fragment (Ward et al., *Nature* 341:544-546, 1989) which consists of a V_H domain.

[00297] "Linear antibodies" comprise a pair of tandem Fd segments (V_H -C_H1-V_H -C_H1) which form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific (Zapata et al. Protein Eng. 8:1057-62 (1995)).

[00298] A "minibody" consisting of scFv fused to CH3 via a peptide linker (hingeless) or via an IgG hinge has been described in Olafsen, et al., Protein Eng Des Sel. 2004 Apr;17(4):315-23.

[00299] The term "maxibody" refers to bivalent scFvs covalently attached to the Fc region of an immunoglobulin, see, for example, Fredericks et al, Protein Engineering, Design & Selection, 17:95-106 (2004) and Powers et al., Journal of Immunological Methods, 251:123-135 (2001).

[00300] Functional heavy-chain antibodies devoid of light chains are naturally occurring in certain species of animals, such as nurse sharks, wobbegong sharks and *Camelidae*, such as camels, dromedaries, alpacas and llamas. The antigen-binding site is reduced to a single domain, the VH_H domain, in these animals. These antibodies form antigen-binding regions using only heavy chain variable region, i.e., these functional antibodies are homodimers of heavy chains only having the structure H_2L_2 (referred to as "heavy-chain antibodies" or "HCAbs"). Camelized V_{HH} reportedly recombines with IgG2 and IgG3 constant regions that contain hinge, CH2, and CH3 domains and lack a CH1 domain. Classical V_H-only fragments are

difficult to produce in soluble form, but improvements in solubility and specific binding can be obtained when framework residues are altered to be more VH_H-like. (See, e.g., Reichman, etal., J Immunol Methods 1999, 231:25-38.) Camelized V_{HH} domains have been found to bind to antigen with high affinity (Desmyter et al., *J. Biol. Chem.* 276:26285-90, 2001) and possess high stability in solution (Ewert et al., *Biochemistry* 41:3628-36, 2002). Methods for generating antibodies having camelized heavy chains are described in, for example, in U.S. Patent Publication Nos. 2005/0136049 and 2005/0037421. Alternative scaffolds can be made from human variable-like domains that more closely match the shark V-NAR scaffold and may provide a framework for a long penetrating loop structure.

[00301] Because the variable domain of the heavy-chain antibodies is the smallest fully functional antigen-binding fragment with a molecular mass of only 15 kDa, this entity is referred to as a nanobody (Cortez-Retamozo et al., *Cancer Research* 64:2853-57, 2004). A nanobody library may be generated from an immunized dromedary as described in Conrath et al., (*Antimicrob Agents Chemother* 45: 2807-12, 2001).

[00302] Intrabodies are single chain antibodies which demonstrate intracellular expression and can manipulate intracellular protein function (Biocca, et al., *EMBO J.* 9:101–108, 1990; Colby et al., *Proc Natl Acad Sci U S A.* 101:17616-21, 2004). Intrabodies, which comprise cell signal sequences which retain the antibody contruct in intracellular regions, may be produced as described in Mhashilkar et al (*EMBO J* 14:1542-51, 1995) and Wheeler et al. (*FASEB J.* 17:1733-5. 2003). Transbodies are cell-permeable antibodies in which a protein transduction domains (PTD) is fused with single chain variable fragment (scFv) antibodies Heng et al., (*Med Hypotheses.* 64:1105-8, 2005).

[00303] Further encompassed by the invention are antibodies that are SMIPs or binding domain immunoglobulin fusion proteins specific for target protein. These constructs are single-chain polypeptides comprising antigen binding domains fused to immunoglobulin domains necessary to carry out antibody effector functions. See

e.g., WO03/041600, U.S. Patent publication 20030133939 and US Patent Publication 20030118592.

[00304] Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies, but can also be produced directly by recombinant host cells. See, for example, Better et al., Science 240: 1041-1043 (1988); Skerra et al. Science 240: 1038-1041 (1988); Carter et al., Bio/Technology 10:163-167 (1992).

[00305] Other Embodiments of Antigen Binding Proteins: Multivalent Antibodies

[00306] In some embodiments, it may be desirable to generate multivalent or even a multispecific (e.g. bispecific, trispecific, etc.) monoclonal antibody. Such antibody may have binding specificities for at least two different epitopes of the target antigen, or alternatively it may bind to two different molecules, e.g. to the target antigen and to a cell surface protein or receptor. For example, a bispecific antibody may include an arm that binds to the target and another arm that binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (e.g., CD2 or CD3), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the target-expressing cell. As another example, bispecific antibodies may be used to localize cytotoxic agents to cells which express target antigen. These antibodies possess a target-binding arm and an arm which binds the cytotoxic agent (e.g., saporin, anti-interferon-60, vinca alkaloid, ricin A chain, methotrexate or radioactive isotope hapten). Multispecific antibodies can be prepared as full length antibodies or antibody fragments.

[00307] Additionally, the anti-DNP or anti-KLH antibodies of the present invention can also be constructed to fold into multivalent forms, which may improve binding affinity, specificity and/or increased half-life in blood. Multivalent forms of anti-DNP or anti-KLH can be prepared by techniques known in the art.

[00308] Bispecific or multispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the

heteroconjugate can be coupled to avidin, the other to biotin. Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Pat. No. 4,676,980, along with a number of cross-linking techniques. Another method is designed to make tetramers by adding a streptavidin-coding sequence at the C-terminus of the scFv. Streptavidin is composed of four subunits, so when the scFv-streptavidin is folded, four subunits associate to form a tetramer (Kipriyanov et al., Hum Antibodies Hybridomas 6(3): 93-101 (1995), the disclosure of which is incorporated herein by reference in its entirety).

[00309] According to another approach for making bispecific antibodies, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. One interface comprises at least a part of the C_H3 domain of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g., tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g., alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers. See WO 96/27011 published Sep. 6, 1996.

[00310] Techniques for generating bispecific or multispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific or trispecific antibodies can be prepared using chemical linkage. Brennan et al., Science 229:81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate $F(ab')_2$ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with

mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes. Better et al., Science 240: 1041-1043 (1988) disclose secretion of functional antibody fragments from bacteria (*see, e.g.,* Better et al., Skerra et al. Science 240: 1038-1041 (1988)). For example, Fab'-SH fragments can be directly recovered from E. coli and chemically coupled to form bispecific antibodies (Carter et al., Bio/Technology 10:163-167 (1992); Shalaby et al., J. Exp. Med. 175:217-225 (1992)).

[00311] Shalaby et al., J. Exp. Med. 175:217-225 (1992) describe the production of a fully humanized bispecific antibody $F(ab')_2$ molecule. Each Fab' fragment was separately secreted from *E.coli* and subjected to directed chemical coupling *in vitro* to form the bispecfic antibody.

[00312] Various techniques for making and isolating bispecific or multispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers, e.g. GCN4. (See generally Kostelny et al., J. Immunol. 148(5):1547-1553 (1992).) The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers.

[00313] Diabodies, described above, are one example of a bispecific antibody. See, for example, Hollinger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993). Bivalent diabodies can be stabilized by disulfide linkage.

[00314] Stable monospecific or bispecific Fv tetramers can also be generated by noncovalent association in $(scFv_2)_2$ configuration or as bis-tetrabodies. Alternatively, two different scFvs can be joined in tandem to form a bis-scFv.

[00315] Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. *See* Gruber et al., J. Immunol. 152: 5368 (1994). One approach has been to link two scFv antibodies with linkers or disulfide bonds (Mallender and Voss, J. Biol. Chem. 269:199-2061994, WO 94/13806, and U.S. Patent No. 5,989,830, the disclosures of which are incorporated herein by reference in their entireties).

[00316] Alternatively, the bispecific antibody may be a "linear antibody" produced as described in Zapata et al. Protein Eng. 8(10):1057-1062 (1995). Briefly, these antibodies comprise a pair of tandem Fd segments (V_H -C_H1-V_H -C_H1) which form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

[00317] Antibodies with more than two valencies are also contemplated. For example, trispecific antibodies can be prepared. (Tutt et al., J. Immunol. 147:60 (1991)).

[00318] A "chelating recombinant antibody" is a bispecific antibody that recognizes adjacent and non-overlapping epitopes of the target antigen, and is flexible enough to bind to both epitopes simultaneously (Neri et al., *J Mol Biol.* 246:367-73, 1995).

[00319] Production of bispecific Fab-scFv ("bibody") and trispecific Fab-(scFv)(2) ("tribody") are described in Schoonjans et al. (*J Immunol.* 165:7050-57, 2000) and Willems et al. (*J Chromatogr B Analyt Technol Biomed Life Sci.* 786:161-76, 2003). For bibodies or tribodies, a scFv molecule is fused to one or both of the VL-CL (L) and VH-CH_1 (Fd) chains, e.g., to produce a tribody two scFvs are fused to C-term of Fab while in a bibody one scFv is fused to C-term of Fab.

[00320] In yet another method, dimers, trimers, and tetramers are produced after a free cysteine is introduced in the parental protein. A peptide-based cross linker with variable numbers (two to four) of maleimide groups was used to cross link the protein of interest to the free cysteines (Cochran et al., Immunity 12(3): 241-50 (2000), the disclosure of which is incorporated herein in its entirety).

[00321] Other Embodiments of Antigen Binding Proteins

[00322] Inventive antigen binding proteins also include peptibodies. The term "peptibody" refers to a molecule comprising an antibody Fc domain attached to at least one peptide. The production of peptibodies is generally described in PCT publication WO 00/24782, published May 4, 2000. Any of these peptides may be linked in tandem (i.e., sequentially), with or without linkers. Peptides containing a cysteinyl residue may be cross-linked with another Cys-containing peptide, either or both of which may be linked to a vehicle. Any peptide having more than one Cys residue may form an intrapeptide disulfide bond, as well. Any of these peptides may be derivatized, for example the carboxyl terminus may be capped with an amino group, cysteines may be cappe, or amino acid residues may substituted by moieties other than amino acid residues (see, e.g., Bhatnagar et al., J. Med. Chem. 39: 3814-9 (1996), and Cuthbertson et al., J. Med. Chem. 40: 2876-82 (1997), which are incorporated by reference herein in their entirety). The peptide sequences may be optimized, analogous to affinity maturation for antibodies, or otherwise altered by alanine scanning or random or directed mutagenesis followed by screening to identify the best binders. Lowman, Ann. Rev. Biophys. Biomol. Struct. 26: 401-24 (1997). Various molecules can be inserted into the antigen binding protein structure, e.g., within the peptide portion itself or between the peptide and vehicle portions of the antigen binding proteins, while retaining the desired activity of antigen binding protein. One can readily insert, for example, molecules such as an Fc domain or fragment thereof, polyethylene glycol or other related molecules such as dextran, a fatty acid, a lipid, a cholesterol group, a small carbohydrate, a peptide, a detectable moiety as described herein (including fluorescent agents, radiolabels such as radioisotopes), an oligosaccharide, oligonucleotide, a polynucleotide, interference (or other) RNA, enzymes, hormones, or the like. Other molecules suitable for insertion in this fashion will be appreciated by those skilled in the art, and are encompassed within the scope of the invention. This includes insertion of, for example, a desired molecule in between two consecutive amino acids, optionally joined by a suitable linker.

[00323] Linkers. A "linker" or "linker moiety", as used interchangeably herein, refers to a biologically acceptable peptidyl or non-peptidyl organic group that is covalently bound to an amino acid residue of a polypeptide chain (e.g., an immunoglobulin HC or immunoglobulin LC or immunoglobulin Fc domain) contained in the inventive composition, which linker moiety covalently joins or conjugates the polypeptide chain to another peptide or polypeptide chain in the molecule, or to a therapeutic moiety, such as a biologically active small molecule or oligopeptide, or to a half-life extending moiety, e.g., see, Sullivan et al., Toxin Peptide Therapeutic Agents, US2007/0071764; Sullivan et al., Toxin Peptide Therapeutic Agents, PCT/US2007/022831, published as WO 2008/088422; and US Provisional Application Serial No. 61/210,594, filed March 20, 2009, which are all incorporated herein by reference in their entireties.

[00324] The presence of any linker moiety in the antigen binding proteins of the present invention is optional. When present, the linker's 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 antigen binding protein. The presence of a linker moiety can be useful in optimizing pharamcologial activity of some embodiments of the inventive antigen binding protein (including antibodies and antibody fragments). 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 antigen binding protein.

[00325] As stated above, the linker moiety, if present (whether within the primary amino acid sequence of the antigen binding protein, or as a linker for attaching a therapeutic moiety or half-life extending moiety to the inventive antigen binding protein), 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:148), wherein X_1, X_2,X_4 and X_5 are each independently any amino acid residue.

[00326] 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:149), $(Gly)_5$ (SEQ ID NO:150) and $(Gly)_7$ (SEQ ID NO:151), as well as, poly$(Gly)_4$Ser (SEQ ID NO:152), poly$(Gly\text{-}Ala)_{2-4}$ and poly$(Ala)_{1-8}$. Other specific examples of peptidyl linkers include $(Gly)_5$Lys (SEQ ID NO:154), and $(Gly)_5$LysArg (SEQ ID NO:155). Other examples of useful peptidyl linkers are: Other examples of useful peptidyl linkers are:

[00327] $(Gly)_3Lys(Gly)_4$ (SEQ ID NO:159);

[00328] $(Gly)_3AsnGlySer(Gly)_2$ (SEQ ID NO:156);

[00329] $(Gly)_3Cys(Gly)_4$ (SEQ ID NO:157); and

[00330] GlyProAsnGlyGly (SEQ ID NO:158).

[00331] To explain the above nomenclature, for example, $(Gly)_3Lys(Gly)_4$ means Gly-Gly-Gly-Lys-Gly-Gly-Gly-Gly (SEQ ID NO:159). Other combinations of Gly and Ala are also useful.

[00332] Commonly used linkers include those which may be identified herein as "L5" (GGGGS; or "G_4S"; SEQ ID NO:152), "L10" (GGGGSGGGGS; SEQ ID NO:153), "L25" (GGGGSGGGGSGGGGSGGGGSGGGGS; SEQ ID NO:146) and any linkers used in the working examples hereinafter.

[00333] 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:

[00334] GGEGGG (SEQ ID NO:160);

[00335] GGEEEGGG (SEQ ID NO:161);

[00336] GEEEG (SEQ ID NO:162);

[00337] GEEE (SEQ ID NO:163);

[00338] GGDGGG (SEQ ID NO:164);

[00339] GGDDDGG (SEQ ID NO:165);

[00340] GDDDG (SEQ ID NO:166);

[00341] GDDD (SEQ ID NO:167);

[00342] GGGGSDDSDEGSDGEDGGGGS (SEQ ID NO:168);

[00343] WEWEW (SEQ ID NO:169);

[00344] FEFEF (SEQ ID NO:170);

[00345] EEEWWW (SEQ ID NO:171);

[00346] EEEFFF (SEQ ID NO:172);

[00347] WWEEEWW (SEQ ID NO:173); or

[00348] FFEEEFF (SEQ ID NO:174).

[00349] In other embodiments, the linker constitutes a phosphorylation site, e.g., $X_1X_2YX_4X_5G$ (SEQ ID NO:175), 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:176), 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:177), wherein X_1, X_2, X_4 and X_5 are each independently any amino acid residue.

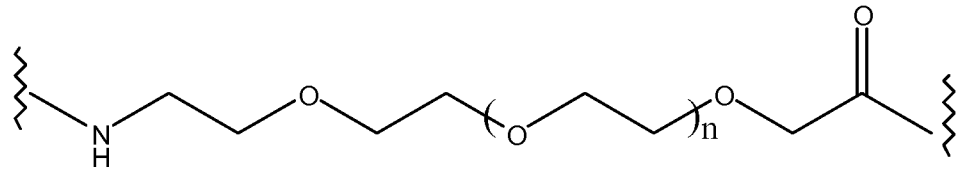
[00350] The linkers shown here are exemplary; peptidyl linkers within the scope of this invention may be much longer and may include other residues. A peptidyl linker can contain, e.g., a cysteine, another thiol, or nucleophile for conjugation with a half-life extending moiety. In another embodiment, 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.

[00351] Another useful peptidyl linker is a large, flexible linker comprising a random Gly/Ser/Thr sequence, for example: GSGSATGGSGSTASSGSGSATH (SEQ ID NO:178) or HGSGSATGGSGSTASSGSGSAT (SEQ ID NO:179), 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:180). 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.

[00352] 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 polyethylene glycol (PEG) linkers (e.g., shown below):

[00353] (I)



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.

[00354] In one embodiment, the non-peptidyl linker is aryl. The linkers may be altered to form derivatives in the same manner as described in the art, e.g., in Sullivan et al., Toxin Peptide Therapeutic Agents, US2007/0071764; Sullivan et al., Toxin Peptide Therapeutic Agents, PCT/US2007/022831, published as WO 2008/088422; and US Provisional Application Serial No. 61/210,594, filed March 20, 2009, which are all incorporated herein by reference in their entireties.

[00355] 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.

[00356] "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.

[00357] "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.

[00358] 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.

[00359] 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.

[00360] Production of Antigen Binding Protein Variants. As noted above, 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 compositions. For example, polypeptides 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 HEK-293 cells, and others noted herein or otherwise known in the art. 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 specific binding agent or antigen binding protein, e.g., an immunoglobulin protein. 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 specific binding agents of the invention, see, e.g., Sullivan et al., Toxin Peptide Therapeutic Agents, US2007/0071764; Sullivan et al., Toxin Peptide Therapeutic Agents, PCT/US2007/022831, published as WO 2008/088422; and US Provisional Application Serial No. 61/210,594, filed March 20, 2009, which are all incorporated herein by reference in their entireties.

[00361] Amino acid sequence variants of the desired antigen binding protein may be prepared by introducing appropriate nucleotide changes into the encoding DNA, or by peptide synthesis. Such variants include, for example, deletions and/or insertions and/or substitutions of residues within the amino acid sequences of the antigen binding proteins or antibodies. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the antigen binding protein, such as changing the number or position of glycosylation sites. In certain instances, antigen binding protein

variants are prepared with the intent to modify those amino acid residues which are directly involved in epitope binding. In other embodiments, modification of residues which are not directly involved in epitope binding or residues not involved in epitope binding in any way, is desirable, for purposes discussed herein. Mutagenesis within any of the CDR regions and/or framework regions is contemplated. Covariance analysis techniques can be employed by the skilled artisan to design useful modifications in the amino acid sequence of the antigen binding protein, including an antibody or antibody fragment. (E.g., Choulier, et al., Covariance Analysis of Protein Families: The Case of the Variable Domains of Antibodies, Proteins: Structure, Function, and Genetics 41:475-484 (2000); Demarest et al., Optimization of the Antibody C_H3 Domain by Residue Frequency Analysis of IgG Sequences, J. Mol. Biol. 335:41-48 (2004); Hugo et al., VL position 34 is a key determinant for the engineering of stable antibodies with fast dissociation rates, Protein Engineering 16(5):381-86 (2003); Aurora et al., Sequence covariance networks, methods and uses thereof, US 2008/0318207 A1; Glaser et al., Stabilized polypeptide compositions, US 2009/0048122 A1; Urech et al., Sequence based engineering and optimization of single chain antibodies, WO 2008/110348 A1; Borras et al., Methods of modifying antibodies, and modified antibodies with improved functional properties, WO 2009/000099 A2). Such modifications determined by covariance analysis can improve potency, pharmacokinetic, pharmacodynamic, and/or manufacturability characteristics of an antigen binding protein.

[00362] Nucleic acid molecules encoding amino acid sequence variants of the antigen binding protein or antibody are prepared by a variety of methods known in the art. Such methods include oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the antigen binding protein.

[00363] Substitutional mutagenesis within any of the hypervariable or CDR regions or framework regions is contemplated. A useful method for identification of certain residues or regions of the antigen binding protein that are preferred locations for mutagenesis is called "alanine scanning mutagenesis," as described by

Cunningham and Wells Science, 244:1081-1085 (1989). Here, a residue or group of target residues are identified (e.g., charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation per se need not be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted at the target codon or region and the expressed variants are screened for the desired activity.

[00364] Some embodiments of the antigen binding proteins 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.

[00365] In further describing any of the antigen binding proteins herein, as well as variants, 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 4). 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 4. 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

[00366] 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 an original 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 original sequence of interest. Another example, "W101F" symbolizes a substitution of a tryptophan residue by a phenylalanine residue at amino acid position 101, relative to the original sequence of interest.

[00367] Non-canonical amino acid residues can be incorporated into a polypeptide 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 5 below, and derivatized forms of any of these as described herein. Table 5 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 5. 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 5 differs from another abbreviation for the same substance disclosed elsewhere herein, both abbreviations are understood to be applicable. The amino acids listed in Table 5 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
methinine 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^{α}-MeHoCit

N^{α}-methylhomolysine	NMeHoK
N^{α}-methylleucine	N^{α}-MeL; NMeL; NMeLeu; NMe-Leu
N^{α}-methyllysine	NMe-Lys
Nε-methyl-lysine	N-eMe-K
Nε-ethyl-lysine	N-eEt-K
Nε-isopropyl-lysine	N-eIPr-K
N^{α}-methylnorleucine	NMeNle; NMe-Nle
N^{α}-methylornithine	N^{α}-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

[00368] 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.

[00369] The one or more useful modifications to peptide domains of the inventive antigen binding protein 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.

[00370] 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^aR^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^aR^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.

[00371] Additional useful embodiments of can result from conservative modifications of the amino acid sequences of the 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 polypeptides disclosed herein are also contemplated as being an embodiment of the present invention.

[00372] 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.

[00373] 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).

[00374] 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.

[00375] 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.

[00376] 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.

[00377] 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.

[00378] 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).

[00379] 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.

[00380] 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.

[00381] 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."

[00382] 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 6 below.

Table 6. 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

[00383] Ordinarily, amino acid sequence variants of the antigen binding protein will have an amino acid sequence having at least 60% amino acid sequence identity with the original antigen binding protein or antibody amino acid sequences of either the heavy or the light chain variable region, or at least 65%, or at least 70%, or at least 75% or at least 80% identity, more preferably at least 85% identity, even more preferably at least 90% identity, and most preferably at least 95% identity, including for example, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and 100%. Identity or homology with respect to this sequence is defined herein as the percentage of amino acid residues in

the candidate sequence that are identical with the original sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. None of N-terminal, C-terminal, or internal extensions, deletions, or insertions into the antigen binding protein or antibody sequence shall be construed as affecting sequence identity or homology.

[00384] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intra-sequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antigen binding protein with an N-terminal methionyl residue or the antigen binding protein (including antibody or antibody fragment) fused to an epitope tag or a salvage receptor binding epitope. Other insertional variants of the antigen binding protein or antibody molecule include the fusion to a polypeptide which increases the serum half-life of the antigen binding protein, e.g. at the N-terminus or C-terminus.

[00385] Examples of epitope tags include the flu HA tag polypeptide and its antibody 12CA5 [Field et al., Mol. Cell. Biol. 8: 2159-2165 (1988)]; the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto [Evan et al., Mol. Cell. Biol. 5(12): 3610-3616 (1985)]; and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody [Paborsky et al., Protein Engineering 3(6): 547-553 (1990)]. Other exemplary tags are a poly-histidine sequence, generally around six histidine residues, that permits isolation of a compound so labeled using nickel chelation. Other labels and tags, such as the FLAG® tag (Eastman Kodak, Rochester, NY) are well known and routinely used in the art.

[00386] Some particular, non-limiting, embodiments of amino acid substitution variants of the inventive antigen binding proteins, including antibodies and antibody fragments are exemplified below.

[00387] Any cysteine residue not involved in maintaining the proper conformation of the antigen binding protein also may be substituted, generally with serine, to

improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) may be added to the antigen binding protein to improve its stability (particularly where the antigen binding protein is an antibody fragment such as an Fv fragment).

[00388] In certain instances, antigen binding protein variants are prepared with the intent to modify those amino acid residues which are directly involved in epitope binding. In other embodiments, modification of residues which are not directly involved in epitope binding or residues not involved in epitope binding in any way, is desirable, for purposes discussed herein. Mutagenesis within any of the CDR regions and/or framework regions is contemplated.

[00389] In order to determine which antigen binding protein amino acid residues are important for epitope recognition and binding, alanine scanning mutagenesis can be performed to produce substitution variants. See, for example, Cunningham et al., Science, 244:1081-1085 (1989), the disclosure of which is incorporated herein by reference in its entirety. In this method, individual amino acid residues are replaced one-at-a-time with an alanine residue and the resulting anti-DNP or anti-KLH antigen binding protein is screened for its ability to bind its specific epitope relative to the unmodified polypeptide. Modified antigen binding proteins with reduced binding capacity are sequenced to determine which residue was changed, indicating its significance in binding or biological properties.

[00390] Substitution variants of antigen binding proteins can be prepared by affinity maturation wherein random amino acid changes are introduced into the parent polypeptide sequence. See, for example, Ouwehand et al., Vox Sang 74 (Suppl 2):223-232, 1998; Rader et al., Proc. Natl. Acad. Sci. USA 95:8910-8915, 1998; Dall'Acqua et al., Curr. Opin. Struct. Biol. 8:443-450, 1998, the disclosures of which are incorporated herein by reference in their entireties. Affinity maturation involves preparing and screening the anti-DNP or anti-KLH antigen binding proteins, or variants thereof and selecting from the resulting variants those that have modified biological properties, such as increased binding affinity relative to the

parent anti-DNP or anti-KLH antigen binding protein. A convenient way for generating substitutional variants is affinity maturation using phage display. Briefly, several hypervariable region sites are mutated to generate all possible amino substitutions at each site. The variants thus generated are expressed in a monovalent fashion on the surface of filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (*e.g.*, binding affinity). See e.g., WO 92/01047, WO 93/112366, WO 95/15388 and WO 93/19172.

[00391] Current antibody affinity maturation methods belong to two mutagenesis categories: stochastic and nonstochastic. Error prone PCR, mutator bacterial strains (Low et al., *J. Mol. Biol.* 260, 359-68, 1996), and saturation mutagenesis (Nishimiya et al., *J. Biol. Chem.* 275:12813-20, 2000; Chowdhury, P. S. *Methods Mol. Biol.* 178, 269-85, 2002) are typical examples of stochastic mutagenesis methods (Rajpal et al., *Proc Natl Acad Sci U S A.* 102:8466-71, 2005). Nonstochastic techniques often use alanine-scanning or site-directed mutagenesis to generate limited collections of specific muteins. Some methods are described in further detail below.

[00392] *Affinity maturation via panning methods*—Affinity maturation of recombinant antibodies is commonly performed through several rounds of panning of candidate antibodies in the presence of decreasing amounts of antigen. Decreasing the amount of antigen per round selects the antibodies with the highest affinity to the antigen thereby yielding antibodies of high affinity from a large pool of starting material. Affinity maturation via panning is well known in the art and is described, for example, in Huls et al. (*Cancer Immunol Immunother.* 50:163-71, 2001). Methods of affinity maturation using phage display technologies are described elsewhere herein and known in the art (see e.g., Daugherty et al., *Proc Natl Acad Sci U S A.* 97:2029-34, 2000).

[00393] Look-through mutagenesis—Look-through mutagenesis (LTM) (Rajpal et al., Proc Natl Acad Sci U S A. 102:8466-71, 2005) provides a method for rapidly mapping the antibody-binding site. For LTM, nine amino acids, representative of the

major side-chain chemistries provided by the 20 natural amino acids, are selected to dissect the functional side-chain contributions to binding at every position in all six CDRs of an antibody. LTM generates a positional series of single mutations within a CDR where each "wild type" residue is systematically substituted by one of nine selected amino acids. Mutated CDRs are combined to generate combinatorial single-chain variable fragment (scFv) libraries of increasing complexity and size without becoming prohibitive to the quantitative display of all muteins. After positive selection, clones with improved binding are sequenced, and beneficial mutations are mapped.

[00394] Error-prone PCR—Error-prone PCR involves the randomization of nucleic acids between different selection rounds. The randomization occurs at a low rate by the intrinsic error rate of the polymerase used but can be enhanced by error-prone PCR (Zaccolo et al., J. Mol. Biol. 285:775-783, 1999) using a polymerase having a high intrinsic error rate during transcription (Hawkins et al., J Mol Biol. 226:889-96, 1992). After the mutation cycles, clones with improved affinity for the antigen are selected using routine methods in the art.

[00395] Techniques utilizing gene shuffling and directed evolution may also be used to prepare and screen anti-DNP or anti-KLH antigen binding proteins, or variants thereof, for desired activity. For example, Jermutus et al., Proc Natl Acad Sci U S A., 98(1):75-80 (2001) showed that tailored *in vitro* selection strategies based on ribosome display were combined with *in vitro* diversification by DNA shuffling to evolve either the off-rate or thermodynamic stability of scFvs; Fermer et al., Tumour Biol. 2004 Jan-Apr;25(1-2):7-13 reported that use of phage display in combination with DNA shuffling raised affinity by almost three orders of magnitude. Dougherty et al., Proc Natl Acad Sci U S A. 2000 Feb. 29; 97(5):2029-2034 reported that (i) functional clones occur at an unexpectedly high frequency in hypermutated libraries, (ii) gain-of-function mutants are well represented in such libraries, and (iii) the majority of the scFv mutations leading to higher affinity correspond to residues distant from the binding site.

[00396] Alternatively, or in addition, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and antigen, or to use computer software to model such contact points. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, they are subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

[00397] Antigen binding proteins with modified carbohydrate

[00398] Antigen binding protein variants can also be produced that have a modified glycosylation pattern relative to the parent polypeptide, for example, adding or deleting one or more of the carbohydrate moieties bound to the antigen binding protein, and/or adding or deleting one or more glycosylation sites in the antigen binding protein.

[00399] Glycosylation of polypeptides, including antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. The presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. Thus, N-linked glycosylation sites may be added to a antigen binding protein by altering the amino acid sequence such that it contains one or more of these tripeptide sequences. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used. O-linked glycosylation sites may be added to a antigen binding protein by inserting or substituting one or more serine or threonine residues to the sequence of the original antigen binding protein or antibody.

[00400] Altered Effector Function

[00401] Cysteine residue(s) may be removed or introduced in the Fc region of an antibody or Fc-containing polypeptide, thereby eliminating or increasing interchain disulfide bond formation in this region. A homodimeric antigen binding protein thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron et al., J. Exp Med. 176: 1191-1195 (1992) and Shopes, B. J. Immunol. 148: 2918-2922 (1992). Homodimeric antigen binding proteins or antibodies may also be prepared using heterobifunctional cross-linkers as described in Wolff et al., Cancer Research 53: 2560-2565 (1993). Alternatively, a antigen binding protein can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson et al., Anti-CancerDrug Design 3: 219-230 (1989).

[00402] It has been shown that sequences within the CDR can cause an antibody to bind to MHC Class II and trigger an unwanted helper T-cell response. A conservative substitution can allow the antigen binding protein to retain binding activity yet reduce its ability to trigger an unwanted T-cell response. It is also contemplated that one or more of the N-terminal 20 amino acids of the heavy or light chain are removed.

[00403] Modifications to increase serum half-life also may desirable, for example, by incorporation of or addition of a salvage receptor binding epitope (e.g., by mutation of the appropriate region or by incorporating the epitope into a peptide tag that is then fused to the antigen binding protein at either end or in the middle, e.g., by DNA or peptide synthesis) (see, e.g., WO96/32478) or adding molecules such as PEG or other water soluble polymers, including polysaccharide polymers.

[00404] The salvage receptor binding epitope preferably constitutes a region wherein any one or more amino acid residues from one or two loops of a Fc domain are transferred to an analogous position of the antigen binding protein or fragment. Even more preferably, three or more residues from one or two loops of the Fc domain are transferred. Still more preferred, the epitope is taken from the CH2

domain of the Fc region (e.g., of an IgG) and transferred to the CH1, CH3, or VH region, or more than one such region, of the antigen binding protein or antibody. Alternatively, the epitope is taken from the CH2 domain of the Fc region and transferred to the C_L region or V_L region, or both, of the antigen binding protein fragment. See also International applications WO 97/34631 and WO 96/32478 which describe Fc variants and their interaction with the salvage receptor.

[00405] Other sites and amino acid residue(s) of the constant region have been identified that are responsible for complement dependent cytotoxicity (CDC), such as the C1q binding site, and/or the antibody-dependent cellular cytotoxicity (ADCC) [see, e.g., Molec. Immunol. 29 (5): 633-9 (1992); Shields et al., J. Biol. Chem., 276(9):6591-6604 (2001); Lazar et al., Proc. Nat'l. Acad. Sci. 103(11): 4005 (2006) which describe the effect of mutations at specific positions, each of which is incorporated by reference herein in its entirety]. Mutation of residues within Fc receptor binding sites can result in altered (i.e. increased or decreased) effector function, such as altered affinity for Fc receptors, altered ADCC or CDC activity, or altered half-life. As described above, potential mutations include insertion, deletion or substitution of one or more residues, including substitution with alanine, a conservative substitution, a non-conservative substitution, or replacement with a corresponding amino acid residue at the same position from a different subclass (e.g. replacing an IgG1 residue with a corresponding IgG2 residue at that position).

[00406] The invention also encompasses production of antigen binding protein molecules, including antibodies and antibody fragments, with altered carbohydrate structure resulting in altered effector activity, including antibody molecules with absent or reduced fucosylation that exhibit improved ADCC activity. A variety of ways are known in the art to accomplish this. For example, ADCC effector activity is mediated by binding of the antibody molecule to the FcγRIII receptor, which has been shown to be dependent on the carbohydrate structure of the N-linked glycosylation at the Asn-297 of the CH2 domain. Non-fucosylated antibodies bind this receptor with increased affinity and trigger FcγRIII-mediated effector functions more efficiently than native, fucosylated antibodies. For example, recombinant

production of non-fucosylated antibody in CHO cells in which the alpha-1,6-fucosyl transferase enzyme has been knocked out results in antibody with 100-fold increased ADCC activity (Yamane-Ohnuki et al., Biotechnol Bioeng. 2004 Sep 5;87(5):614-22). Similar effects can be accomplished through decreasing the activity of this or other enzymes in the fucosylation pathway, e.g., through siRNA or antisense RNA treatment, engineering cell lines to knockout the enzyme(s), or culturing with selective glycosylation inhibitors (Rothman et al., Mol Immunol. 1989 Dec;26(12):1113-23). Some host cell strains, e.g. Lec13 or rat hybridoma YB2/0 cell line naturally produce antibodies with lower fucosylation levels. Shields et al., J Biol Chem. 2002 Jul 26;277(30):26733-40; Shinkawa et al., J Biol Chem. 2003 Jan 31;278(5):3466-73. An increase in the level of bisected carbohydrate, e.g. through recombinantly producing antibody in cells that overexpress GnTIII enzyme, has also been determined to increase ADCC activity. Umana et al., Nat Biotechnol. 1999 Feb;17(2):176-80. It has been predicted that the absence of only one of the two fucose residues may be sufficient to increase ADCC activity. (Ferrara et al., J Biol Chem. 2005 Dec 5).

[00407] Other Covalent Modifications of Antigen Binding Proteins

[00408] Other particular covalent modifications of the anti-DNP or anti-KLH antigen binding protein, are also included within the scope of this invention. They may be made by chemical synthesis or by enzymatic or chemical cleavage of the antigen binding protein or antibody, if applicable. Other types of covalent modifications can be introduced by reacting targeted amino acid residues with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues.

[00409] Cysteinyl residues most commonly are reacted with α-haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyl residues also are derivatized by reaction with bromotrifluoroacetone, .alpha.-bromo-β-(5-imidozoyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-

pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.

[00410] Histidyl residues are derivatized by reaction with diethylpyrocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1 M sodium cacodylate at pH 6.0.

[00411] Lysinyl and amino-terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing 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.

[00412] Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pK_a of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine epsilon-amino group.

[00413] The specific modification of tyrosyl residues may be made, 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. Tyrosyl residues are iodinated using ^{125}I or ^{131}I to prepare labeled proteins for use in radioimmunoassay.

[00414] Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides (R-N.dbd.C.dbd.N-R'), where R and R' are different alkyl groups, 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 are converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

[00415] Glutaminyl and asparaginyl residues are frequently deamidated to the corresponding glutamyl and aspartyl residues, respectively. These residues are deamidated under neutral or basic conditions. The deamidated form of these residues falls within the scope of this invention.

[00416] Other modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the .alpha.-amino groups of lysine, arginine, and histidine side chains (T. E. Creighton, Proteins: Structure and Molecular Properties, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)), acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

[00417] Another type of covalent modification involves chemically or enzymatically coupling glycosides to the antigen binding protein (e.g., antibody or antibody fragment). These procedures are advantageous in that they do not require production of the antigen binding protein in a host cell that has glycosylation capabilities for N- or O-linked glycosylation. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described in WO87/05330 published 11 Sep. 1987, and in Aplin and Wriston, CRC Crit. Rev. Biochem., pp. 259-306 (1981).

[00418] Removal of any carbohydrate moieties present on the antigen binding protein may be accomplished chemically or enzymatically. Chemical deglycosylation requires exposure of the antigen binding protein to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or

N-acetylgalactosamine), while leaving the antigen binding protein intact. Chemical deglycosylation is described by Hakimuddin, et al. Arch. Biochem. Biophys. 259: 52 (1987) and by Edge et al. Anal. Biochem., 118: 131 (1981). Enzymatic cleavage of carbohydrate moieties on a antigen binding protein can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura et al. Meth. Enzymol. 138: 350 (1987).

[00419] Another type of covalent modification of the antigen binding proteins of the invention (including antibodies and antibody fragments) comprises linking the antigen binding protein to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol, polypropylene glycol, polyoxyethylated polyols, polyoxyethylated sorbitol, polyoxyethylated glucose, polyoxyethylated glycerol, polyoxyalkylenes, or polysaccharide polymers such as dextran. Such methods are known in the art, see, e.g. U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192, 4,179,337, 4,766,106, 4,179,337, 4,495,285, 4,609,546 or EP 315 456.

[00420] Isolated nucleic acids

[00421] Another aspect of the present invention is an isolated nucleic acid that encodes an antigen binding protein of the invention, such as, but not limited to, an isolated nucleic acid that encodes an antibody or antibody fragment of the invention. Such nucleic acids are made by recombinant techniques known in the art and/or disclosed herein.

[00422] For example, the isolated nucleic acid encodes an antigen binding protein comprising an immunoglobulin heavy chain variable region comprising an amino acid sequence at least 95 % identical to SEQ ID NO:250, SEQ ID NO:252, SEQ ID NO:254, SEQ ID NO:256, SEQ ID NO:258, or SEQ ID NO:260.

[00423] In other embodiments, the isolated nucleic acid encodes an antigen binding protein comprising an immunoglobulin light chain variable region

comprising an amino acid sequence at least 95 % identical to SEQ ID NO:232, SEQ ID NO:234, SEQ ID NO:236, SEQ ID NO:238, or SEQ ID NO:240.

[00424] Other examples of the isolated nucleic acid include such that encodes an immunoglobulin heavy chain variable region, wherein the isolated nucleic acid comprises coding sequences for three complementarity determining regions, designated CDRHl, CDRH2 and CDRH3, and wherein:

[00425] (a) CDRHl comprises the amino acid sequence of SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, or SEQ ID NO:191;

[00426] (b) CDRH2 comprises the amino acid sequence of SEQ ID NO:192, SEQ ID NO:193, SEQ ID NO:194, or SEQ ID NO:195; and

[00427] (c) CDRH3 comprises the amino acid sequence of SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, or SEQ ID NO:201.

[00428] Still other examples of the isolated nucleic acid include such that encodes an immunoglobulin light chain variable region, wherein the isolated nucleic acid comprises coding sequences for three complementarity determining regions, designated CDRLl, CDRL2 and CDRL3, and wherein:

[00429] (a) CDRL1 comprises the amino acid sequence of SEQ ID NO:202, SEQ ID NO:203, SEQ ID NO:204, or SEQ ID NO:205;

[00430] (b) CDRL2 comprises the amino acid sequence of SEQ ID NO:206 or SEQ ID NO:207; and

[00431] (c) CDRL3 comprises the amino acid sequence of SEQ ID NO:208, SEQ ID NO:209, SEQ ID NO:210, SEQ ID NO:211, or SEQ ID NO:212.

[00432] In other embodiments the isolated nucleic acid encodes an antigen binding protein comprising an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO:77, SEQ ID NO:107, SEQ ID NO:111, SEQ ID NO:113,

SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:123, SEQ ID NO:129, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:181, SEQ ID NO:182, SEQ ID NO:183, SEQ ID NO:184, or SEQ ID NO:185, or comprising any one of the foregoing sequences from which one, two, three, four or five amino acid residues are lacking from the N-terminal or C-terminal, or both.

[00433] And in some embodiments the isolated nucleic acid encodes an antigen binding protein comprising an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO:105, SEQ ID NO:109, SEQ ID NO:121; SEQ ID NO:125, or SEQ ID NO:127, or comprising any one of the foregoing sequences from which one, two, three, four or five amino acid residues are lacking from the N-terminal or C-terminal, or both.

[00434] For another example, the isolated nucleic acid encodes an antigen binding protein comprising an immunoglobulin heavy chain variable region comprising an amino acid sequence at least 95 % identical to the sequence of SEQ ID NO:262, SEQ ID NO:264, or SEQ ID NO:266.

[00435] In other embodiments, the isolated nucleic acid encodes an antigen binding protein comprising an immunoglobulin light chain variable region comprising an amino acid sequence at least 95 % identical to SEQ ID NO:242, SEQ ID NO:244, SEQ ID NO:246, or SEQ ID NO:248.

[00436] Other examples of the isolated nucleic acid include such that encodes an immunoglobulin heavy chain variable region, wherein the isolated nucleic acid comprises coding sequences for three complementarity determining regions, designated CDRHl, CDRH2 and CDRH3, and wherein:

[00437] (a) CDRHl comprises the amino acid sequence of SEQ ID NO:213, SEQ ID NO:214, or SEQ ID NO:215;

[00438] (b) CDRH2 comprises the amino acid sequence of SEQ ID NO:216, SEQ ID NO:217, or SEQ ID NO:218; and

[00439] (c) CDRH3 comprises the amino acid sequence of SEQ ID NO:219, SEQ ID NO:220, or SEQ ID NO:221.

[00440] Still other examples of the isolated nucleic acid include such that encodes an immunoglobulin light chain variable region, wherein the isolated nucleic acid comprises coding sequences for three complementarity determining regions, designated CDRLl, CDRL2 and CDRL3, and wherein:

[00441] (a) CDRL1 comprises the amino acid sequence of SEQ ID NO:204, SEQ ID NO:222, SEQ ID NO:223, or SEQ ID NO:224;

[00442] (b) CDRL2 comprises the amino acid sequence of SEQ ID NO:206, SEQ ID NO:225, or SEQ ID NO:226; and

[00443] (c) CDRL3 comprises the amino acid sequence of SEQ ID NO:227, SEQ ID NO:228, SEQ ID NO:229, or SEQ ID NO:230.

[00444] In other embodiments the isolated nucleic acid encodes an antigen binding protein comprising an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO:46, SEQ ID NO:133, SEQ ID NO:139, SEQ ID NO:143, SEQ ID NO:186, or comprising any one of the foregoing sequences from which one, two, three, four or five amino acid residues are lacking from the N-terminal or C-terminal, or both.

[00445] And in some embodiments the isolated nucleic acid encodes an antigen binding protein comprising an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO:28, SEQ ID NO:131, SEQ ID NO:135, SEQ ID NO:137; or SEQ ID NO:141, or comprising any one of the foregoing sequences from which one, two, three, four or five amino acid residues are lacking from the N-terminal or C-terminal, or both.

[00446] The present invention is also directed to vectors, including expression vectors, that comprise any of the inventive isolated nucleic acids. An isolated host cell that comprises the expression vector is also encompassed by the present invention, which is made by molecular biological techniques known in the art and/or disclosed herein. The invention is also directed to a method involving:

[00447] (a) culturing the host cell in a culture medium under conditions permitting expression of the antigen binding protein encoded by the expression vector; and

[00448] (b) recovering the antigen binding protein from the culture medium. Recovering the antigen binding protein is accomplished by known methods of antbody purification, such as but not limited to, antibody purification techniques disclosed in Example 1 and elsewhere herein.

[00449] Gene Therapy

[00450] Delivery of a therapeutic antigen binding protein to appropriate cells can be effected via gene therapy ex vivo, in situ, or in vivo by use of any suitable approach known in the art. For example, for in vivo therapy, a nucleic acid encoding the desired antigen binding protein or antibody, either alone or in conjunction with a vector, liposome, or precipitate may be injected directly into the subject, and in some embodiments, may be injected at the site where the expression of the antigen binding protein compound is desired. For ex vivo treatment, the subject's cells are removed, the nucleic acid is introduced into these cells, and the modified cells are returned to the subject either directly or, for example, encapsulated within porous membranes which are implanted into the patient. See, e.g. U.S. Pat. Nos. 4,892,538 and 5,283,187.

[00451] There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cells in vitro, or in vivo in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells in vitro include the use of liposomes, electroporation, microinjection, cell fusion, chemical

treatments, DEAE-dextran, and calcium phosphate precipitation. Other in vivo nucleic acid transfer techniques include transfection with viral vectors (such as adenovirus, Herpes simplex I virus, adeno-associated virus or retrovirus) and lipid-based systems. The nucleic acid and transfection agent are optionally associated with a microparticle. Exemplary transfection agents include calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, quaternary ammonium amphiphile DOTMA ((dioleoyloxypropyl) trimethylammonium bromide, commercialized as Lipofectin by GIBCO-BRL))(Felgner et al, (1987) Proc. Natl. Acad. Sci. USA 84, 7413-7417; Malone et al. (1989) Proc. Natl Acad. Sci. USA 86 6077-6081); lipophilic glutamate diesters with pendent trimethylammonium heads (Ito et al. (1990) Biochem. Biophys. Acta 1023, 124-132); the metabolizable parent lipids such as the cationic lipid dioctadecylamido glycylspermine (DOGS, Transfectam, Promega) and dipalmitoylphosphatidyl ethanolamylspermine (DPPES)(J. P. Behr (1986) Tetrahedron Lett. 27, 5861-5864; J. P. Behr et al. (1989) Proc. Natl. Acad. Sci. USA 86, 6982-6986); metabolizable quaternary ammonium salts (DOTB, N-(1-[2,3-dioleoyloxy]propyl)-N,N,N-trimethylammonium methylsulfate (DOTAP)(Boehringer Mannheim), polyethyleneimine (PEI), dioleoyl esters, ChoTB, ChoSC, DOSC)(Leventis et al. (1990) Biochim. Inter. 22, 235-241); 3beta[N-(N', N'-dimethylaminoethane)-carbamoyl]cholesterol (DC-Chol), dioleoylphosphatidyl ethanolamine (DOPE)/3beta[N-(N',N'-dimethylaminoethane)-carbamoyl]cholesterolDC-Chol in one to one mixtures (Gao et al., (1991) Biochim. Biophys. Acta 1065, 8-14), spermine, spermidine, lipopolyamines (Behr et al., Bioconjugate Chem, 1994, 5: 382-389), lipophilic polylysines (LPLL) (Zhou et al., (1991) Biochim. Biophys. Acta 939, 8-18), [[(1,1,3,3-tetramethylbutyl)cresoxy]ethoxy]ethyl]dimethylbenzylammonium hydroxide (DEBDA hydroxide) with excess phosphatidylcholine/cholesterol (Ballas et al., (1988) Biochim. Biophys. Acta 939, 8-18), cetyltrimethylammonium bromide (CTAB)/DOPE mixtures (Pinnaduwage et al, (1989) Biochim. Biophys. Acta 985, 33-37), lipophilic diester of glutamic acid (TMAG) with DOPE, CTAB, DEBDA, didodecylammonium bromide (DDAB), and stearylamine in admixture with phosphatidylethanolamine (Rose et al., (1991) Biotechnique 10, 520-525), DDAB/DOPE (TransfectACE, GIBCO BRL),

and oligogalactose bearing lipids. Exemplary transfection enhancer agents that increase the efficiency of transfer include, for example, DEAE-dextran, polybrene, lysosome-disruptive peptide (Ohmori N I et al, Biochem Biophys Res Commun Jun. 27, 1997;235(3):726-9), chondroitan-based proteoglycans, sulfated proteoglycans, polyethylenimine, polylysine (Pollard H et al. J Biol Chem, 1998 273 (13):7507-11), integrin-binding peptide CYGGRGDTP (SEQ ID NO:235), linear dextran nonasaccharide, glycerol, cholesteryl groups tethered at the 3'-terminal internucleoside link of an oligonucleotide (Letsinger, R. L. 1989 Proc Natl Acad Sci USA 86: (17):6553-6), lysophosphatide, lysophosphatidylcholine, lysophosphatidylethanolamine, and 1-oleoyl lysophosphatidylcholine.

[00452] In some situations it may be desirable to deliver the nucleic acid with an agent that directs the nucleic acid-containing vector to target cells. Such "targeting" molecules include antigen binding proteins specific for a cell-surface membrane protein on the target cell, or a ligand for a receptor on the target cell. Where liposomes are employed, proteins which bind to a cell-surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake. Examples of such proteins include capsid proteins and fragments thereof tropic for a particular cell type, antigen binding proteins for proteins which undergo internalization in cycling, and proteins that target intracellular localization and enhance intracellular half-life. In other embodiments, receptor-mediated endocytosis can be used. Such methods are described, for example, in Wu et al., 1987 or Wagner et al., 1990. For review of the currently known gene marking and gene therapy protocols, see Anderson 1992. See also WO 93/25673 and the references cited therein. For additional reviews of gene therapy technology, see Friedmann, Science, 244: 1275-1281 (1989); Anderson, Nature, supplement to vol. 392, no 6679, pp. 25-30 (1998); Verma, Scientific American: 68-84 (1990); and Miller, Nature, 357: 455460 (1992).

[00453] Administration and Preparation of Pharmaceutical Formulations

[00454] The anti-DNP or anti-KLH antigen binding proteins or antibodies used in the practice of a method of the invention may be formulated into pharmaceutical compositions and medicaments comprising a carrier suitable for the desired delivery method. Suitable carriers include any material which, when combined with the anti-DNP or anti-KLH antigen binding protein or antibody, retains the high-affinity binding of DNP or KLH, respectively, and is nonreactive with the subject's immune systems. Examples include, but are not limited to, any of a number of standard pharmaceutical carriers such as sterile phosphate buffered saline solutions, bacteriostatic water, and the like. A variety of aqueous carriers may be used, e.g., water, buffered water, 0.4% saline, 0.3% glycine and the like, and may include other proteins for enhanced stability, such as albumin, lipoprotein, globulin, etc., subjected to mild chemical modifications or the like.

[00455] Exemplary antigen binding protein concentrations in the formulation may range from about 0.1 mg/ml to about 180 mg/ml or from about 0.1 mg/mL to about 50 mg/mL, or from about 0.5 mg/mL to about 25 mg/mL, or alternatively from about 2 mg/mL to about 10 mg/mL. An aqueous formulation of the antigen binding protein may be prepared in a pH-buffered solution, for example, at pH ranging from about 4.5 to about 6.5, or from about 4.8 to about 5.5, or alternatively about 5.0. Examples of buffers that are suitable for a pH within this range include acetate (e.g. sodium acetate), succinate (such as sodium succinate), gluconate, histidine, citrate and other organic acid buffers. The buffer concentration can be from about 1 mM to about 200 mM, or from about 10 mM to about 60 mM, depending, for example, on the buffer and the desired isotonicity of the formulation.

[00456] A tonicity agent, which may also stabilize the antigen binding protein, may be included in the formulation. Exemplary tonicity agents include polyols, such as mannitol, sucrose or trehalose. Preferably the aqueous formulation is isotonic, although hypertonic or hypotonic solutions may be suitable. Exemplary

concentrations of the polyol in the formulation may range from about 1% to about 15% w/v.

[00457] A surfactant may also be added to the antigen binding protein formulation to reduce aggregation of the formulated antigen binding protein and/or minimize the formation of particulates in the formulation and/or reduce adsorption. Exemplary surfactants include nonionic surfactants such as polysorbates (e.g. polysorbate 20, or polysorbate 80) or poloxamers (e.g. poloxamer 188). Exemplary concentrations of surfactant may range from about 0.001% to about 0.5%, or from about 0.005% to about 0.2%, or alternatively from about 0.004% to about 0.01% w/v.

[00458] In one embodiment, the formulation contains the above-identified agents (i.e. antigen binding protein, buffer, polyol and surfactant) and is essentially free of one or more preservatives, such as benzyl alcohol, phenol, m-cresol, chlorobutanol and benzethonium Cl. In another embodiment, a preservative may be included in the formulation, e.g., at concentrations ranging from about 0.1% to about 2%, or alternatively from about 0.5% to about 1%. One or more other pharmaceutically acceptable carriers, excipients or stabilizers such as those described in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980) may be included in the formulation provided that they do not adversely affect the desired characteristics of the formulation. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed and include; additional buffering agents; co-solvents; antoxidants including ascorbic acid and methionine; chelating agents such as EDTA; metal complexes (e.g. Zn-protein complexes); biodegradable polymers such as polyesters; and/or salt-forming counterions such as sodium.

[00459] Therapeutic formulations of the antigen binding protein are prepared for storage by mixing the antigen binding protein having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers,

excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, maltose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.*, Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

[00460] In one embodiment, a suitable formulation of the claimed invention contains an isotonic buffer such as a phosphate, acetate, or Tris buffer in combination with a tonicity agent such as a polyol, Sorbitol, sucrose or sodium chloride which tonicifies and stabilizes. One example of such a tonicity agent is 5% Sorbitol or sucrose. In addition, the formulation could optionally include a surfactant such as to prevent aggregation and for stabilization at 0.01 to 0.02% wt/vol. The pH of the formulation may range from 4.5-6.5 or 4.5 to 5.5. Other exemplary descriptions of pharmaceutical formulations for antibodies may be found in US 2003/0113316 and US patent no. 6,171,586, each incorporated herein by reference in its entirety.

[00461] The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide an immunosuppressive agent. Such molecules are

suitably present in combination in amounts that are effective for the purpose intended.

[00462] The active ingredients may also be entrapped in microcapsule prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsule and poly-(methylmethacylate) microcapsule, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

[00463] Suspensions and crystal forms of antigen binding proteins are also contemplated. Methods to make suspensions and crystal forms are known to one of skill in the art.

[00464] The formulations to be used for in vivo administration must be sterile. The compositions of the invention may be sterilized by conventional, well known sterilization techniques. For example, sterilization is readily accomplished by filtration through sterile filtration membranes. The resulting solutions may be packaged for use or filtered under aseptic conditions and lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration.

[00465] The process of freeze-drying is often employed to stabilize polypeptides for long-term storage, particularly when the polypeptide is relatively unstable in liquid compositions. A lyophilization cycle is usually composed of three steps: freezing, primary drying, and secondary drying; Williams and Polli, Journal of Parenteral Science and Technology, Volume 38, Number 2, pages 48-59 (1984). In the freezing step, the solution is cooled until it is adequately frozen. Bulk water in the solution forms ice at this stage. The ice sublimes in the primary drying stage, which is conducted by reducing chamber pressure below the vapor pressure of the ice, using a vacuum. Finally, sorbed or bound water is removed at the secondary drying stage under reduced chamber pressure and an elevated shelf temperature. The

process produces a material known as a lyophilized cake. Thereafter the cake can be reconstituted prior to use.

[00466] The standard reconstitution practice for lyophilized material is to add back a volume of pure water (typically equivalent to the volume removed during lyophilization), although dilute solutions of antibacterial agents are sometimes used in the production of pharmaceuticals for parenteral administration; Chen, Drug Development and Industrial Pharmacy, Volume 18, Numbers 11 and 12, pages 1311-1354 (1992).

[00467] Excipients have been noted in some cases to act as stabilizers for freeze-dried products; Carpenter et al., Developments in Biological Standardization, Volume 74, pages 225-239 (1991). For example, known excipients include polyols (including mannitol, sorbitol and glycerol); sugars (including glucose and sucrose); and amino acids (including alanine, glycine and glutamic acid).

[00468] In addition, polyols and sugars are also often used to protect polypeptides from freezing and drying-induced damage and to enhance the stability during storage in the dried state. In general, sugars, in particular disaccharides, are effective in both the freeze-drying process and during storage. Other classes of molecules, including mono- and di-saccharides and polymers such as PVP, have also been reported as stabilizers of lyophilized products.

[00469] For injection, the pharmaceutical formulation and/or medicament may be a powder suitable for reconstitution with an appropriate solution as described above. Examples of these include, but are not limited to, freeze dried, rotary dried or spray dried powders, amorphous powders, granules, precipitates, or particulates. For injection, the formulations may optionally contain stabilizers, pH modifiers, surfactants, bioavailability modifiers and combinations of these.

[00470] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antigen binding protein, which matrices are in the form of

shaped articles, *e.g.*, films, or microcapsule. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Patent No. 3,773,919), copolymers of L-glutamic acid and y ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the Lupron Depot™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated polypeptides remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C., resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S--S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

[00471] The formulations of the invention may be designed to be short-acting, fast-releasing, long-acting, or sustained-releasing as described herein. Thus, the pharmaceutical formulations may also be formulated for controlled release or for slow release.

[00472] Specific dosages may be adjusted depending on conditions of disease, the age, body weight, general health conditions, sex, and diet of the subject, dose intervals, administration routes, excretion rate, and combinations of drugs. Any of the above dosage forms containing effective amounts are well within the bounds of routine experimentation and therefore, well within the scope of the instant invention.

[00473] The antigen binding protein is administered by any suitable means, including parenteral, subcutaneous, intraperitoneal, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions

include intravenous, intraarterial, intraperitoneal, intramuscular, intradermal or subcutaneous administration. In addition, the antigen binding protein is suitably administered by pulse infusion, particularly with declining doses of the antigen binding protein or antibody. Preferably the dosing is given by injections, most preferably intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Other administration methods are contemplated, including topical, particularly transdermal, transmucosal, rectal, oral or local administration e.g. through a catheter placed close to the desired site. Most preferably, the antigen binding protein of the invention is administered intravenously in a physiological solution at a dose ranging between 0.01 mg/kg to 100 mg/kg at a frequency ranging from daily to weekly to monthly (e.g. every day, every other day, every third day, or 2, 3, 4, 5, or 6 times per week), preferably a dose ranging from 0.1 to 45 mg/kg, 0.1 to 15 mg/kg or 0.1 to 10 mg/kg at a frequency of 2 or 3 times per week, or up to 45mg/kg once a month.

[00474] The invention is illustrated by the following examples, which are not intended to be limiting in any way.

[00475] EXAMPLES

[00476] **Example 1**

[00477] **Generation of antibodies to DNP or KLH and screening**

[00478] 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.

[00479] 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).

[00480] 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.

[00481] 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.

[00482] 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.

[00483] 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.

[00484] Cloning and Engineering of Carrier Antibodies anti-KLH and anti-DNP. The sequences for the Xenomouse derived human anti-KLH antibodies were obtained by the polymerase chain reaction (PCR) amplification technique known as 5' RACE (rapid amplification of cDNA ends). Total RNA was isolated from three hybridomas expressing KLH binding monoclonal antibodies; 16.3.1, 108.1.2 and 120.6, using TRIzol reagent (Invitrogen) followed by a further purification using the RNeasy Mini Kit (Qiagen). Mixed random and oligo-dT primed first strand, RACE ready cDNAs were prepared using the GeneRacer Kit (Invitrogen). PCR amplifications of the cDNAs were performed with Advantage HF2 DNA polymerase (Clontech) with the forward primer, GeneRacer™ nested primer:

[00485] 5'- GGA CAC TGA CAT GGA CTG AAG GAG TA -3'// (SEQ ID NO:271); and the reverse primers:

[00486] 5'- CTC CTG GGA GTT ACC CGA TTG -3'// (SEQ ID NO:272, for the light chain, and 5'- GAT GGG CCC TTG GTG GAG GCT GAG GAG ACG GTG ACC GTG G -3'// (SEQ ID NO:273), for the heavy chain. The PCR reaction cycles consisted of a 30 second denaturation of the cDNA at 94°C, followed by three cycles of amplification with each cycles consisting of 20 seconds at 94°C; 30 seconds at 55°C; and 90 seconds at 72°C plus an additional 27 cycles consisting of 20 seconds at 94°C; 30 seconds at 65°C; and 90 seconds at 72°C. The reactions were then incubated for 7 minutes at 72°C following the last PCR cycle to insure complete elongation. The RACE PCR products were cloned into pCR4-TOPO (Invitrogen) and their sequences determined using ABI DNA sequencing instruments (Perkin Elmer). Consensus sequences were determined using Vector NTI 8.0 software (Invitrogen) and used to design primers for full-length antibody chain PCR amplification.

[00487] To obtain the complete coding region sequences for the expression of anti-KLH antibodies, using 16.3.1 as an example, PCR was again used. The light chain 5' PCR primer encoded the amino terminus of the signal sequence, a SalI restriction enzyme site, and an optimized Kozak sequence was:

[00488] 5'- AAG CTC GAG GTC GAC TAG ACC ACC ATG GAC ATG AGG -3'// (SEQ ID NO:274), and the 3' primer that encoded the carboxyl terminus and termination codon, as well as a NotI restriction site was:

[00489] 5'- AAC CGT TTA AAC GCG GCC GCT CAA CAC TCT CCC CTG TTG AA -3'// (SEQ ID NO:275).

[00490] The heavy chain 5' PCR primer encoded the amino terminus of the signal sequence, a SalI restriction enzyme site, and an optimized Kozak sequence was:

[00491] 5'- AAG CTC GAG GTC GAC TAG ACC ACC ATG GAA TTG GGA CTG AG -3'// (SEQ ID NO:276), and the 3' primer encoded the carboxyl terminus and termination codon, as well as a NotI restriction site was:

[00492] 5'- AAC CGT TTA AAC GCG GCC GCT CAT TTA CCC GGA GAC AGG GA -3'// (SEQ ID NO:277).

[00493] The PCRs were performed using Advantage HF2 DNA polymerase and the reaction cycles consisted of a 30 second denaturation of the cDNA at 94°C, followed by 30 cycles consisting of 20 seconds at 94°C; 30 seconds at 65°C; and 90 seconds at 72°C. The reactions were then incubated for 7 minutes at 72°C following the last PCR cycle to insure complete elongation. The resulting PCR products were gel isolated, purified using QIAquick spin columns (Qiagen), digested with SalI (NEBL) and NotI (NEBL), gel isolated and purified using QIAquick spin columns, and then ligated into the mammalian expression vector pTT5.

[00494] The sequences for the XenoMouse®-derived human anti-DNP antibody variable regions were obtained by sequencing reverse transcription PCR products. PCR was then used to adapt the variable region sequence ends to make them compatible with the ends of pTT5 vectors containing a VK1 signal peptide and the appropriate antibody constant region. As example, anti-DNP 3A4 light chain was cloned into pTT5 using the unique BssHII site at the end of a Vk1 signal peptide and the unique BsiW1 site at the beginning of the human kappa constant region. To add

the BssHII and the BsiWI sites to the ends of the 3A4 variable region was amplified by PCR using 5' primer:

[00495] 5' TTT TTT TTG CGC GCT GTG ACA TCC AGA TGA CCC AGT C 3'// (SEQ ID NO:278),

[00496] and 3' primer 5' AAA AAA CGT ACG TTT GAT ATC CAC TTT GGT CC 3'// (SEQ ID NO:279).

[00497] The anti-DNP 3A4 contained a tryptophan in the variable region of the heavy chain. The tryptophan codon was mutated to a phenylalanie by PCR using (+) strand primer:

[00498] 5' CTG TGT ATT ACT GTG CGA GGT ATA ACT TCA ACT ACG GTA TGG ACG TCT GG 3'// (SEQ ID NO:280) and (-) strand primer:

[00499] 5' CCA GAC GTC CAT ACC GTA GTT GAA GTT ATA CCT CGC ACA GTA ATA CAC AG 3'// (SEQ ID NO:281) and to a tyrosine by PCR using (+) strand primer:

[00500] 5' CTG TGT ATT ACT GTG CGA GGT ATA ACT ACA ACT ACG GTA TGG ACG TCT GG 3'// (SEQ ID NO:282) and (-) strand primer:

[00501] 5' CCA GAC GTC CAT ACC GTA GTT GTA GTT ATA CCT CGC ACA GTA ATA CAC AG 3'// (SEQ ID NO:283) in conjunction with the heavy chain 5' end primer:

[00502] 5' AAG CTC GAG GTC GAC TAG ACC ACC ATG GAC ATG AGG GTG CCC GCT CAG CTC CTG GGG CT 3'// (SEQ ID NO:284) and the heavy chain 3' primer:

[00503] 5' AAC CGT TTA AAC GCG GCC GCT CAT TTA CCC GGA GAC AGG GA 3'// (SEQ ID NO:285). Also, to reduce disulfide scrambling in the hinge region of the 3A4 IgG2 heavy chain , as example, the hinge cysteines 219 and 220 (EU numbering) were mutated by PCR using (+) strand primer:

[00504] 5' GGA CAA GAC AGT TGA GCG CAA ATC TTC TGT CGA GTG CCC ACC GTG CCC AG 3'// (SEQ ID NO:286) and (-) strand primer:

[00505] 5' CTG GGC ACG GTG GGC ACT CGA CAG AAG ATT TGC GCT CAA CTG TCT TGT CC 3'// (SEQ ID NO:287) in conjunction with the heavy chain 5' end primer:

[00506] 5' AAG CTC GAG GTC GAC TAG ACC ACC ATG GAC ATG AGG GTG CCC GCT CAG CTC CTG GGG CT 3'// (SEQ ID NO:288) and the heavy chain 3' primer:

[00507] 5' AAC CGT TTA AAC GCG GCC GCT CAT TTA CCC GGA GAC AGG GA 3'// (SEQ ID NO:289).

[00508] 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.).

[00509] 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.

[00510] 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.

[00511] The aDNP 3A4-F and aDNP 3B1 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.

[00512] 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.

[00513] The lead candidates were then selected based on the product behavior by SDS-PAGE. The aDNP 3B1, 3H4, 3C2, 3A1 and 3A4 antibodies from both transient and stable expression mammalian cell lines were analyzed for product quality on a 1.0-mm Tris-glycine 4-20% SDS-PAGE (Novex) using reducing loading buffer (Figure 11). Using these data, the aDNP 3H4 antibody produced a heterogenous product from the stable cell line, which indicated that it was not a good candidate as a carrier antibody, since a homogenous product is desirable. The aDNP 3A1, 3A4, 3C2, and 3B1 and aKLH 120.6 antibodies were analyzed for product quality on a 1.0-mm Tris-glycine 4-20% SDS-PAGE (Novex) using non-reducing loading buffer (Figure 12A-B). The aDNP 3C2 antibody produced a heterogenous product with exceptional high molecular mass material, indicating it was not an ideal candidate as a carrier antibody, since a product containing high molecular mass material is not desirable. In addition, the aDNP 3B1 antibody showed a doublet under these conditions. The aDNP 3B1 and aDNP 3A1 antibodies were then compared using both Tris-glycine SDS-PAGE as well as bis-Tris NuPAGE systems under non-reducing conditions (Figure 13A-B). It was found that the aDNP 3B1 antibody clearly produces a doublet not observed with aDNP 3A1 on the Tris-glycine SDS-PAGE; however, the aDNP 3B1 antibody appeared more homogenous than the aDNP 3A1 antibody when analyzed by bis-Tris NuPAGE, indicating that the doublet may be an artifact of the method of analysis. When the aDNP 3B1 antibody was analyzed by Tris-glycine SDS-PAGE after treatment with non-reducing sample buffer at room temperature, 85 °C, or 100 °C, the doublet was not eliminated (Figure 14A). However, when the aDNP 3B1 antibody was examined by Tris-glycine SDS-PAGE using 0.4% SDS in the gel running buffer rather than the

usual 0.1%, the doublet was greatly reduced (Figure 14B), offering additional evidence that the doublet was an artifact of the system of analysis.

[00514] Antibodies were further analyzed for homogeneity using two size exclusion columns (TSK-GEL G3000SWXL, 5 mm particle size, 7.8 x 300 mm, TosohBioscience, 08541) in series with a 100 mM sodium phosphate, 250 mM NaCl, pH 6.8, mobile phase flowed at 0.5 mL/min (Figure 15). The aDNP 3C2 antibody displayed a substantial post-peak shoulder, which was deemed undesirable, so this antibody was demoted as a candidate carrier antibody. In addition, it was observed that the aDNP 3C2 and aDNP 3A4 antibodies eluted later than expected indicating a potential interactions with the stationary phase of the chromatography column.

[00515] Antibodies (aDNP 3A1, aDNP 3C2 and aDNP 3A4) were tested for resistance to photodegradation. The antibodies were either exposed to fluorescent light at 4 °C for 3 weeks or were protected from light by covering samples of each with aluminum foil. The antibody samples were then analyzed using two size exclusion columns (TSK-GEL G3000SWXL, 5 mm particle size, 7.8 x 300 mm, TosohBioscience, 08541) in series with a 100 mM sodium phosphate, 250 mM NaCl, pH 6.8, mobile phase flowed at 0.5 mL/min (Figure 16). The aDNP 3C2 and aDNP 3A4 antibodies showed substantial peak broadening after light exposure, which is consistent with oxidation of a susceptible tryptophan. To reduce the oxidation susceptibility of the aDNP 3A4 antibody, several variants with the CDR3 tryptophan mutated to either tyrosine or phenylalanine were constructed (aDNP 3A4, aDNP 3A4-Y, aDNP 3A4-F, aDNP 3A4-YSS and aDNP 3A4-FSS). These antibodies were then evaluated by SEC for resistance to photodegradation after two days of light exposure (336W/m2 UV light and 331 k-lux for fluorescent light) at 6 °C, by analysis using two size exclusion columns (TSK-GEL G3000SWXL, 5 mm particle size, 7.8 x 300 mm, TosohBioscience, 08541) in series with a 100 mM sodium phosphate, 250 mM NaCl at pH 6.8 mobile phase flowed at 0.5 mL/min (Figure 17A-B).

[00516] All four of the aDNP 3A4 variants showed substantially less peak broadening than the wild type molecule, indicating that the CDR3 tryptophan was responsible for this undesirable phenomenon. Furthermore, the retention time extension on SEC was also greatly reduced with the variants indicating less interaction with the stationary phase of the column. Anti-DNP 3A4 antibodies with various mutations (aDNP 3A4, aDNP 3A4-Y, aDNP 3A4-F, aDNP 3A4-YSS and aDNP 3A4-FSS) were analyzed for homogeneity using a Tosohaas SP-5PW column (10-μm particle, 7.5 mm ID X 7.5 cm long) using Buffer A (10 mM sodium acetate, pH 5.0) and Buffer B (10 mM sodium acetate, 600 mM NaCl, pH 5.0) flowed at 1 ml/min with a programmed linear gradient (1 min 0%B, 10 min 35% B, 30 min 70% B, 3 min 90%B and 3 min 0%B) (Figure 18). The aDNP 3A4 antibody with the CDR3 tryptophan converted to phenylalanine produced a more desirable narrower elution peak than the wild type or tyrosine variant; therefore, the aDNP 3A4-F variant was deemed to be the superior molecule. The aDNP 3B1, aDNP 3A4-F, and aDNP 3A4-FSS antibodies were analyzed by non-reducing CE-SDS (Figure 19A-C). All CE SDS experiments were performed using Beckman PA800 CE system (Fullerton, CA) equipped with UV diode detector. 221 nm and 220 nm wavelength were employed. A bare-fused silica capillary 50 μm x 30.2 cm was used for the separation analysis. Buffer vial preparation and loading as well as Install Capillary Cartridge were described in the Beckman Coulter manual for IgG Purity/Heterogeneity. The running conditions for reduced and non-reduced CE-SDS were similar to those described in Beckman Coulter manual for IgG Purity/Heterogeneity with some modifications which are briefly described below. For non-reducing conditions, the antibody sample (150 μg) was added 20 μl of SDS reaction buffer and 5 μl of 70 mM N-ethylmaleimide. Water was then added to make final volume 35 μl and the protein concentration was brought to 4.3 mg/ml. The SDS reaction buffer was made of 4% SDS, 0.01 M citrate phosphate buffer (Sigma) and 0.036 M sodium phosphate dibasic. The preparation was vortexed thoroughly, and heated at 45°C for 5 min. The preparation was then added additional 115 μl of 4% SDS. After being vortexed and centrifuged, the preparation was placed in a 200 μl PCR vial and then loaded onto the PA800 instrument. The

sample was injected at the anode with reverse polarity using -10 kV for 30 sec, and was then separated at -15 kV with 20 psi pressure at both ends of capillary during 35 min separation. The aDNP 3B1 antibody produced the most desirable profile with the highest level of uniformity under non-reducing conditions. The aDNP 3B1, aDNP 3A4-F, and aDNP 3A4-FSS antibodies were analyzed by reducing CE-SDS (Figure 20A-C herein). For reducing conditions, the antibody sample was diluted to 2.1 mg/ml by adding purified H_2O, and 95 µl of the antibody was added 105 µl of SDS sample buffer (Beckman) with 5.6% beta mercaptoethanol. The preparation was then vortexed thoroughly and then heated at 70°C for 10 min. After being centrifuged, the supernatant was placed in a 200 µl PCR vial and then loaded onto the PA800 instrument. The sample was injected at the anode with reverse polarity using -5 kV for 20 sec, and was then separated at -15 kV with 20 psi pressure at both ends of capillary during 30 min separation. The aDNP 3A4-F produced the most desirable uniform peaks under reducing conditions.

[00517] The aDNP 3A4-F, aDNP 3A4-FSS and aDNP 3B1 antibodies were analyzed for thermoresistance by DSC using a MicrCal VP-DSC where the samples were heated from 20°C to 95°C at a rate of 1°C per minute. The proteins were at 0.5 mg/ml in 10 mM sodium acetate, 9% sucrose, pH 5.0 (Figure 21). The aDNP 3B1 and aDNP 3A4-F antibodies produced the most desirable melting profiles, with a higher temperature for the initial transition. The aDNP 3B1 and aDNP 3A4-F antibodies were differentiated by the presence of a single melting transition for the aDNP 3B1 antibody and a double transition for the aDNP 3A4-F antibody.

[00518] ELISA assays. ELISA assays were conducted as follows. Costar 3072 medium binding 384 well plates (Corning Life Sciences) were coated with DNP-BSA (BioSearch Technologies, Novato, CA) at 5 µg/ml in 1XPBS/0.05% Azide, (40 µl/well). The plates were incubated at 4°C overnight. The plates were then washed using 3-cycle wash on a Titertek M384 plate washer (Titertek, Huntsville, AL). The plates were blocked with 90 µl of 1XPBS/1% milk and incubated approximately 30 minutes at room temperature. The plates were then washed using a 3-cycle wash on a Titertek plate washer. 10ul antibody samples were added to 40ul 1XPBS/1% milk.

The plates were then incubated for 1 hour at room temperature. Next, plates were then washed using 3-cycle wash on a Titertek M384 plate washer (Titertek, Huntsville, AL). Goat anti Human IgG Fc HRP was then added at 100 ng/ml (1:4000) in 1XPBS/1%milk/10mM Ca^{2+} (50 µl/well) was added to the plate and was incubated 1 hour at room temperature. The plates were washed once again, using a 3-cycle wash. The plates were then patted dry with paper towel. Finally, 1 step TMB (Neogen, Lexington, Kentucky) (50 µl/well) was added to the plate and was quenched with 1N hydrochloric acid (50 µl/well) after 30 minutes at room temperature. OD's were read immediately at 450 nm using a Titertek plate reader.

[00519] **Example 2**

[00520] **Pharmacokinetic (PK) & Pharmacodynamic (PD) Studies of Anti-DNP Antibody Embodiments of the Invention**

[00521] The pharmacokinetic profile of the aDNP 3A4-F, aDNP 3A4-FSS and aDNP 3B1 antibodies was determined in adult Sprague-Dawley rats (8-12 weeks old) by injecting 5 mg/kg subcutaneously and collecting approximately 250 µL of blood in Microtainer® serum separator tubes at 0, 0.25, 1, 4, 24, 48, 72, 96, 168, 336, 504, 672, 840 and 1008 hours post-dose from the lateral tail vein (Figure 22). Each sample was maintained at room temperature following collection, and following a 30-40 minute clotting period, samples were centrifuged at 2-8°C at 11,500 rpm for about 10 minutes using a calibrated Eppendorf 5417R Centrifuge System (Brinkmann Instruments, Inc., Westbury, NY). The collected serum was then transferred into a pre-labeled (for each rat), cryogenic storage tube and stored at -60°C to -80°C for analysis. To measure the serum sample concentrations from the PK study samples, the following method was used: ½ area black plate (Corning 3694) was coated with 2 µg/ml of Anti-hu FC, Ab 1.35.1 in 1x PBS and then incubated overnight at 4°C. The plate was washed and blocked with I-Block™ (Applied Biosystems) overnight at 4°C. If samples needed to be diluted, then they were diluted in Rat SD serum. The standards and samples were diluted 1: 20 in I-

Block™ + 5% BSA into 380 µl of diluting buffer. The plate was washed and 50-µl samples of pretreated standards and samples were transferred into an Ab 1.35.1 coated plate and incubated for 1.5 h at room temperature. The plate was washed, then 50 µl of 100 ng/ml of anti-hu FC Ab 21.1-HRP conjugate in I-Block™ +5% BSA were added and incubated for 1.5 h. The plate was washed, then 50 µl of Pico substrate were added, after which the plate was immediately analyzed with a luminometer. The pharmacokentic profile was good for all antibodies, but the aDNP 3B1 showed the best overall profile.

[00522] The pharmacokinetic profile of the aDNP 3A4-F antibody was determined in 6 male cynomologous monkeys (3-7 kg) by injecting 6 mg/kg bolus dose intravenously and taking blood samples at 0 and 30 minutes and 2, 7, 9, 11, 14, 21, 28, 35, 42, 49, 56 and 63 days (Figure 23). The pharmacokinetic profile of the aKLH 120.6 antibody was determined in 4 male cynomologous monkeys (2-4 kg) by injecting 3 mg/kg bolus dose intravenously and taking blood samples at 0, 0.25, 1, 4, 8, 12, 24, 72, 168, 240, 336, 408, 504, 576, 672, 744, 840, 1008, 1176 and 1344 hours (Figure 23). To measure the serum sample concentrations from the PK study samples, the same method as mentioned above for the rat pharmacokinetic study was employed. The pharmacokinetic profile for both antibodies in cynomologous monkeys was good, but the dose normalized profile for the aKLH 120.6 was marginally better than that of the aDNP 3A4-F.

[00523] **Example 3**

[00524] **Human tissue cross-reactivity assessment**

[00525] In general accordance with the guidance laid out in Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use (U.S. Department of Health and Human Services, Food and Drug Administraton, Center for Biologics Evaluation and Research (1997)), a preliminary non-GLP study was carried out to determine cross-reactivity of inventive antibodies with a variety of human tissues. If an antibody is intended for drug development, a more extensive

testing under GLP conditions is required. The tissue cross-reactivity of antibodies aDNP 3A4-F and aKLH 120.6 was evaluated (Charles River Laboratories, Preclinical Services, Reno, NV) with cryosections of selected human tissues using Alexa Fluor 488 labeled forms of the test articles. Normal human tissues from two unique individuals (unless otherwise indicated) were obtained from the Special Pathology Services Human Tissue Bank collected by the National Disease Research Interchange (NDRI, Philadelphia, PA), Cureline, Inc. (Burlingame, CA), Cybrdi (Rockville, MD), or Rocky Mountain Lions Eye Bank (Aurora, CO). Tissues tested included human cerebellum, lung, cerebral cortex, ovary (from mature female), eye, placenta, gastrointestinal tract (small intestine), skin (1 individual), heart, spleen, kidney (1 individual), thyroid, liver, testis. Sections of fresh-frozen human tissues and control bead blocks (DNP[31]-bovine serum albumin [BSA] beads [positive], and human serum albumin [HSA] beads [negative]) were cut on the cryostat and thaw mounted onto capillary gap slides. The tissue and control bead slides were fixed in cold acetone for approximately 10 minutes at -10°C to -25°C. The fixed slides were allowed to dry for at least one hour (to overnight). If stored frozen, fixed slides were removed from the freezer on the day prior to an experiment and allowed to thaw overnight prior to use. All the following steps were performed at room temperature unless otherwise specified. The slides were incubated with 1X Morphosave™ for approximately 15 minutes to preserve tissue morphology then washed two times for approximately 5 minutes each in1X phosphate-buffered saline (PBS). To block endogenous peroxidase, the slides were incubated in a glucose oxidase solution for approximately 1 hour at approximately 37°C. The slides were washed two times in 1X PBS for approximately 5 minutes each. Endogenous biotin was blocked by sequential incubation (approximately 15 minutes each) in avidin and biotin solutions. Following the incubation in biotin, the tissue sections were blocked with a blocking antibody solution for approximately 25 minutes. Alexa Fluor 488-Ab 3A4 W101F (anti-DNP), and Alexa Fluor 488 anti-KLH (anti-KLH Ab) were applied to sections at the optimal concentration (2.0 μg/mL) or 5 times the optimal concentration (10.0 μg/mL) for approximately 25 minutes. Slides were washed 3 times with wash buffer and then incubated with the secondary antibody (rabbit anti-

Alexa Fluor 488) for approximately 25 minutes. Following incubation with the secondary antibody, slides were washed 4 times with wash buffer then incubated with the tertiary antibody (horseradish peroxidase conjugated goat anti-rabbit IgG antibody) for approximately 25 minutes and binding visualized with a diaminobenzidine (DAB) chromogen substrate. DNP(31)-BSA beads were used as a positive control in all experiments. HSA beads were used as a negative control. Tissues were qualified as adequate for immunohistochemistry via staining with an antibody against CD31 (anti-CD31) i.e., platelet endothelial cell adhesion molecule (PECAM-1). There was no specific staining in any human tissue examined at either 2.0 or 10.0 μg/mL concentration for any of the tested antibodies.

[00526] **Example 4**

[00527] **Expression and Purification of Monovalent or Multivalent Immunoglobulin- and/or Fc domain-Toxin Peptide Analog Fusions**

[00528] An assortment of monovalent, bivalent and trivalent structures were expressed and purified for comparison, including exemplary embodiments of the invention. Those included aKLH IgG2/Fc-ShK variants (see schematic representation of Figure 1E: "hemibody" configuration), and anti-KLH IgG2-ShK variants (see Figure 1F-L). For example, bivalent Fc-L10-ShK[1-35], monovalent anti-Keyhole Limpet Hemocyanin (KLH) immunoglobulin heavy chain-[Lys16]ShK fusion antibody (designated "aKLH HC-[Lys16]ShK Ab"; see Figure 1F), and monovalent anti-KLH immunoglobulin light chain-[Lys16]ShK antibody (designated "aKLH LC-[Lys16]ShK Ab"; see Figure 1J). IgG2 Fc/Fc-ShK variants (see Figure 1A), bivalent Fc-L10-ShK[2-35], monovalent Fc/Fc-L10-ShK[2-35] were made for comparison, by recombinant methods as described in Sullivan et al., WO 2008/088422 A2, and in particular Examples 1, 2, and 56 therein, incorporated by reference in its entirety, or as modified herein.

[00529] 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 (6 mM) and Geneticin (25 μg/ml) at 37°C, 5% CO_2, 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.

[00530] 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.

[00531] 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:

MEWSWVFLFFLSVTTGVHSERKVECPPCPAPPVAGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVL
TVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKL
TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK// (SEQ ID NO:1),
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 therein). 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 therein). These ShK constructs have the following modified VH21 Signal peptide amino acid sequence of MEWSWVFLFFLSVTTGVHSERKVECPPCP// SEQ ID NO:2 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:3); and
5'- CA CGG TGG GCA CTC GAC TTT GCG CTC GGA GTG GAC ACC -3' (SEQ ID NO:4).

[00532] Wild Type ShK[2-35] with N-terminal linker extension (amino acid sequence GGGGSGGGGSSCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC// SEQ ID NO:6) was encoded by the DNA sequence below:
GGAGGAGGAGGATCCGGAGGAGGAGGAAGCAGCTGCATCGACACCATC CCCAAGAGCCGCTGCACCGCCTTCCAGTGCAAGCACAGCATGAAGTACC GCCTGAGCTTCTGCCGCAAGACCTGCGGCACCTGC// (SEQ ID NO:5). A fragment containing this coding sequence (SEQ ID NO:5) was generated using the oligos below (SEQ ID NO:7 and SEQ ID NO:8)-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 therein, incorporated by reference):

5'-GTC CAC TCC GAG CGC AAA GTC GAG TGC CCA CCG TGC C-3' (SEQ ID NO:7); and
5'- TCC TCC TCC TTT ACC CGG AGA CAG GGA GAG -3'// (SEQ ID NO:8).

[00533] 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:9); and
5'- GCT GTG CTT GCA CTT GAA GGC GGT GCA GC -3' (SEQ ID NO:10); 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-B therein) resulting in the DNA coding sequence
GGAGGAGGAGGATCCGGAGGAGGAGGAAGCAGCTGCATCGACACCATC
CCCAAGAGCCGCTGCACCGCCTTCAAGTGCAAGCACAGCATGAAGTACC
GCCTGAGCTTCTGCCGCAAGACCTGCGGCACCTGC// (SEQ ID NO:11),
which encodes the amino acid sequence Shk(2-35, K16) with a N-terminal linker extension: GGGGSGGGGSSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCGTC// SEQ ID NO:12).

[00534] 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 therein) and oligos:

5'-GTC CAC TCC GAG CGC AAA GTC GAG TGC CCA CCG TGC C-3' (SEQ ID NO:7); and
5'- TCC TCC TCC TTT ACC CGG AGA CAG GGA GAG -3' (SEQ ID NO:8).

[00535] The IgG2Fc region was generated using oligos:

5'-CCG GGT AAA GGA GGA GGA GGA TCC GGA G-3' (SEQ ID NO:13); and
5'- CAT GCG GCC GCT CAT TAG CAG GTG -3' (SEQ ID NO:14), and the pSelexis Vh21-hIgG2-Fc template resulting in a fragment containing the following DNA coding sequence:
GCACCACCTGTGGCAGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAA
GGACACCCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGG
ACGTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGG
CGTGGAGGTGCATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAAC
AGCACGTTCCGTGTGGTCAGCGTCCTCACCGTTGTGCACCAGGACTGGCT
GAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCAGCC
CCCATCGAGAAAACCATCTCCAAAACCAAAGGGCAGCCCCGAGAACCAC
AGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGT
CAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGG
AGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACACCTCC
CATGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGG

ACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCA
TGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGG
GTAAA // SEQ ID NO:15, which encodes the amino acid sequence
APPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVE
VHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEK
TISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGK SEQ ID NO:16).

[00536] 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:3); and
5'- CAT GCG GCC GCT CAT TAG CAG GTG -3' (SEQ ID NO:14).

[00537] 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:

MEWSWVFLFFLSVTTGVHSERKVECPPCPAPPVAGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVL
TVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKL

TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSSCID TIPKSRCTAFQCKHSMKYRLSFCRKTCGTC// (SEQ ID NO:17).

[00538] The pTT14-VH21SP-IgG2-Fc-L10-ShK(2-35,Q16K) construct encoded a IgG2-Fc L10-ShK(2-35, Q16K) fusion polypeptide sequence:

MEWSWVFLFFLSVTTGVHSERKVECPPCPAPPVAGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVL TVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSSCID TIPKSRCTAFKCKHSMKYRLSFCRKTCGTC// SEQ ID NO:18;

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: MEWSWVFLFFLSVTTGVHSERKVECPPCPAPPVAGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVL TVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSRSCI DTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC// (SEQ ID NO:19).

[00539] 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:20); and 5'- CA CGG TGG GCA CTC GAC TTT GCG CTC GGA GTG GAC ACC -3' (SEQ ID NO:4), and using the pSelexis template as noted above.

[00540] 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:7); and

5'- CAT GGA TCC TCA TTT ACC CGG AGA CAG GGA G -3' (SEQ ID NO:21).

[00541] 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:1 above).

[00542] Anti-KLH 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

GGATCCGGAGGAGGAGGAAGCCGCAGCTGCATCGACACCATCCCCAAGA
GCCGCTGCACCGCCTTCAAGTGCAAGCACAGCATGAAGTACCGCCTGAG
CTTCTGCCGCAAGACCTGCGGCACCTGCTAATGAGCGGCCGCTCGAGGCC
GGCAAGGCCGGATCC// (SEQ ID NO:22)

was cut out using BamHI/BamHI. This coding sequence (SEQ ID NO:23) encodes ShK(1-35, Q16K) with an N-terminal linker sequence:
GSGGGGSRSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCGTC// (SEQ ID NO:23).

[00543] 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

ATGGAATGGAGCTGGGTCTTTCTCTTCTTCCTGTCAGTAACGACTGGTGT
CCACTCCGAGCGCAAAGTCGAGTGCCCACCGTGCCCAGCACCACCTGTG
GCAGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCAT
GATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGACGTGAGCCAC
GAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCGTGGAGGTGC
ATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGCACGTTCCG
TGTGGTCAGCGTCCTCACCGTTGTGCACCAGGACTGGCTGAACGGCAAG
GAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCAGCCCCCATCGAGA
AAACCATCTCCAAAACCAAAGGGCAGCCCCGAGAACCACAGGTGTACAC
CCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACC
TGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGA
GCAATGGGCAGCCGGAGAACAACTACAAGACCACACCTCCCATGCTGGA
CTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCA
GGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTG
CACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAGGAG
GAGGA // (SEQ ID NO:24), encoding the amino acid sequence
MEWSWVFLFFLSVTTGVHSERKVECPPCPAPPVAGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVL
TVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKL
TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGG// (SEQ ID
NO:25).

[00544] 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-L10-ShK(1-35, Q16K) fusion protein sequence:

MEWSWVFLFFLSVTTGVHSERKVECPPCPAPPVAGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVL
TVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKL
TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSRSCI
DTIPKSRCTAFKCKHSMKYRLSFCRKTCGTC// (SEQ ID NO:26).

[00545] 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 by Figure 1E) included:

[00546] (a) aKLH 120.6 kappa LC (SEQ ID NO:28, below), which incorporates a N-terminal VK-1 SP signal peptide sequence(SEQ ID NO:103):

MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSLSASVGDRVTITCRASQGIR
NDLGWYQQKPGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQPEDF
ATYYCLQHNSYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL
NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE
KHKVYACEVTHQGLSSPVTKSFNRGEC// (SEQ ID NO:28);

[00547] (b) aKLH 120.6 IgG2 HC (SEQ ID NO:29, below), which incorporates a N-terminal VK-1 SP signal peptide sequence(SEQ ID NO:103):

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASGY
TFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSI
STAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSASTKGPS
VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAP
PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEV

HNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKT
ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGK// (SEQ ID NO:29);

[00548] and

[00549] (c) IgG2 Fc-L10-ShK(1-35):

MEWSWVFLFFLSVTTGVHSERKVECPPCPAPPVAGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVL
TVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKL
TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSRSCI
DTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC// (SEQ ID NO:30).

[00550] The desired aKLH IgG2/Fc-ShK product contained one copy of each of components (a)-(c), immediately above, configured as in Figure 1E. 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.

[00551] Monovalent aKLH 120.6 IgG2-ShKand ShK peptide analog fusions.

[00552] The components of the aKLH 120.6 IgG2-ShK fusion antibody (schematically represented in Figure 1F) included monomers:

[00553] (a) aKLH 120.6 kappa LC (SEQ ID NO:28, above);

[00554] (b) aKLH 120.6 IgG2 HC (SEQ ID NO:29, above); and

[00555] (c) aKLH 120.6 IgG2-ShK fusion having the following HC sequence:

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASGY
TFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSI

STAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSASTKGPS
VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAP
PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEV
HNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKT
ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGGGGGSGGGGSRSCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCG
TC// (SEQ ID NO:31).
[00556]

[00557] The components of a monovalent aKLH 120.6 IgG2-ShK[1-35, Q16K] fusion antibody (schematically represented in Figure 1F) included monomers:

[00558] (a) aKLH 120.6 kappa LC (SEQ ID NO:28, above);

[00559] (b) aKLH 120.6 IgG2 HC (SEQ ID NO:29, above); and

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

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASGY
TFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSI
STAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSASTKGPS
VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAP
PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEV
HNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKT
ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGGGGGSGGGGSRSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCG
TC// (SEQ ID NO:32).

[00561] The components of the monovalent aKLH 120.6 HC-ShK[1-35, R1A, I4A, Q16K] fusion antibody (schematically represented in Figure 1F) included the following monomers:

[00562] (a) aKLH 120.6 kappa LC (SEQ ID NO:28);

[00563] (b) aKLH 120.6 IgG2 HC (SEQ ID NO:29); and

[00564] (c) aKLH 120.6 IgG2 HC-ShK[1-35, R1A, I4A, Q16K] fusion having the following amino acid sequence:

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASGY
TFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSI
STAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSASTKGPS
VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAP
PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEV
HNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKT
ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGGGGGSGGGGSASCADTIPKSRCTAFKCKHSMKYRLSFCRKTCG
TC// (SEQ ID NO:304).

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 aKLH 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.

[00565] The components of the monovalent aKLH 120.6 HC-ShK[1-35, R1A, Q16K, K30E] fusion antibody (schematically represented in Figure 1F) included the following monomers:

[00566] (a) aKLH 120.6 kappa LC (SEQ ID NO:28);

[00567] (b) aKLH 120.6 IgG2 HC (SEQ ID NO:29); and

[00568] (c) aKLH 120.6 IgG2-ShK[1-35, R1A, Q16K, K30E] fusion having the following sequence:

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASGY TFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSI STAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAP PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEV HNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKT ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGGGGGSGGGGSASCIDTIPKSRCTAFKCKHSMKYRLSFCRETCGT C// (SEQ ID NO:305).

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 aKLH 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.

[00569] The components of the monovalent aKLH 120.6 HC (IgG2)-ShK[1-35, R1H, I4A, Q16K] fusion antibody (schematically represented in Figure 1F) included monomers:

[00570] (a) aKLH 120.6 kappa LC (SEQ ID NO:28);

[00571] (b) aKLH 120.6 IgG2 HC (SEQ ID NO:29); and

[00572] (c) aKLH 120.6 HC IgG2-ShK[1-35, R1H, I4A, Q16K] fusion having the following amino acid sequence:

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASGY
TFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSI
STAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSASTKGPS
VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAP
PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEV
HNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKT
ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGGGGGSGGGGSHSCADTIPKSRCTAFKCKHSMKYRLSFCRKTCG
TC// (SEQ ID NO:306).

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 aKLH 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.

[00573] The components of the monovalent aKLH 120.6 HC-ShK[1-35, R1H, Q16K, K30E] fusion antibody(schematically represented in Figure 1F) included the monomers:

[00574] (a) aKLH 120.6 kappa LC (SEQ ID NO:28);

[00575] (b) aKLH 120.6 IgG2 HC (SEQ ID NO:29); and

[00576] (c) aKLH 120.6 IgG2-ShK[1-35, R1H, Q16K, K30E] fusion having the following sequence:

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASGY
TFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSI
STAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSASTKGPS
VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAP
PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEV
HNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKT
ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGGGGGSGGGGSHSCIDTIPKSRCTAFKCKHSMKYRLSFCRETCGT
C// (SEQ ID NO:307).

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 aKLH 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.

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

[00578] (a) aKLH 120.6 kappa LC (SEQ ID NO:28);

[00579] (b) aKLH 120.6 IgG2 HC (SEQ ID NO:29); and

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

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASGY
TFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSI
STAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSASTKGPS
VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAP
PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEV
HNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKT
ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGGGGGSGGGGSKSCADTIPKSRCTAFKCKHSMKYRLSFCRKTCG
TC// (SEQ ID NO:308).

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 aKLH 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.

[00581] The components of the monovalent aKLH 120.6 HC-ShK[1-35, R1K, Q16K, K30E] fusion antibody (schematically represented in Figure 1F) included the monomers:

[00582] (a) aKLH 120.6 kappa LC (SEQ ID NO:28);

[00583] (b) aKLH 120.6 IgG2 HC (SEQ ID NO:29); and

[00584] (c) aKLH 120.6 IgG2-ShK[1-35, R1K, Q16K, K30E] fusion having the following amino acid sequence:

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASGY
TFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSI
STAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSASTKGPS
VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAP
PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEV
HNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKT
ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGGGGGSGGGGSKSCIDTIPKSRCTAFKCKHSMKYRLSFCRETCGT
C// (SEQ ID NO:309).

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 aKLH 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.

[00585] The components of a monovalent aKLH 120.6 IgG2-ShK[2-35, Q16K] fusion antibody (schematically represented in Figure 1F) included monomers:

[00586] (a) aKLH 120.6 kappa LC (SEQ ID NO:28, above);

[00587] (b) aKLH 120.6 IgG2 HC (SEQ ID NO:29, above); and

[00588] (c) aKLH 120.6 IgG2-ShK[2-35, Q16K] fusion having the following HC sequence:

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASGY
TFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSI
STAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSASTKGPS
VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAP
PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEV
HNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKT
ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGGGGGSGGGGSSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCGT
C// (SEQ ID NO:33).

[00589] The desired aKLH 120.6 IgG2-ShK analog product was a full antibody with the ShK peptide fused to the C-terminus of one heavy chain, configured as in Figure 1F. 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, and bivalent aKLH 120.6 IgG2-ShK. The monovalent aKLH 120.6 IgG2-toxin peptide (or toxin peptide analog) fusion antibody was isolated from the mix using cation exchange chromatography, as described herein.

[00590] Anti-KLH IgG1-loop-ShK. The aKLH IgG1-loop-ShK 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 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 antibody include

[00591] (a) aKLH 120.6 kappa LC (SEQ ID NO:28, above);

[00592] (b) aKLH 120.6 IgG1 HC:

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASGY
TFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSI
STAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSASTKGPS
VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPC
PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH
YTQKSLSLSPGK// (SEQ ID NO:34);
[00593] and

[00594] (c) aKLH 120.6 IgG1-loop-ShK:

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASGY
TFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSI
STAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSASTKGPS
VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPC
PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSRDELGGRSCIDTIPKSRCTAFKCKHSMKYR
LSFCRKTCGTCGGTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK//
(SEQ ID NO:35).

[00595] 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, and bivalent aKLH 120.6 IgG1-loop-ShK. The monovalent aKLH 120.6 IgG1-loop-ShK fusion antibody (represented schematically by Figure 1N) was isolated from the mix using cation exchange chromatography as described herein.

[00596] 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 antibody (schematically represented in Figure 1J) included the monomers:

[00597] (a) aKLH 120.6 IgG2 HC (SEQ ID NO:29);

[00598] (b) aKLH 120.6 kappa LC (SEQ ID NO:28); and

[00599] (c) aKLH 120.6 kappa LC-ShK[1-35, Q16K] fusion having the following sequence:

[00600] MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSLSASVGDRVTITC
RASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTIS
SLQPEDFATYYCLQHNSYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGT
ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT
LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSGGGGSRSCIDTIP
KSRCTAFKCKHSMKYRLSFCRKTCGTC// (SEQ ID NO:267).

This embodiment of monovalent aKLH 120.6 IgG2 LC-ShK[1-35, Q16K] product was a full antibody with the ShK peptide fused to the C-terminus of one light chain as shown in Figure 1J. 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 aKLH 120.6 IgG2 LC-ShK[1-35, Q16K], and bivalent aKLH 120.6 IgG2 LC-ShK[1-35, Q16K]. The monovalent aKLH 120.6 IgG2 LC-toxin peptide fusion-containing antibody was isolated from the mix using cation exchange chromatography, as described herein.

[00601] 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 antibodies (schematically represented in Figure 1J) included the monomers:

[00602] (a) aKLH 120.6 IgG2 HC (SEQ ID NO:29);

[00603] (b) aKLH 120.6 kappa LC (SEQ ID NO:28); and

[00604] (c) aKLH 120.6 kappa LC-ShK[2-35, Q16K] fusion having the following sequence:

[00605] MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSLSASVGDRVTITC RASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTIS SLQPEDFATYYCLQHNSYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSGGGGSSCIDTIPK SRCTAFKCKHSMKYRLSFCRKTCGTC// (SEQ ID NO:268).

This embodiment of monovalent aKLH 120.6 IgG2 LC-ShK[2-35, Q16K] product was a full antibody with the ShK peptide fused to the C-terminus of one light chain as shown in Figure 1J. 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 aKLH 120.6 IgG2, monovalent aKLH 120.6 IgG2 LC-ShK[2-35, Q16K], and bivalent aKLH 120.6 IgG2 LC-ShK[2-35, Q16K]. The monovalent aKLH 120.6 IgG2 LC-toxin peptide fusion-containing antibody was isolated from the mix using cation exchange chromatography, as described herein.

[00606] 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 antibodies (schematically represented in Figure 1K) included the monomers:

[00607] (a) aKLH 120.6 IgG2 HC (SEQ ID NO:29); and

[00608] (b) aKLH 120.6 kappa LC-ShK[1-35, Q16K] fusion (SEQ ID NO:267), above.

This embodiment of bivalent aKLH 120.6 IgG2 LC-ShK[1-35, Q16K] antibody product was a full antibody with the ShK peptide fused to the C-terminus of both light chains as shown in Figure 1K. 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]. The bivalent aKLH 120.6 IgG2 LC-ShK[1-35, Q16K] peptide fusion-containing antibody molecule was isolated from the mix using cation exchange chromatography, as described herein.

[00609] 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 antibodies (schematically represented in Figure 1K) included the monomers:

[00610] (a) aKLH 120.6 IgG2 HC (SEQ ID NO:29); and

[00611] (b) aKLH 120.6 kappa LC-ShK[2-35, Q16K] fusion (SEQ ID NO:268), above.

This embodiment of bivalent aKLH 120.6 IgG2 LC-ShK[2-35, Q16K] product was a full antibody with the ShK peptide fused to the C-terminus of both light chains as shown in Figure 1K. 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] antibody. The bivalent aKLH 120.6 IgG2 LC-ShK[2-35, Q16K] toxin fusion-containing antibody was isolated from the mix using cation exchange chromatography, as described herein.

[00612] 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 antibodies (schematically represented in Figure 1L) included the monomers:

[00613] a) aKLH 120.6 IgG2 HC (SEQ ID NO:29, above);

[00614] (b) aKLH 120.6 IgG2 HC-Shk[1-35, Q16K] fusion having the amino acid of SEQ ID NO:32, above; and

[00615] (c) aKLH 120.6 kappa LC-ShK[1-35, Q16K] fusion having the amino acid sequence of SEQ ID NO:267, above.

This embodiment of trivalent aKLH 120.6 IgG2 LC-ShK 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 1L. 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] antibody, trivalent aKLH 120.6 IgG2 LC-ShK[1-35, Q16K] antibody, and tetravalent aKLH 120.6 IgG2 LC-ShK[1-35, Q16K] antibody. The trivalent aKLH 120.6 IgG2 LC-toxin peptide fusion-containing antibody molecule was isolated from the mix using cation exchange chromatography, as described herein.

[00616] Trivalent aKLH 120.6 kappa LC-ShK[2-35, Q16K] fusion. The components of the trivalent aKLH 120.6 kappa LC-ShK[2-35, Q16K] fusion antibody (schematically represented in Figure 1L) included the monomers:

[00617] a) aKLH 120.6 IgG2 HC (SEQ ID NO:29);

[00618] (b) aKLH 120.6 IgG2 HC-Shk[2-35, Q16K] fusion (SEQ ID NO:33), above; and

[00619] (c) aKLH 120.6 kappa LC-ShK[2-35, Q16K] fusion (SEQ ID NO:268), above.

This embodiment of trivalent aKLH 120.6 IgG2 LC-ShK[2-35, Q16K] antibody 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 1L. 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] antibody, trivalent aKLH 120.6 IgG2 LC-ShK[2-35, Q16K] antibody, and

tetravalent aKLH 120.6 IgG2 LC-ShK[2-35, Q16K] antibody. The trivalent aKLH 120.6 IgG2 LC-toxin peptide fusion-containing antibody molecule was isolated from the mix using cation exchange chromatography, as described herein.

[00620] Anti-KLH 120.6 Antibody Light Chain mammalian expression. The XenoMouse® hybridoma expressing KLH 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:36) 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:37) and the reverse primer was 5'- AAC CGT TTA AAC GCG GCC GCT CAA CAC TCT CCC CTG TTG AA -3' (SEQ ID NO:38). 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.

[00621] 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:39). 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:38). 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.

[00622] 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:40) and 5'-TCA TCT GGA TGT CAC ATC TGG CAC C –3' (SEQ ID NO:41). The primers used for the mature light chain peptide were 5'-GGT GCC AGA TGT GAC ATC CAG ATG A –3' (SEQ ID NO:42) and (SEQ ID NO:38). The resulting fragments were joined by overlap PCR using primers (SEQ ID NO:40) and (SEQ ID NO:38). The sequence of the resulting clone encodes the following immunoglobulin kappa LC sequence:

MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSLSASVGDRVTITCRASQGIR NDLGWYQQKPGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQPEDF ATYYCLQHNSYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC// (SEQ ID NO:28).

[00623] 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:26), described above, as a template and the oligos:

5'- AAC AGG GGA GAG TGT GGA GGA GGA GGA TCC GGA G -3' (SEQ ID NO:269); and

5'- CAT GCG GCC GCT CAT TAG CAG G -3' (SEQ ID NO:270).

[00624] The light chain fragment and ShK PCR product were then amplified by PCR using the outside primers CAT TCT AGA ACC ACC ATG GAC ATG AGG GTG// (SEQ ID NO:343) and SEQ ID NO:270. 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:267).

[00625] 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:269 and SEQ ID NO:270.

[00626] The light chain and ShK PCR products were amplified by PCR using the outside primers SEQ ID NO:343 and SEQ ID NO:270. 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-aKLH 120.6-VK1SP-LC-L10-ShK[2-35, Q16K] construct encoded an IgG2-LC-L10-ShK[2-35, Q16K] fusion polypeptide monomer (SEQ ID NO:268).

[00627] aKLH -IgG2 Heavy Chain-L10-ShK[1-35] and aKLH-IgG2 Heavy Chain-L10-ShK peptide analogs in mammalian expression.

Using oligos
5'-CAT TCT AGA CCC ACC ATG GAC ATG AGG GTG-3' (SEQ ID NO:43); and
5'-GGA TCC TCC TCC TCC ACC CGG AGA CAG GGA GAG G-3' (SEQ ID NO:44),

the aKLH-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:45; below), encoding aKLH 120.6-VK1SP-IgG2 Heavy Chain (SEQ ID NO:46; below):

ATGGACATGAGGGTGCCCGCTCAGCTCCTGGGGCTCCTGCTGCTGTGGCT
GAGAGGTGCCAGATGTCAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTG
AAGAAGCCTGGGGCCTCAGTGAAGGTCTCCTGCAAGGCTTCTGGATACA
CCTTCACCGGCTACCACATGCACTGGGTGCGACAGGCCCCTGGACAAGG
GCTTGAGTGGATGGGATGGATCAACCCTAACAGTGGTGGCACAAACTAT
GCACAGAAGTTTCAGGGCAGGGTCACCATGACCAGGGACACGTCCATCA
GCACAGCCTACATGGAGCTGAGCAGGCTGAGATCTGACGACACGGCCGT
GTATTACTGTGCGAGAGATCGTGGGAGCTACTACTGGTTCGACCCCTGGG
GCCAGGGAACCCTGGTCACCGTCTCCTCAGCCTCCACCAAGGGCCCATCG
GTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCGGC
CCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGT
GGAACTCAGGCGCTCTGACCAGCGGCGTGCACACCTTCCCAGCTGTCCTA
CAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAG
CAACTTCGGCACCCAGACCTACACCTGCAACGTAGATCACAAGCCCAGC
AACACCAAGGTGGACAAGACAGTTGAGCGCAAATGTTGTGTCGAGTGCC
CACCGTGCCCAGCACCACCTGTGGCAGGACCGTCAGTCTTCCTCTTCCCC
CCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACGT
GCGTGGTGGTGGACGTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTG
GTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCACGGGAG
GAGCAGTTCAACAGCACGTTCCGTGTGGTCAGCGTCCTCACCGTTGTGCA
CCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAA
GGCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAACCAAAGGGCAGC
CCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGAC
CAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGC
GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTAC
AAGACCACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTCCTCTACAG
CAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCA

TGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCC
TCTCCCTGTCTCCGGGT// (SEQ ID NO:45),
encoding the amino acid sequence
MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASGY
TFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSI
STAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSASTKGPS
VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAP
PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEV
HNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKT
ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPG// (SEQ ID NO:46).

[00628] 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:47); and 5'- CAT GCG GCC GCT CAT TAG CAG GTG -3' (SEQ ID NO:14) 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:
MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASGY
TFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSI
STAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSASTKGPS
VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAP
PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEV
HNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKT
ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGGGGGSGGGGSRSCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCG
TC// (SEQ ID NO:48).

[00629] 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:9); and
5'- GCT GTG CTT GCA CTT GAA GGC GGT GCA GC -3' (SEQ ID NO:10),.
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:
MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASGY
TFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSI
STAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSASTKGPS
VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAP

PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEV
HNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKT
ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGGGGGSGGGGSRSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCG
TC// (SEQ ID NO:49).

[00630] 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:

ATGGACATGAGGGTGCCCGCTCAGCTCCTGGGGCTCCTGCTGCTGTGGCT
GAGAGGTGCCAGATGTCAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTG
AAGAAGCCTGGGGCCTCAGTGAAGGTCTCCTGCAAGGCTTCTGGATACA
CCTTCACCGGCTACCACATGCACTGGGTGCGACAGGCCCCTGGACAAGG
GCTTGAGTGGATGGGATGGATCAACCCTAACAGTGGTGGCACAAACTAT
GCACAGAAGTTTCAGGGCAGGGTCACCATGACCAGGGACACGTCCATCA
GCACAGCCTACATGGAGCTGAGCAGGCTGAGATCTGACGACACGGCCGT
GTATTACTGTGCGAGAGATCGTGGGAGCTACTACTGGTTCGACCCCTGGG
GCCAGGGAACCCTGGTCACCGTCTCCTCAGCCTCCACCAAGGGCCCATCG
GTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCGGC
CCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGT
GGAACTCAGGCGCTCTGACCAGCGGCGTGCACACCTTCCCAGCTGTCCTA
CAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAG
CAACTTCGGCACCCAGACCTACACCTGCAACGTAGATCACAAGCCCAGC
AACACCAAGGTGGACAAGACAGTTGAGCGCAAATGTTGTGTCGAGTGCC
CACCGTGCCCAGCACCACCTGTGGCAGGACCGTCAGTCTTCCTCTTCCCC
CCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACGT
GCGTGGTGGTGGACGTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTG
GTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCACGGGAG

GAGCAGTTCAACAGCACGTTCCGTGTGGTCAGCGTCCTCACCGTTGTGCA
CCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAA
GGCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAACCAAAGGGCAGC
CCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGAC
CAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGC
GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTAC
AAGACCACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTCCTCTACAG
CAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCA
TGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCC
TCTCCCTGTCTCCGGGTGGAGGAGGA // (SEQ ID NO:50),

encoding the amino acid sequence

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASGY
TFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSI
STAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSASTKGPS
VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAP
PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEV
HNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKT
ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGGGG// (SEQ ID NO:51).

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):

MEWSWVFLFFLSVTTGVHSERKVECPPCPAPPVAGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVL
TVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKL
TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSSCID
TIPKSRCTAFKCKHSMKYRLSFCRKTCGTC// (SEQ ID NO:18),

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:52),

encoding the amino acid sequence GSGGGGSSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCGTC (SEQ ID NO:53), 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:

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASGY TFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSI STAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAP PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEV HNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKT ISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGGGGGSGGGGSSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCGT C// (SEQ ID NO:54).

[00631] 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:

[00632] 5'- AGG AGG AGG AAG CGC CAG CTG CGC CGA CAC CAT CCC C -3'// (SEQ ID NO:310); and

[00633] 5'- GGG GAT GGT GTC GGC GCA GCT GGC GCT TCC TCC TCC T -3'// (SEQ ID NO:311).

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:304).

[00634] The Shk[1-35, R1A, Q16K, K30E] fragment was generated as described above using the following four oligos:

[00635] 5'- GAG GAG GAG GAA GCG CCA GCT GCA TCG ACA -3'// (SEQ ID NO:312);

[00636] 5'- GAG CTT CTG CCG CGA GAC CTG CGG CAC -3'// (SEQ ID NO:313);

[00637] 5'- CGA TGC AGC TGG CGC TTC CTC CTC CTC -3'// (SEQ ID NO:314); and

[00638] 5'- GTG CCG CAG GTC TCG CGG CAG AAG CTC -3'// (SEQ ID NO:315).

The final pTT5-aKLH 120.6-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:305).

[00639] 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:

[00640] 5'- GGA GGA GGA AGC CAC AGC TGC GCC GAC ACC ATC CCC -3'// (SEQ ID NO:316); and

[00641] 5'- GGG GAT GGT GTC GGC GCA GCT GTG GCT TCC TCC TCC -3'// (SEQ ID NO:317).

Site-directed mutagenesis was performed using the Stratagene Quikchange Multi site Directed Mutagenesis Kit (Cat#200531), per manufacturer's instructions. The final pTT5-aKLH 120.6-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:306).

[00642] The Shk[1-35, R1H, Q16K, K30E] fragment was generated as described above using the following four oligos:

[00643] 5'- GGA GGA GGA AGC CAC AGC TGC ATC GAC -3'// (SEQ ID NO:318) and SEQ ID NO:313;

[00644] 5'- GTC GAT GCA GCT GTG GCT TCC TCC TCC -3'// (SEQ ID NO:319) and SEQ ID NO:315.

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, R1H, Q16K, K30E] fusion polypeptide (SEQ ID NO:307).

[00645] 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:

[00646] 5'- CCG GAG GAG GAG GAA GCA AGA GCT GCG CCG ACA CCA TCC CCA AGA -3'// (SEQ ID NO:320); and

[00647] 5'- TCT TGG GGA TGG TGT CGG CGC AGC TCT TGC TTC CTC CTC CTC CGG -3'// (SEQ ID NO:321).

Site-directed mutagenesis was performed using the Stratagene Quikchange Multi site Directed Mutagenesis Kit (Cat#200531), per manufacturer's instructions. The final pTT5-aKLH 120.6-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:308).

[00648] The Shk[1-35, R1K, Q16K, K30E] fragment was generated as described above using the following four oligos:

[00649] 5'- CGG AGG AGG AGG AAG CAA GAG CTG CAT CGA CAC CA -3'// (SEQ ID NO:322) and SEQ ID NO:313;

[00650] 5'- TGG TGT CGA TGC AGC TCT TGC TTC CTC CTC CTC CG -3'// (SEQ ID NO:323) and SEQ ID NO:315.

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 (SE Q ID NO:309).

[00651] 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 24-26A-B, 27-29A-B, 30-32A-B, and 33-35. The non-reducing SDS-PAGE analysis (Figures 24, 28, 30 and 33) 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 25, 28, 31 and 34) show that the majority of the purified product is in the desired non-aggregated state. Finally, the mass spectral analysis (Figures 26A-B, 29A-B, 32A-B and 35) 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.

[00652] 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.

[00653] 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).

[00654] 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:

TGCAGAAGCTTCTAGACCACCATGGAATGGAGCTGGGTCTTTCTCTTCTT CCTGTCAGTAACGACTGGTGTCCACTCCCGCAGCTGCATCGACACCATCC CCAAGAGCCGCTGCACCGCCTTCCAGT// (SEQ ID NO:55); and

Reverse primer:

CTCCGGATCCTCCTCCTCCGCAGGTGCCGCAGGTCTTGCGGCAGA AGCTCAGGCGGTACTTCATGCTGTGCTTGCACTGGAAGGCGGTGCAGCG GCTCTTGGGGATGGTGTCGAT// (SEQ ID NO:56).

[00655] 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).

[00656] 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:57); and

Reverse primer:

CGAGCGGCCGCTTACTATTTACCCGGAGACAGGGA// (SEQ ID NO:58).

[00657] 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).

[00658] 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

ATGGAATGGAGCTGGGTCTTTCTCTTCTTCCTGTCAGTAACGACTGGTGT
CCACTCCCGCAGCTGCATCGACACCATCCCCAAGAGCCGCTGCACCGCCT
TCCAGTGCAAGCACAGCATGAAGTACCGCCTGAGCTTCTGCCGCAAGAC
CTGCGGCACCTGCGGAGGAGGAGGATCCGGAGGAGGAGGAAGCGACAA
AACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCG
TCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCG
GACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCT
GAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCA
AGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAG
CGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAG
TGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTC
CAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCA
TCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCA
AAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCA
GCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGC
TCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCA
GGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACT

ACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATAGTAA// (SEQ ID NO:59),

encoding VH21 SP-ShK(1-35)-L10-IgG1 Fc amino acid sequence MEWSWVFLFFLSVTTGVHSRSCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCG TCGGGGSGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK// (SEQ ID NO:60).

[00659] 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:9); and 5'- GCT GTG CTT GCA CTT GAA GGC GGT GCA GC -3' (SEQ ID NO:10). 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 (VH21 SP)-ShK[1-35, Q16K]-L10-IgG1-Fc fusion polypeptide: MEWSWVFLFFLSVTTGVHSRSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCG TCGGGGSGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK// (SEQ ID NO:61).

[00660] 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:62);

5'- CAG CTG CAC CTG GCT TCC TCC TCC TCC GG -3' (SEQ ID NO:63);
and template pCMVi-N-terminus-ShK[1-35, Q16K]-L10-IgG1-Fc, resulted in a fragment containing the coding sequence
ATGGAATGGAGCTGGGTCTTTCTCTTCTTCCTGTCAGTAACGACTGGTGT
CCACTCCCGCAGCTGCATCGACACCATCCCCAAGAGCCGCTGCACCGCCT
TCAAGTGCAAGCACAGCATGAAGTACCGCCTGAGCTTCTGCCGCAAGAC
CTGCGGCACCTGCGGAGGAGGAGGATCCGGAGGAGGAGGAAGC// (SEQ ID NO:64),
encoding the VH21 SP-ShK(1-35, Q16K)-L10 amino acid sequence
MEWSWVFLFFLSVTTGVHSRSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCG
TCGGGGSGGGGS// (SEQ ID NO:65).

[00661] 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:66);
5'- CAT GCG GCC GCT CAT TTA CCC -3' (SEQ ID NO:67);
and template pTT5-aKLH 120.6-HC, resulting in a DNA fragment containing the coding sequence
CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCT
CAGTGAAGGTCTCCTGCAAGGCTTCTGGATACACCTTCACCGGCTACCAC
ATGCACTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAT
GGATCAACCCTAACAGTGGTGGCACAAACTATGCACAGAAGTTTCAGGG
CAGGGTCACCATGACCAGGGACACGTCCATCAGCACAGCCTACATGGAG
CTGAGCAGGCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGAG
ATCGTGGGAGCTACTACTGGTTCGACCCCTGGGGCCAGGGAACCCTGGTC
ACCGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCGCC
CTGCTCCAGGAGCACCTCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTC
AAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCTCT
GACCAGCGGCGTGCACACCTTCCCAGCTGTCCTACAGTCCTCAGGACTCT
ACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCCAG
ACCTACACCTGCAACGTAGATCACAAGCCCAGCAACACCAAGGTGGACA

AGACAGTTGAGCGCAAATGTTGTGTCGAGTGCCCACCGTGCCCAGCACC
ACCTGTGGCAGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACA
CCCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGACGTG
AGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCGTGG
AGGTGCATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGCAC
GTTCCGTGTGGTCAGCGTCCTCACCGTTGTGCACCAGGACTGGCTGAACG
GCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCAGCCCCCAT
CGAGAAAACCATCTCCAAAACCAAAGGGCAGCCCCGAGAACCACAGGTG
TACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCC
TGACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGG
GAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACACCTCCCATGC
TGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAG
AGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGG
CTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA
TGA// (SEQ ID NO:68),

encoding amino acid sequence

QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYHMHWVRQAPGQGLEWM
GWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARD
RGSYYWFDPWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDY
FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCN
VDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPE
VTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTV
VHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK// (SEQ ID NO:69).

[00662] 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:62) and (SEQ ID NO:67). 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 VH21 SP-ShK[1-35, Q16K]-L10-aKLH120.6-HC fusion polypeptide:

MEWSWVFLFFLSVTTGVHSRSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCG
TCGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYTFTGYHMHWV
RQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLR
SDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSASTKGPSVFPLAPCSRSTS
ESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV
PSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQ
FNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQ
VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPML
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK//
(SEQ ID NO:70).

[00663] Lastly, the N-terminus-ShK[1-35, Q16K]-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:62); and
5'- CAT CTG GAT GTC GCT TCC TCC TCC TCC GG -3' (SEQ ID NO:71);
and template pCMVi-N-terminus-ShK[1-35Q16K]-L10-IgG1-Fc, resulting in a DNA fragment containing the coding sequence
ATGGAATGGAGCTGGGTCTTTCTCTTCTTCCTGTCAGTAACGACTGGTGT
CCACTCCCGCAGCTGCATCGACACCATCCCCAAGAGCCGCTGCACCGCCT
TCAAGTGCAAGCACAGCATGAAGTACCGCCTGAGCTTCTGCCGCAAGAC
CTGCGGCACCTGCGGAGGAGGAGGATCCGGAGGAGGAGGAAGC// (SEQ

ID NO:64),

encoding the amino acid sequence for a signal peptide (VH21 SP)-ShK(1-35, Q16K)-L10 linker:

MEWSWVFLFFLSVTTGVHSRSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCG TCGGGGSGGGGS// (SEQ ID NO:65).

Using template and oligos:
5'-GGA GGA GGA AGC GAC ATC CAG ATG ACC CAG TC-3' (SEQ ID NO:72); and

5'- CAT CTC GAG CGG CCG CTC AAC -3' (SEQ ID NO:73).

The resulting cloned PCR fragment contained the coding sequence

ATGGAATGGAGCTGGGTCTTTCTCTTCTTCCTGTCAGTAACGACTGGTGT
CCACTCCCGCAGCTGCATCGACACCATCCCCAAGAGCCGCTGCACCGCCT
TCAAGTGCAAGCACAGCATGAAGTACCGCCTGAGCTTCTGCCGCAAGAC
CTGCGGCACCTGCGGAGGAGGAGGATCCGGAGGAGGAGGAAGCGACAT
CCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAG
TCACCATCACTTGCCGGGCAAGTCAGGGCATTAGAAATGATTTAGGCTGG
TATCAGCAGAAACCAGGGAAAGCCCCTAAACGCCTGATCTATGCTGCAT
CCAGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGCGGCAGTGGATCTGG
GACAGAATTCACTCTCACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAA
CTTATTACTGTCTACAGCATAATAGTTACCCGCTCACTTTCGGCGGAGGG
ACCAAGGTGGAGATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCTT
CCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCC
TGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGA
TAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGAC
AGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAG
CAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGG
CCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTGA//

(SEQ ID NO:74) was generated,

encoding the amino acid sequence for N-terminus VH21 SP-ShK[1-35, Q16K]-L10-aKLH120.6 Light Chain (LC) with an N-terminal signal peptide:

MEWSWVFLFFLSVTTGVHSRSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCG

TCGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKP
GKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSY
PLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ
WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC// (SEQ ID NO:75).

[00664] Both PCR fragments (DNA fragment containing the coding sequence (SEQ ID NO:64) and aKLH 120.6 Light Chain LC fragment containing the coding sequence (SEQ ID NO:74) 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:62) and (SEQ ID NO:73). The resulting PCR fragment was cut using XbaI and NotI and cleaned with Qiagen PCR Cleanup Kit.

[00665] 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:75).

[00666] Mammalian expression of aDNP 3A4 (W101F) IgG2-Shk[1-35].

[00667] 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:76) were constructed using standard cloning technology. Plasid pTT5 – aDNP 3A4 (W101F) IgG2 -Shk[1-35, Q16K] 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 the amino acid sequence:

MDMRVPAQLLGLLLLWLRGARCQVQLVESGGGVVQPGRSLRLSCAASGFT
FSSYGMHWVRQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCARYNFNYGMDVWGQGTTVTVSSASTKGPSVFP
LAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPV
AGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHN
AKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISK
TKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPG// (SEQ ID NO:77);
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
ATGGACATGAGGGTGCCCGCTCAGCTCCTGGGGCTCCTGCTGCTGTGGCT
GAGAGGTGCGCGCTGTCAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTG
GTCCAGCCTGGGAGGTCCCTGAGACTCTCCTGTGCAGCGTCTGGATTCAC
CTTCAGTAGCTATGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGG
CTGGAGTGGGTGGCAGTTATATGGTATGATGGAAGTAATAAATACTATG
CAGACTCCGTGAAGGGCCGATTCACTATCTCCAGAGACAATTCCAAGAA

CACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTG
TATTACTGTGCGAGGTATAACTTCAACTACGGTATGGACGTCTGGGGCCA
AGGGACCACGGTCACCGTCTCTAGTGCCTCCACCAAGGGCCCATCGGTCT
TCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCGGCCCTG
GGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGA
ACTCAGGCGCTCTGACCAGCGGCGTGCACACCTTCCCAGCTGTCCTACAG
TCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAA
CTTCGGCACCCAGACCTACACCTGCAACGTAGATCACAAGCCCAGCAAC
ACCAAGGTGGACAAGACAGTTGAGCGCAAATGTTGTGTCGAGTGCCCAC
CGTGCCCAGCACCACCTGTGGCAGGACCGTCAGTCTTCCTCTTCCCCCCA
AAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGT
GGTGGTGGACGTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTAC
GTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCACGGGAGGAG
CAGTTCAACAGCACGTTCCGTGTGGTCAGCGTCCTCACCGTTGTGCACCA
GGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGC
// (SEQ ID NO:78), encoding amino acid sequence
MDMRVPAQLLGLLLLWLRGARCQVQLVESGGGVVQPGRSLRLSCAASGFT
FSSYGMHWVRQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCARYNFNYGMDVWGQGTTVTVSSASTKGPSVFP
LAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPV
AGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHN
AKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKG// (SEQ ID
NO:79).

The second insert was digested out using StuI/NotI and contained the coding sequence

CTCCCAGCCCCCATCGAGAAAACCATCTCCAAAACCAAAGGGCAGCCCC
GAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAA
GAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGCGAC
ATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAG
ACCACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAA

GCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGC
TCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTC
CCTGTCTCCGGGTAAAGGAGGAGGAGGATCCGGAGGAGGAGGAAGCCG
CAGCTGCATCGACACCATCCCCAAGAGCCGCTGCACCGCCTTCAAGTGCA
AGCACAGCATGAAGTACCGCCTGAGCTTCTGCCGCAAGACCTGCGGCAC
CTGCTAATGA// (SEQ ID NO:80),
encoding the following truncated IgG2 Fc-L10-ShK(1-35, Q16K) amino acid sequence
LPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGKGGGGSGGGGSRSCIDTIPKSRCTAFKCKHSMKYRL
SFCRKTCGTC// (SEQ ID NO:81).

[00668] 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 HC-L10-Shk[1-35, Q16K] having the following amino acid sequence:

MDMRVPAQLLGLLLLWLRGARCQVQLVESGGGVVQPGRSLRLSCAASGFT
FSSYGMHWVRQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCARYNFNYGMDVWGQGTTVTVSSASTKGPSVFP
LAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPV
AGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHN
AKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISK
TKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN

YKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGGGGGSGGGGSRSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCGTC// (SEQ ID NO:82).

[00669] 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:83) 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:84). 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:

MDMRVPAQLLGLLLLWLRGARCDIQMTQSPSSVSASVGDRVTITCRASQGIS RRLAWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDF ATYYCQQANSFPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC// (SEQ ID NO:109).

[00670] 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 immunoglobulin-toxin peptide analog fusion proteins are shown in Figure 3A-C, Figure 4A-C, Figure 5A-C, Figure 6A-C and Figures 24-35.

[00671] 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. Reducing and non-reducing (+iodoacetamide) analysis was done on 4-12% or 4-20% SDS-PAGE Tris-glycine gels (Invitrogen) with 0.5 μg, 2 μg, and 10 μg of protein, stained with QuickBlue (Boston Biologicals). Analytical SEC was done using a Biosep SEC-S3000 column (Phenomenex) and an isocratic elution of 50 mM sodium phosphate, 250 mM NaCl, pH 6.9, over 18 min. Exemplary purifications of immunoglobulin-toxin peptide analog fusion proteins are shown in Figure 24-26A-B, 27-29A-B, 30-32A-B, and 33-35.

[00672] **Example 5**

[00673] **Pharmacokinetic/Pharmacodynamic Evaluation of Monovalent Fc/Fc-L10-ShK[2-35] Heterodimers and Monovalent or Bivalent Fc/Fc-ShK(1-35 Q16K)(IgG2) Heterodimers and Immunoglobulin Fusion Proteins of the Invention**

[00674] Embodiments of the antigen binding proteins of the present invention, used as immunoglobulin carriers for pharmacologically active polypeptides were demonstrated to provide favorable pharmacokinetic and pharmacodynamic properties. 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 proteins, monovalent Fc-ShK(1-35 Q16K)/KLH Ab heterotrimer, and other exemplary embodiments listed in Table 7H, were expressed, isolated and purified by methods described in Example 4. PEGylated and un-PEGylated toxin peptide comparators in Table 7H were prepared synthetically as follows:

[00675] 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 (RSCIDTIPKSRCTAFQCKHSMKYRLSFCRKTCGTC// SEQ ID NO:361), [Lys16]ShK (RSCIDTIPKSRCTAFKCKHSMKYRLSFCRKTCGTC// SEQ ID NO:76), 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.

[00676] 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.

[00677] Peptide Folding. The dry crude linear peptide (about 600 mg), for example [Lys16]ShK peptide (SEQ ID NO:76) or [Lys16]ShK-Ala (also known as [Lys16, Ala36]-ShK; SEQ ID NO:362) 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).

[00678] 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.

[00679] 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.

[00680] PEGylation, Purification and Analysis. Peptide, e.g., [Lys16]ShK (SEQ ID NO:76) or [Lys16]ShK-Ala (SEQ ID NO:362), 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 (data not shown). 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.

[00681] Fusion Proteins. Generally, Figure 1A and Figure 1B 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:153) 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.

[00682] In Examples 1 and 2 of Sullivan et al., WO 2008/088422A2, were described 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, was 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 7H). 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, Examples 4), 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.

[00683] PK assays

[00684] Antibodies to ShK. Rabbit polyclonal and mouse monoclonal antibodies to ShK (SEQ ID NO:361) 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.

[00685] Pharmacokinetic (PK) studies on 20kDa-PEG-ShK (SEQ ID NO:363) and 20 kDa-PEG-[Lys16]ShK (SEQ ID NO:364) peptide conjugates in rats and monkeys. Single subcutaneous doses were delivered to animals and serum was collected at various time points after injection. Studies in rats 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) 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).

[00686] A brief description of the ELISA protocol for detecting serum levels of PEG-ShK and PEG-[Lys16]ShK is provided below:

[00687] (1) Protocol 1, (a)-(g) below, detects PEG-ShK and PEG-[Lys16]ShK, as well as the ShK and [Lys16]ShK peptides alone:

[00688] (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.

[00689] (b) Plates were washed three times with KPL wash buffer (Kirkegaard & Perry Laboratories).

[00690] (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.)

[00691] (d) Plates were washed three times with KPL wash buffer.

[00692] (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.

[00693] (f) Plates were again washed three times with KPL wash buffer and the Femto [Thermo Scientific] substrate was added.

[00694] (g) The plate was read with a Lmax II 384 (Molecular Devices) luminometer.

[00695] 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:

[00696] (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:

[00697] (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;

[00698] (b) Plates were washed three times with KPL wash buffer (Kirkegaard & Perry Laboratories)

[00699] (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.);

[00700] (d) Plates were washed three times with KPL wash buffer;

[00701] (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.

[00702] (f) Plates were washed three times with KPL wash buffer and the Femto [Thermo Scientific] substrate was added;

[00703] (g) The plate was read with a Lmax II 384 [Molecular Devices] luminometer.

[00704] (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:

[00705] (a) Costar 3590 96-well EIA/RIA plates were coated with 0.1 mL/well of 2 μg/mL Goat anti-HuFc, Fab2, (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;

[00706] (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;

[00707] (c) Plates were washed with a KP Wash Solution (Cat #50-63-00, KPL, Gaithersburg, MD);

[00708] (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;

[00709] (e) Plates were washed with a KP Wash Solution (Cat #50-63-00, KPL, Gaithersburg, MD);

[00710] (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;

[00711] (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;

[00712] (h) At appropriate times after substrate addition and shaking, the plate was read with a SpectraMax340 [Molecular Devices] plate reader.

[00713] 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 4 herein also describes cloning and mammalian expression 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 4 herein). The monovalent Fc-L10-ShK(2-35) molecule had an IC50 of 2.1 nM (Table 7H) 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 10) 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.

[00714] Monovalent Fc/Fc-ShK(1-35 Q16K) heterodimer (IgG2). ShK[Lys16] toxin peptide analog (SEQ ID NO:76) shows significant Kv1.3 selectivity over neuronal Kv1.1 (Table 7H). 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 4). A schematic representation of this monovalent construct is provided in Figure 1A. The monovalent Fc/Fc-L10-ShK(1-35 Q16K) heterodimer [also referred to as monovalent Fc/Fc-ShK(1-35, Q16K)] potently blocked T cell inflammation in whole blood, suppressing IL-2 secretion with an IC50 of 0.16 nM (Table 7H). 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:76) peptide alone showed about 18-fold selectivity for Kv1.3 versus Kv1.1 (Table 7H), the monovalent Fc/Fc-L10-ShK(1-35 Q16K) heterodimer was about 1225-fold more active in blocking Kv1.3 versus Kv1.1. 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:364) also showed enhanced Kv1.3 selectivity (Table 7H) relative to the peptide alone, the combined data suggests that the [Lys16]-ShK (SEQ ID NO:76) 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.

[00715] To assess the pharmacokinetics and stability of the molecule in vivo, as a basis of comparison for the inventive molecules, 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 (of monomers SEQ ID NOS:1 and 26) exhibited

an extended half-life in vivo (Figure 7). Since the sandwich ELISA used to measure serum levels of the molecule ("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:76), 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 7, open squares; Table 7I 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:361) peptide alone that was reported to have a half-life of 20-30 min (C. Beeton et al., PNAS 98:13942 (2001)).

[00716] 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:26). A schematic representation of this bivalent construct is provided in Figure 1B. The molecule (homodimer of SEQ ID NO:26) was cloned, expressed and purified as described in Example 4 herein. 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 7H). 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:76) peptides at its end, is less stable and/or interferes with Kv1.3 channel binding to some extent.

[00717] Monovalent and bivalent aKLH HC-ShK(1-35, Q16K) Ab. The monovalent anti-KLH Heavy Chain (HC) fusion antibody (Ab) construct embodiment of the present invention contained, from N- to C-terminus: human anti-KLH Ab Heavy Chain-peptidyl linker-[Lys16]ShK molecule (SEQ ID NO:32), that was co-expressed with the human aKLH Heavy Chain alone (SEQ ID NO:29) and the human aKLH light chain (SEQ ID NO:28) to form a monovalent aKLH Ab-[Lys16]ShK molecule (heterotetramer of SEQ ID NO:28; SEQ ID NO:29; SEQ ID NO:28; and SEQ ID NO:32). A schematic representation of this monovalent

construct is provided in Figure 1F. The monovalent aKLH HC-ShK(1-35, Q16K) Ab potently blocked T cell inflammation in whole blood, suppressing IL-2 secretion with an IC50 of 0.274 nM (Table 7H). 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:28; SEQ ID NO:29; SEQ ID NO:28; and SEQ ID NO:32) had significantly better Kv1.3 selectivity than the [Lys16]ShK (SEQ ID NO:76) peptide alone. This monovalent Ab-ShK conjugate was about 1458-fold more active in blocking Kv1.3 versus Kv1.1 (Table 7H and Figure 2A-B).

[00718] 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 aKLH HC-ShK(1-35 Q16K) Ab conjugate exhibited an extended half-life in vivo (Figure 7, closed circles). Since the sandwich ELISA used to measure serum levels of the molecule ("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:76), 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 (Figure7, Figure 8, and Table 7J). The bivalent aKLH HC-ShK(1-35, Q16K) Ab molecule (schematically represented by Figure 1G) given at the same 6 mg/kg dose, showed a similarly slow elimination rate (Figure 8), but provided about 37 times less exposure (as measured by AUC_{0-t}, Table 7J) relative to the monovalent molecule (Figure 8). 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 7J).

[00719] Monovalent aKLH HC-ShK(2-35 Q16K) Ab. This monovalent aKLH Heavy Chain (HC) fusion antibody (Ab) construct embodiment of the present invention contained from N- to C-terminus: human anti-KLH Ab Heavy Chain – linker – [desArg1, Lys16]ShK molecule (SEQ ID NO:33), that was co-expressed with the human aKLH Heavy Chain (SEQ ID NO:29) and the human aKLH light chain (SEQ ID NO:28) to form a monovalent aKLH Ab-[desArg1, Lys16]ShK

molecule. A schematic representation of this monovalent construct is provided in Figure 1F. The monovalent aKLH HC-ShK(2-35, Q16K) Ab (heterotetramer of SEQ ID NO:28; SEQ ID NO:29; SEQ ID NO:28; and SEQ ID NO:33) potently blocked T cell inflammation in whole blood, suppressing IL-2 secretion with an IC50 of 0.570 nM (Table 7H) and unexpectedly was about 1576 fold more potent in blocking the T-cell potassium channel Kv1.3 than the neuronal channel Kv1.1.

[00720] Monovalent Fc-ShK(1-35 Q16K)/KLH Ab Heterotrimer. The monovalent Fc-ShK(1-35, Q16K)/KLH Ab heterotrimer or hemibody embodiment of the present invention contained from N- to C-terminus: human Fc (IgG2) – L10 linker – [Lys16]ShK molecule (SEQ ID NO:26), that was co-expressed with the human aKLH Heavy Chain (IgG2) (SEQ ID NO:29) and the human aKLH light chain (SEQ ID NO:28). A schematic representation of this monovalent construct is provided in Figure 1E. The monovalent Fc-ShK(1-35, Q16K)/KLH Ab heterotrimer (SEQ ID NO:28; SEQ ID NO:29; SEQ ID NO:26) potently blocked T cell inflammation in human whole blood, suppressing IL-2 secretion with an IC50 of 0.245 nM (Table 7H). Surpisingly, studies examining the Kv1.3 versus Kv1.1 selectivity of the molecule revealed that the monovalent Fc-ShK(1-35, Q16K)/KLH Ab heterotrimer had significantly better Kv1.3 selectivity than the [Lys16]ShK peptide alone (SEQ ID NO:76). This monovalent heterotrimer was about 1935 fold more active in blocking Kv1.3 versus Kv1.1 (Table 7H).

[00721] Although we have not examined the pharmacokinetics (PK) of the Kv1.3 selective monovalent Fc-ShK(1-35, Q16K)/KLH Ab heterotrimer or hemibody, we have examined the PK profile of a similar hemibody, that being the Fc-ShK(2-35)/KLH Ab heterotrimer. A schematic of the structure of this molecule is provided in Figure 1E, 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)/KLH Ab heterotrimer (also referred to as monovalent Fc-ShK/KLH Ab heterotrimer) exhibited an extended half-life in rats (Figure 10). Since the sandwich ELISA used to measure serum levels of the molecule ("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 10, Table 7K). The large, about 103 kDa monovalent Fc-ShK(2-35)KLH Ab heterotrimer or hemibody showed greater exposure and about 2-fold less clearance than the about 56 kDa monovalent Fc/Fc-ShK heterodimer (Figure 10, Table 7K). 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)/KLH Ab heterotrimer (CL/F = 22.6 mL $h^{-1}kg^{-1}$) molecule.

[00722] Monovalent and bivalent anti-KLH AbLoop-[Lys16]ShK fusion proteins. Recombinant monovalent and bivalent anti-KLH AbLoop-[Lys16]ShK fusion proteins embodiments of the present invention were constructed as described in Example 4 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 1N. Linker sequences of differing amino acid composition and length were examined. The monovalent anti-KLH AbLoop-[Lys16]ShK fusion protein was a selective inhibitor of Kv1.3 activity (over Kv1.1; >121-fold more selective for Kv1.3; Table 7H and Figure 2A-B). The monovalent KLH-AbLoop-[Lys16]ShK molecule exhibited the slowest clearance in rats of all the novel toxin-conjugates that we have examined (Figure 7 and Figure 9 and Table 7L).

[00723] 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 1M. 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 9). Exposure as measured by AUC_{0-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 7L). Therefore, our novel monovalent forms show an unexpected and vastly better pharmacokinetic profile in vivo compared to typical bivalent forms of the same molecule.

[00724] 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:153) 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 1C. The molecule was cloned, expressed and purified as described Example 4 herein. 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 7H). 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 7H). 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 7H) 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 (SEQ ID NO:362) 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 7H). 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 domain or immunoglobulin light chain or heavy chain to improve the conjugates Kv1.3 selectivity.

[00725] Monovalent ShK(1-35, Q16K)-HC aKLH Ab. The monovalent ShK(1-35, Q16K)-HC aKLH Ab embodiment of the present invention 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 1I. 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 7H). 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.

[00726] Monovalent aDNP HC-ShK(1-35, Q16K) Ab. The monovalent aDNP Heavy Chain (HC) fusion antibody (Ab) construct embodiment of the present invention 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 1F. The monovalent aDNP HC-ShK(1-35, Q16K) Ab potently blocked T cell inflammation in human whole blood, suppressing IL-2 secretion with an IC50 of 0.278 nM (Table 7H). 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 7H).

Table 7H Data demonstrating various conjugates of [Lys16]ShK having improved Kv1.3 selectivity. Toxin peptides and toxin peptide analogs were PEGylated as described in Example 4 herein. Immunoglobulin-containing compounds were recombinantly expressed and purified as described in Example 4. Electrophysiology was by PatchXpress® (PX), except asterisks indicate data from whole cell patch clamp (see, Examples 6 and 8 herein). Human whole blood ("WB") assays of IL-2 and interferon-gamma ("IFNg") were conducted as described in Example 7 herein).

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)
361	none	ShK(1-35)	0.062	0.087	1.40	0.067	0.078	1.00
76	none	[Lys16]ShK	0.207	3.677	17.76	0.110	0.158	1.64
362	none	[Lys16]ShK-Ala	0.06	15.726	262.10	0.138	0.266	2.06
363	PEG	20kDa-PEG-ShK	0.299*	1.628*	5.44	0.380	0.840	5.67
364	PEG	20kDa-PEG-[Lys16]ShK	0.94	997	1060.64	0.092	0.160	1.37
365	PEG	20kDa-PEG-[Lys16]ShK-Ala	0.596	2156	3617.45	0.754	1.187	11.25
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

Table 7H continued:

1; 26	IgG2	Monovalent Fc/Fc-ShK(1-35 Q16K) heterodimer	2.73	3344	**1224.91**	0.160	0.499	**2.39**
26; 26	IgG2	Bivalent Fc-ShK(1-35 Q16K) homodimer	ND	ND	ND	1.850	3.140	27.61
28; 29; 26	IgG2	Monovalent Fc-ShK(1-35 Q16K)/KLH Ab Heterotrimer	0.98	1896	**1934.69**	0.245	0.665	**3.66**
109; 82; 109; 77	IgG2	Monovalent aDNP HC-ShK(1-35 Q16K) Ab	0.574	>3333	**>5806.62**	0.278	0.660	**4.15**
28; 29; 28; 32	IgG2	Monovalent aKLH HC-ShK(1-35 Q16K) Ab	3.96	5774	**1458.08**	0.274	0.657	**4.09**
28; 32; 28; 32	IgG2	Bivalent aKLH HC-ShK(1-35 Q16K) Ab	ND	ND	ND	1.392	3.568	20.78
28; 29; 28; 33	IgG2	Monovalent aKLH HC-ShK(2-35 Q16K) Ab	1.66	2617	**1576.51**	0.570	0.820	**8.51**
28; 29; 28; 70	IgG2	Monovalent ShK(1-35 Q16K)-HC aKLH Ab	ND	ND	ND	0.214	0.332	**3.19**
28; 35; 28; 34	IgG1	Monovalent aKLH HC-loop-ShK(1-35 Q16K) Ab	8.264	>1000	**>121.01**	1.604	5.386	23.94
28; 35; 28; 35	IgG1	Bivalent aKLH HC-loop-ShK(1-35 Q16K) Ab	ND	ND	ND	3.910	55.235	58.36

Table 7I. Pharmacokinetics of monovalent Fc/Fc-[Lys16]ShK 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 7J. 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 7K. Pharmacokinetic data for recombinant monovalent Fc/Fc-ShK heterodimer, monovalent Fc-ShK/KLH 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 7L. 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

[00727] **Example 6**

[00728] **Kv1.3 and Kv1.1 Electrophysiology**

[00729] Cell lines expressing Kv1.1 through Kv1.7. CHO-K1 cells were stably transfected with human Kv1.3, or for counterscreens (see, Example 8 herein), 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).

[00730] 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.

[00731] 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 7M in Example 8 herein. 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 400ms incubation time. At the end, excess of a specific benchmark ion channel inhibitor (Table 7M in Example 8) 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 7M in Example 8. Each individual set of traces or trial were visually inspected and either accepted or rejected. The general criteria for acceptance were:

[00732] 1. Baseline current must be stable

[00733] 2. Initial peak current must be >300 pA

[00734] 3. Intitial Rm and final Rm must >300 Ohm

[00735] 4. Peak current must achieve a steady-state prior to first compound addition.

[00736] 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.

[00737] 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:

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

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.

[00738] **Example 7**

[00739] **Measuring Bioactivity in Human Whole Blood**

[00740] 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.

[00741] 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.

[00742] **Example 8**

[00743] **Ion channel counterscreens**

[00744] Kv1.1, Kv1.2, Kv1.3, Kv1.4, Kv1.6 and Kv1.7, PatchXpress®, planar patch-clamp electrophysiology. Ion channel currents can be recorded at room temperature using PatchXpress® 7000A electrophysiology system from MDC using methods and cells

described in Example 6 above. The voltage protocols for each channel are shown in Table 7M, below.

Table 7M. 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

[00745] Cardiac ion channel counterscreens (hERG, hKvLQt1/hminK, hNav1.5, hKv1.5, hCav1.2, hKv4.3).

[00746] 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.

[00747] 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:

[00748] 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.

[00749] 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.

[00750] 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.

[00751] 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.

[00752] 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.

[00753] 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.

[00754] 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.

[00755] **Example 9**

[00756] **AMP5-aKLH Fusions**

[00757] The AMP5 TPO-mimetic peptide was genetically fused to anti-KLH antibodies of the invention in all four possible terminal fusion configurations (represented schematically in Figure 1F-1K; Figure 45), i.e., N-terminally fused and C-terminally fused to both immunoglobulin light chain monomers and to both immunoglobulin heavy chain monomers, and was expressed in mammalian (CHO) cells. The fusions were then purified by protein A chromatography (GE Life Sciences) using 10 column volumes of Dulbecco's PBS without divalent cations as the wash buffer and 100 mM acetic acid as the elution buffer at 7°C. The elution peak was pooled based on the chromatogram and the pH was raised to ~ 5.0 using 2 M Tris base. The pool was then diluted with at least 4 volumes of water 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 60% 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 the material was dialyzed against >20 volumes of 10 mM

acetic acid, 9% sucrose, pH 5.0 using 10 kDa Slide-A-Lyzers (Pierce) at 4°C. The dialyzed material was then filtered through a 0.22 μm cellulose acetate filter and concentration was determined by the absorbance at 280 nm. Injected 50 μg of each antibody along with an unfused control on to a Phenomenex SEC 3000 column (7.8 x 300 mm) in 50 mM NaH2PO4 pH 6.5, 250 mM NaCl at developed at 1 ml/min observing the absorbance at 280 nm (Figure 39). Each antibody was analyzed using a 1.0 mm Tris-glycine 4-20% SDS-PAGE (Novex) developed at 220V using reducing and non-reducing loading buffers and staining with QuickBlue (Boston Biologicals) (Figure 40A-E), and the masses were determined by LC-MS (Figure 41A-D).

[00758] The components of the various aKLH 120.6 IgG2-AMP5, AMP5-aKLH 120.6 IgG2, aKLH 120.6 hIgG1 (N297Q)-AMP5- Fc(CH3) Loop fusion, and AMP5-aKLH 120.6 Kappa embodiments include the following polypeptide monomers:

[00759] (a) aKLH 120.6 kappa LC (SEQ ID NO:28, above);

[00760] (b) aKLH 120.6 IgG2 HC (SEQ ID NO:29, above);

[00761] (c) aKLH 120.6 IgG1 HC (SEQ ID NO:34, above);

[00762] (c) aKLH 120.6 IgG2 HC-Amp5 having the following amino acid sequence:

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASGYTFTGYH
MHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRS
DDTAVYYCARDRGSYYWFDPWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAAL
GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYT
CNVDHKPSNTKVDKTVERKCCVECWYVDGVEVHNAKTKPREEQFNSTFRVVSVLT
VVHQDWLNGKEYKCKVSPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS
HEDPEVQFNNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSLSLSPGGGGGGQGCSSGGPTLREWQQCRRAQHS// (SEQ ID.
NO:324);

[00763] (d) Amp5-aKLH 120.6 IgG2 HC (SEQ ID NO:332) having the following amino acid sequence:

MDMRVPAQLLGLLLLWLRGARCQGCSSGGPTLREWQQCRRAQHSGGGGGQVQLV
QSGAEVKKPGASVKVSCKASGYTFTGYHMHWVRQAPGQGLEWMGWINPNSGGTN
YAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTL
VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG// (SEQ ID
NO:332).

[00764] (e) aKLH 120.6 hIgG1 N297Q-Amp5 Fc(CH3) Loop having the following amino acid sequence:

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASGYTFTGYH
MHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRS
DDTAVYYCARDRGSYYWFDPWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAAL
GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC
NVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE
VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQD
WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMGGQGCSSGGPT
LREWQQCRRAQHSGGTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG// (SEQ ID
NO:341);

[00765] (f): Amp5-aKLH 120.6 kappa LC polypeptide fusion having the following amino acid sequence:

MDMRVPAQLLGLLLLWLRGARCQGCSSGGPTLREWQQCRRAQHSGGGGGDIQMT
QSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPSRFS
GSGSGTEFTLTISSLQPEDFATYYCLQHNSYPLTFGGGTKVEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL
TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC// (SEQ ID NO:342).

[00766] aKLH 120.6--IgG2 Heavy Chain(HC)-AMP5 Mammalian Expression. The desired aKLH 120.6 IgG2 DesK-AMP5 product is a full antibody with the AMP5 peptide

fused to the C-terminus of one heavy chain, configured as in the schematic representation of Figure 1F, and was assembled by two separate rounds of Polymerase Chain Reaction (PCR) using PFU High Fidelity Ultra, by Stratagene. The first round of PCR generated two fragments: VK1sp-aKLH 120.6 IgG2 HC DesK-G5 and G5-AMP5 fragment. The oligo's and PCR templates that were used to generate these fragments were SEQ ID NO:325 and 326, below. Polymerase Chain Reaction 1(PCR1) generated the VK1sp-aKLH 120.6 IgG2 HC DesK-G5 fragment and existing DNA that coded for the VK1sp-aKLH 120.6 IgG2 DesK HC peptide was used as template.

[00767] Forward primer sequence was:

[00768] AAG CTC GAG GTC GAC TAG ACC ACC ATG GAC ATG AGG GTG CCC GCT CAG CTC CTG GGG CT// (SEQ ID NO:325); and

[00769] Reverse Primer sequence was:

[00770] GCC GCT GCT GCA GCC CTG ACC ACC ACC TCC ACC ACC CGG AGA CAG GGA GAG// (SEQ ID NO:326).

[00771] The amino acid sequence encoded by the VK1sp-aKLH120.6 IgG2 HC DesK-G5 fragment, generated from PCR1 was:

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASGYTFTGYH
MHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRS
DDTAVYYCARDRGSYYWFDPWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAAL
GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYT
CNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNG
KEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP
SDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGGGGGGQGC// (SEQ ID NO:327).

[00772] Polymerase Chain Reaction 2 (PCR2) generated the G5-AMP5 fragment (SEQ ID NO:330), and existing DNA that coded for the AMP5 polypeptide was used as template with the following primers sequence:

[00773] Forward primer sequence was

CTC TCC CTG TCT CCG GGT GGT GGA GGT GGT GGT CAG GGC TGC AGC AGC GGC// (SEQ ID NO:328); and

[00774] Reverse primer sequence was:

CTA CTA GCG GCC GCT CAG CTA TGC TGA GCG CGG CG// (SEQ ID NO:329). The amino acid sequence encoded by the fragment generated from PCR2 was:

[00775] LSLSPGGGGGGQGCSSGGPTLREWQQCRRAQHS// (SEQ ID NO:330).

[00776] The 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 in PCR3 using the outside primers SEQ ID NO:325 and SEQ ID NO:329. The final PCR product was run on a 1% agarose gel. The correct size product was cut out, then gel purified by Qiagen's Gel Purification Kit. The purified gel fragment of VK1sp-aKLH 120.6 IgG2 DesK HC-G5-AMP5 was digested with restriction enzymes SalI and NotI, and then the digested product was purified by Qiagen's PCR Purification Kit. At the same time, pTT5 Vector (an Amgen vector containing a CMV promoter and Poly A tail) was cut by SalI 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 VK1sp-aKLH 120.6 IgG2 DesK HC-G5-AMP5 product was ligated to the large vector fragment and transformed into OneShot® Top10 bacterial cells. The DNAs from transformed bacterial colonies were isolated and submitted for sequence analysis. One correct clone was selected for large scale plasmid purification.

[00777] The final pTT5:VK1sp-aKLH 120.6-IgG2 DesK HC-G5-AMP5 construct encoded the following IgG2 DesK HC-AMP5 polypeptide:

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASGYTFTGYH
MHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRS
DDTAVYYCARDRGSYYWFDPWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAAL
GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYT
CNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNG
KEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP

SDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGGGGGGQGCSSGGPTLREWQQCRRAQHS// (SEQ ID NO:331).

[00778] AMP5-aKLH120.6-IgG2 Heavy Chain(HC) Mammalian Expression.

[00779] The desired AMP5-aKLH 120.6 IgG2 DesK HC product (SEQ ID NO:332, above) including the monomer is a full antibody with the AMP5 peptide fused to the N-terminus of one heavy chain, configured as in schematic representation Figure 1I, and was assembled by two separate rounds of PCR using PFU High Fidelity Ultra, by Stratagene. The first round of PCR generated three fragments: VK1sp-AMP5, AMP5-G5, and G5-aKLH 120.6 IgG2 DesK HC fragment. The oligo's and PCR templates that were used to generate these fragments are listed below. Polyermase Chain Reaction 1 (PCR1) generated the VK1sp-AMP5 and existing DNA which coded for the VK1sp was used as template. Note this fragment was also used in construction of the VK1sp-AMP5-G5-aKLH 120.6 Kappa LC.

[00780] The forward primer sequence was:

[00781] AAG CTC GAG GTC GAC TAG ACC ACC ATG GAC ATG AGG GTG CCC GCT CAG CTC CTG GGG CT// (SEQ ID NO:325); and

[00782] The reverse primer sequence was:

[00783] GCC GCT GCT GCA GCC CTG ACA TCT GGC ACC TCT CAA CC// (SEQ ID NO:333). The amino acid sequence encoded by the fragment generated from PCR1 was:

MDMRVPAQLLGLLLLWLRGARCQGCSSG// (SEQ ID NO:334).

[00784] PCR2 generated the AMP5-G5 and existing DNA which coded for the AMP5 peptide was used as template.

[00785] Forward Primer sequence was:

[00786] GGT TGA GAG GTG CCA GAT GTC AGG GCT GCA GCA GCG GC// (SEQ ID NO:335); and

[00787] The reverse primer sequence was:

[00788] CAG CTG CAC CTG ACC ACC ACC TCC ACC GCT ATG CTG AGC GCG// (SEQ ID NO:336).

[00789] The amino acid sequence encoded by the fragment generated from PCR2 was: WLRGARCQGCSSGGPTLREWQQCRRAQHSGGGGGQVQLV// (SEQ ID NO:337).

[00790] PCR3 generated G5-aKLH 120.6 IgG2 DesK HC, and existing DNA which coded for the aKLH 120.6 IgG2 HC (SEQ ID NO:29) monomer was used as template.

[00791] The forward primer sequence was:

CGC GCT CAG CAT AGC GGT GGA GGT GGT GGT CAG GTG CAG CTG// (SEQ ID NO:338); and

[00792] The reverse primer sequence was:

CTA CTA GCG GCC GCT CAA CCC GGA GAC AGG GAG A// (SEQ ID NO:339).

[00793] The amino acid sequence encoded by the fragment generated from PCR3 was:

RAQHSGGGGGQVQLVQSGAEVKKPGASVKVSCKASGYTFTGYHMHWVRQAPGQG
LEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARD
RGSYYWFDPWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV
TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV
DKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPG// (SEQ ID NO:340).

[00794] The 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 PCR4 using the outside primers SEQ ID NO:325 and SEQ ID NO:339. The final PCR product was run on a 1% agarose gel. The correct size product was cut out, then gel purified by Qiagen's Gel Purification Kit. The purified gel fragment of VK1sp-AMP5-G5-aKLH 120.6 IgG2 DesK HC was digested with restriction enzymes SalI and NotI, and then the digested product was purified by Qiagen's PCR Purification Kit. At the same time, pTT5 Vector (an Amgen vector containing a CMV promoter and Poly A tail) was cut by SalI 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 VK1sp-AMP5-G5-aKLH

120.6 IgG2 DesK HC product was ligated to the large vector fragment and transformed into OneShot® Top10 bacterial cells. The DNA's from transformed bacterial colonies were isolated and submitted for sequence analysis. One correct clone was selected for large scale plasmid purification. The final pTT5:VK1sp-AMP5-G5-aKLH 120.6-IgG2 DesK HC construct encoded the AMP5-IgG2 DesK HC polypeptide (SEQ ID NO:332, above).

[00795] aKLH 120.6 aglycosylated hIgG1-AMP5 Fc(CH3) Loop Heavy Chain(HC) Mammalian Expression. The desired aKLH 120.6 IgG1 aglycosylated (N297Q)-AMP5-Fc HC product comprising HC fusion monomer SEQ ID NO:341 (above) is a full antibody with the Amp5 peptide inserted into the CH3 domain of the IgG1 (N297Q) Fc DesK heavy chain, configured as schematically represented in Figure 1M. The VK1sp-aKLH 120.6 IgG1 (N297Q)-AMP5- Fc DesK HC product was ordered by the synthetic gene company, Blue Heron. The final product was generated by digesting the VK1sp-aKLH 120.6 IgG1(N297Q)-AMP5- Fc DesK HC with its corresponding restriction enzymes, SalI and NotI. The digested product was run on a 1% agarose gel. The fragment was cut out, gel purified by Qiagen's Gel Purification Kit. At the same time, pTT5 Vector (an Amgen vector containing a CMV promoter and Poly A tail) was cut by SalI 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 gel fragment of aKLH 120.6 IgG1 (N297Q)-AMP5- Fc DesK HC was ligated to the large vector fragment and transformed into OneShot® Top10 bacterial cells. The DNA's from transformed bacterial colonies were isolated and submitted for sequence analysis. One correct clone was selected for large scale plasmid purification. The final pTT5:VK1sp-aKLH 120.6 IgG1(N297Q)-AMP5- Fc DesK HC construct encodes for the aKLH 120.6 IgG1 (N297Q)-AMP5-DesK polypeptide fusion monomer (SEQ ID NO:341, above).

[00796] AMP5-G5-aKLH 120.6–Kappa Light Chain(LC) Mammalian Expression. The desired AMP5-aKLH 120.6 Kappa LC product is a full antibody with AMP5 peptide fused to the N-terminus of one light chain fusion monomer (SEQ ID NO:342, above), configured as schematically represented in Figure 1H, and was assembled by two separate rounds of Polyermase Chain Reaction (PCR) using PFU High Fidelity Ultra, by Stratagene. The first round of PCR generated three fragments which included, VK1sp-AMP5, AMP5-G5, and G5-aKLH 120.6 Kappa LC. The oligo's and templates used for PCR reactions to generate the fragments are listed below. The fragment that generated the VK1sp-AMP5 is the same

fragment that was used in construction of the AMP5-aKLH 120.6 IgG2 DesK HC, and is described in that section. Polyermase Chain Reaction 2(PCR2) generated the AMP5-G5 fragment and existing DNA that coded for the AMP5 peptide was used as template. Forward primer sequence was (SEQ ID NO:335, above) and reverse primer sequence was CTG GGT CAT CTG GAT GTC ACC ACC ACC TCC ACC GCT ATG CTG AGC GCG// (SEQ ID NO:344). The amino acid sequence encoded by the fragment generated from PCR2 was: WLRGARCQGCSSGGPTLREWQQCRRAQHSGGGGGDIQMTQ// (SEQ ID NO:345).

[00797] PCR3 generated the G5-aKLH 120.6-Kappa LC fragment and existing DNA that coded for the aKLH 120.6 Kappa LC (SEQ ID NO:28) was used as template.

[00798] The forward primer sequence was:

CGC GCT CAG CAT AGC GGT GGA GGT GGT GGT GAC ATC CAG ATG ACC CAG// SEQ ID NO:346); and

[00799] the reverse primer sequence was:

AAC CGT TTA AAC GCG GCC GCT CAA CAC TCT CCC CTG TTG AA// (SEQ ID NO:347). The peptide sequence of the fragment generated from PCR3 was:

RAQHSGGGGGDIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKR LIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPLTFGGGTKVEI KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESV TEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC// (SEQ ID NO:348).

[00800] The 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 PCR4 using the outside primers SEQ ID NO: 325 and SEQ ID NO:347. The final PCR product was run on a 1% agarose gel. The correct size product was cut out, then gel purified by Qiagen's Gel Purification Kit. The purified gel fragment of VK1sp-AMP5-G5-aKLH 120.6 Kappa LC was digested with restriction enzymes SalI and NotI, and then the digested product was purified by Qiagen's PCR Purification Kit. At the same time, pTT5 Vector (an Amgen vector containing a CMV promoter and Poly A tail) was cut by SalI 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 VK1sp-AMP5-G5-aKLH 120.6 Kappa LC product was ligated to the large vector fragment and transformed into OneShot® Top10 bacteria. DNAs from transformed bacterial colonies were isolated and submitted for sequence analysis. One correct clone was selected for large scaled plasmid purification. The final pTT5:VK1sp-AMP5-G5-aKLH 120.6-Kappa LC construct encoded an AMP5-Kappa LC polypeptide fusion monomer (SEQ ID NO:342, above).

[00801] Transient transfection was carried out in 293-6E cells (NRCC) using PEI (Polyethylenimine, linear, 25 kDa, 1 mg/ml sterile stock solution, pH 7.0, Polysciences). The 293-6E cell density was 1.1 X 10^6 before transfection, then using 500 micrograms of DNA (heavy chain and light chain DNA, 1:1 ratio) per liter of cells transfected. The DNA was added to 50 ml 293 FreeStyle media (Invitrogen) and combined with 1.5 ml of PEI solution, vortexed mildly and then incubated 15 minutes at room temperature. The cells were transfected by adding the whole PEI-DNA mixture to the culture. Cells were then incubated on a shaker (120 rpm) at 37°C containing 5% CO_2 for 24 hours. Tryptone N1 (TekniScience Inc, 20% in FreeStyle media) was then added to a final concentration of 0.5% and the incubation was continued for 5 days. The condition medium was harvested at day 5 by centrifuge at 4000 rpm followed by filtration through a 0.45 μm filter (Corning Inc.).

[00802] The fusions were then purified by protein A chromatography (GE Life Sciences) using 10 column volumes of Dulbecco's PBS without divalent cations as the wash buffer and 100 mM acetic acid 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 4 volumes of water 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 60% 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 the material was dialyzed against >20 volumes of 10 mM acetic acid, 9% sucrose, pH 5.0, using 10 kDa Slide-A-Lyzers (Pierce) at 4°C. The dialyzed material was then filtered through a 0.22 um cellulose acetate filter and concentration was determined by the absorbance at 280 nm. Injected 50 μg of each antibody along with an unfused control on to a Phenomenex SEC 3000 column (7.8 x 300 mm) in 50 mM NaH_2PO_4, pH 6.5, 250 mM NaCl, developed at 1 ml/min, detecting the absorbance at 280 nm (Figure 39). All five antibodies showed the expected retention time for molecules of their size showing that very little aggregate was

present. Each antibody was analyzed using a 1.0 mm Tris-glycine 4-20% SDS-PAGE (Novex) developed at 220V using reducing and non-reducing loading buffers and staining with QuickBlue (Boston Biologicals; Figure 40A-E), and the masses were determined by LC-MS (Figure 41A-D). In a typical experiment, 10 µg of the sample was reduced in 25 µl of 8 M GdHCl 50mM Tris (pH 8.5) for 30 min at 55°C, then the reduced material was chromatographed through a Waters Massprep micro desalting column (2.1 x 5 mm) using an Acquity UPLC system (solvent A was 0.1 % formic acid in water and solvent B was 0.1% formic acid in acetonitrile). The column was equilibrated with 5 % solvent B at a flow rate 0.2 ml per min at 80 °C, and upon sample introduction, the column was washed with 5% B for 1 min before the protein was eluted using a linear gradient from 5 to 40% B over 10 min. The column effluent was introduced into a Waters time-of-flight LCT premier mass spectrometer for mass measurement. CsI ions (3 mg CsI per ml in 50% isopropanol) was used as lock mass. The mass spectrum was deconvoluted using the MaxEnt1 software supplied with the instrument. The SDS-PAGE analysis demonstrated that all the expected quaternary structures were formed, and the mass spectral analysis demonstrates that the expected fusions were present in the purified molecules. Taken together these data indicate that fusions can be made with any of the four possible N-terminal or C-terminal fusion configurations of the monomers of aKLH 120.6 antibody, as well as Fc domain internal loop inserts (see, Figure 1F-1N and Figure 45 schematic representations).

[00803] **Example 10**

[00804] **Ex4-aKLH Ab Fusions**

[00805] The Exendin-4 peptide (HGEGTFTSDL SKQMEEEAVR LFIEWLKNGG PSSGAPPPS// SEQ ID NO:349) was genetically fused to N-terminus of the light chain of the anti-KLH 120.6 antibody through the 1kG linker (designated "Ex-4-1kG-aKLH 120.6-Ab"and expressed in mammalian cells. Figure 42 is a schematic map of the Exendin-4 ("Ex4")-1kG-aKLH 120.6 LC fusion construct.

[00806] The components of the Ex-4-1kG-aKLH 120.6-Ab fusion included the following monomers:

[00807] (a) Ex-4-1kG-aKLH 120.6 kappa LC having the following amino acid sequence:

MDMRVPAQLLGLLLLWLRGARCHGEGTFTSDL SKQMEEEAVR LFIEWLKNGG PSSGAPPPSG SGSATGGSGSGASSGSGSAT GSDIQMTQSP SSLSASVGDR VTITCRASQG IRNDLGWYQQKPGKAPKRLI YAASSLQSGV PSRFSGSGSG TEFTLTISSL QPEDFATYYCLQHNSYPLTF GGGTKVEIKR TVAAPSVFIF PPSDEQLKSG TASVVCLLNNFYPREAKVQW KVDNALQSGN SQESVTEQDS KDSTYSLSST LTLSKADYEKHKVYACEVTH QGLSSPVTKS FNRGEC// (SEQ ID NO:355); and

[00808] (b) aKLH 120.6 IgG2 HC (SEQ ID NO:29, above).

[00809] The desired Ex-4-1kG-aKLH 120.6-Ab product was a full antibody configured with the Ex-4 peptide fused to the N-termini of both light chains (see, schematic representation in Figure 1K). The ratio of Ex-4-light chain:heavy chain was 1:1. The isolation and cloning of the genes encoding XenoMouse® hybridoma expressing aKLH 120.6 monoclonal antibody 120.6 heavy and light chains have been described in Example 1 and Example 4, above. Its native signal peptides have been replaced by the VK1/O12 peptide (MDMRVPAQLLGLLLLWLRGARC// SEQ ID NO:103) as described above. DNA fragments encoding aKLH 120.6 LC (SEQ ID NO:28) and aKLH 120.6 HC IgG2 (SEQ ID NO:29) monomers were individually cloned into mammalian expression vector pTT5 (An Amgen vector containing a CMV promoter and Poly A tail.) to generate pTT5:aKLH120.6-VK1SP-kappa Light Chain(LC) construct and pTT5:aKLH120.6-VK1SP-IgG2 Heavy Chain (HC) construct, respectively.

[00810] A DNA fragment (SEQ ID NO:351, below) flanked by SalI (5') and BamHI (3') that comprises the Kozak sequence and the first part of an ORF that encompasses the VK1/O12 signal peptide (SEQ ID NO:103), the Ex-4 (1-39) peptide (SEQ ID NO:349), and the 1kG linker peptide was synthesized and cloned by GenScript (Piscataway, NJ) according to standard gene synthesis techniques.

SalI

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GTCGACTAGACCACCATGGACATGAGGGTCCCCGCTCAGCTCCTGGGGCTCCTGCTATTGTG
GTTGAGAGGTGCCAGATGTCATGGGGAGGGAACATTTACAAGCGATCTGAGCAAACAAATGG
AGGAAGAGGCAGTTAGACTGTTCATTGAATGGCTCAAGAACGGCGGACCGAGTAGTGGTGCT
CCGCCTCCCAGCGGATCTGGCAGCGCTACTGGTG
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GATCTGGATCGGGTGCATCCTCTGGATCTGGAAGCGCTACCGGATCC//(SEQ ID NO:351)

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BamHI

[00811] The BamHI (5') to NotI (3') fragment (SEQ ID NO:368, below) that covers the latter part of an ORF that consists of the mature aKLH 120.6-Ab LC was amplified from the aKLH 120.6-Ab LC DNA template described above (pTT5-aKLH 120.6-VK1SP-kappa Light Chain(LC) construct) with a pair of oligo primers:

AAT GGA TCC GAC ATC CAG ATG ACC CAG TC/ (SEQ ID NO:352); and AAT GCG GCC GCT CAA CAC TCT CC// (SEQ ID NO:353), according to standard PCR techniques.

BamHI
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GGATCCGACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGT
CACCATCACTTGCCGGGCAAGTCAGGGCATTAGAAATGATTTAGGCTGGTATCAGCAGAAAC
CAGGGAAAGCCCCTAAACGCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCA
AGGTTCAGCGGCAGTGGATCTGGGACAGAATTCACTCTCACAATCAGCAGCCTGCAGCCTGA
AGATTTTGCAACTTATTACTGTCTACAGCATAATAGTTACCCGCTCACTTTCGGCGGAGGGA
CCAAGGTGGAGATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGAT
GAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGA
GGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCA
CAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCA
GACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGT
CACAAAGAGCTTCAACAGGGGAGAGTGTTGAGCGGCCGC// (SEQ ID NO:368)

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NotI

[00812] The synthetic SalI-BamHI fragment and the PCR-amplified BamHI-NotI fragment were digested by corresponding restriction enzymes, isolated from an agarose gel and ligated into the SalI and NotI cloning sites of the pTT5 mammalian transient expression vector according to standard molecular cloning techniques (and described above in Example 4 re aKLH 120.6-HC-[Lys16]ShK Ab) resulting in the expression vector pTT5:Ex-4-1kG-aKLH 120.6 LC containing a clone (SEQ ID NO:354) that encodes the amino acid sequence of the
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Ex-4-1kG-aKLH 120.6 LC monomer (with N-terminal VK1/O12 signal peptide) (SEQ ID NO:355).

SalI

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GTCGACTAGACCACCATGGACATGAGGGTCCCCGCTCAGCTCCTGGGGCTCCTGCTATTGTG
GTTGAGAGGTGCCAGATGTCATGGGGAGGGAACATTTACAAGCGATCTGAGCAAACAAATGG
AGGAAGAGGCAGTTAGACTGTTCATTGAATGGCTCAAGAACGGCGGACCGAGTAGTGGTGCT
CCGCCTCCCAGCGGATCTGGCAGCGCTACTGGTG
GATCTGGATCGGGTGCATCCTCTGGATCTGGAAGCGCTACCGGATCCGACATCCAGATGACC
CAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGCAAG
TCAGGGCATTAGAAATGATTTAGGCTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAACGCC
TGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGCGGCAGTGGATCT
GGGACAGAATTCACTCTCACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTG
TCTACAGCATAATAGTTACCCGCTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAACGAA
CTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACT
GCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGT
GGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACA
GCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTC
TACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGG
AGAGTGTTGAGCGGCCGC// (SEQ ID NO:354)

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NotI

[00813] Transient expression was conducted with these pair of expression vectors (pTT5:Ex-4-1kG-aKLH 120.6 LC and pTT5: aKLH 120.6 HC) to generate conditioned medium for the purification of Ex4-1kG-aKLH 120.6-Ab fusion. 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 6mM L-glutamine (Invitrogen, Carlsbad, CA), 1.1% F-68 Pluronic (Invitrogen, Carlsbad, CA) and 250 ug/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 10^6 cells/ml then transfection complexes were prepared in $1/10^{th}$ 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. Twenty-four 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.).

[00814] The fusions were then purified by protein A chromatography (GE Life Sciences) using 10 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 pH of the fractions were increased by leaving 0.025 volumes of 2 M Tris base in the fraction collector tubes. The elution peak was pooled based on the chromatogram and then dialyzed against >20 volumes of 10 mM acetic acid, 9% sucrose, pH 5.0, using 10 kDa Slide-A-Lyzers (Pierce) at room temperature for 3 hours. The dialyzed material was then filtered through a 0.22 μm cellulose acetate filter and concentration was determined by the absorbance at 280 nm. Samples of 25 μg of the antibody fusion were injected on to a Phenomenex SEC 3000 column (7.8 x 300 mm) in 50 mM NaH_2PO_4 pH 6.5, 250 mM NaCl at developed at 1 ml/min observing the absorbance at 280 nm (Figure 43). Since the fusion protein eluted with the expected retention time for a protein of its expected size, this indicates that the protein was able to form the expected complex without excessive aggregation. The Ex4-aKLH 120.6 antibody was analyzed using a 1.0 mm Tris-glycine 4-20% SDS-PAGE (Novex) developed at 220V using reducing and non-reducing loading buffers and staining with QuickBlue (Boston Biologicals)

(Figure 44). The non-reducing SDS-PAGE indicates that the expected quaternary complex of the fusion protein was formed and fusion of the exendin-4 peptide to the aKLH 120.6 antibody results in a product with the expected structure.

[00815] **Example 11**

[00816] **Avimer-aKLH Fusions**

[00817] The C681 polypeptide is an IL-6 binding polypeptide with a so-called avimer structure. (See, e.g., Kolkman et al., Novel Proteins with Targeted Binding, US 2005/0089932; Baker et al., IL-6 Binding Proteins, US 2008/0281076; Stemmer et al., Protein Scaffolds and Uses Thereof, US 2006/0223114 and US 2006/0234299).

[00818] The components of the C681-aKLH 120.6 IgG2 HC fusion included the monomers:

[00819] (a) aKLH 120.6 kappa LC (SEQ ID NO:28); and

[00820] (b) (VK-1 SP)-C681-(G5)-aKLH 120.6 IgG2 HC fusion having the following amino acid sequence:

MDMRVPAQLLGLLLLWLRGARCSGGSCLPDQFRCGNGQCIPLDWVCDGVNDCPDD
SDEEGCPPRTCAPSQFQCGSGYCISQRWVCDGENDCEDGSDEANCAGSVPTCPSDEF
RCRNGRCIPRAWRCDGVNDCADNSDEEDCTEHTGGGGGQVQLVQSGAEVKKPGAS
VKVSCKASGYTFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTM
TRDTSISTAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSSASTKGPS
VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTF
RVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG// (SEQ ID NO:356).

[00821] The desired C681-aKLH 120.6 IgG2 HC product was a full antibody with the Avimer fused to the N-terminus of both heavy chains. The ratio of C681-heavy chain:light

chain was 1:1. The expected C681-aKLH 120.6 IgG2 HC fusion protein was isolated using ion exchange chromatography, as described herein.

[00822] The C681-aKLH 120.6 IgG2 variable HC fusion was ordered from Blue Heron as a synthetic gene encoding the following amino acid sequence:

[00823] MDMRVPAQLLGLLLLWLRGARCSGGSCLPDQFRCGNGQCIPLDWVCDGV NDCPDDSDEEGCPPRTCAPSQFQCGSGYCISQRWVCDGENDCEDGSDEANCAGSVP TCPSDEFRCRNGRCIPRAWRCDGVNDCADNSDEEDCTEHTGGGGGQVQLVQSGAE VKKPGASVKVSCKASGYTFTGYHMHWVRQAPGQGLEWMGWINPNSGGTNYAQKF QGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARDRGSYYWFDPWGQGTLVTVSS ASTK// (SEQ ID NO:350).

[00824] The fragment was digested with SalI and BsmbI, run out on a 1% agarose gel and the corresponding fragment cut out and purified by Qiagen's Gel Purification Kit. At the same time, a pTT5-VK1SP-aKLH 120.6 IgG2 HC DNA template was digested and purified similarly, yielding a pTT5 vector backbone with the constant HC region. The Avimer fragment was ligated to the pTT5-IgG2 HC constant region and transformed into OneShot® Top10 bacteria. DNAs 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-VK1SP-C681-aKLH 120.6 IgG2 HC construct encoded a C681-(G5)-aKLH 120.6 IgG2 HC fusion polypeptide (SEQ ID NO:356).

[00825] The components of the aKLH 120.6 IgG2 HC-C681 fusion included the monomers:

[00826] (a) aKLH 120.6 kappa LC (SEQ ID NO:28); and

[00827] (b) aKLH 120.6 IgG2 HC-C681 fusion having the following amino acid sequence:

MDMRVPAQLLGLLLLWLRGARCQVQLVQSGAEVKKPGASVKVSCKASGYTFTGYH MHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRS DDTAVYYCARDRGSYYWFDPWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYT CNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTC

VVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNG KEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGGGGGGSGGSCLPDQFRCGNGQCIPLDWVCDGVNDCPDDSD EEGCPPRTCAPSQFQCGSGYCISQRWVCDGENDCEDGSDEANCAGSVPTCPSDEFRC RNGRCIPRAWRCDGVNDCADNSDEEDCTEHT// (SEQ ID NO:357).

[00828] The desired aKLH 120.6 IgG2 HC-C681 product was a full antibody with the Avimer fused to the C-terminus of both heavy chains (schematically represented in Figure 1G). The ratio of heavy chain-C681:light chain was 1:1. The expected aKLH 120.6 IgG2 HC-C681 fusion protein was isolated using ion exchange chromatography, as described herein.

[00829] The C681 fragment with flanking SexAI and NotI restriction sites was ordered from Blue Heron as a synthetic gene encoding the following amino acid sequence:

[00830] MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGGSGGSCLPDQFRC GNGQCIPLDWVCDGVNDCPDDSDEEGCPPRTCAPSQFQCGSGYCISQRWVCDGEND CEDGSDEANCAGSVPTCPSDEFRCRNGRCIPRAWRCDGVNDCADNSDEEDCTEHT// (SEQ ID NO:358).

[00831] The fragment was digested with SexAI and NotI, run out on a 1% agarose gel and the corresponding fragment cut out and purified by Qiagen's Gel Purification Kit. At the same time, a pTT5-VK1SP-aKLH 120.6 IgG2 HC DNA template was digested with SalI and SexAI and purified similarly to generate the DNA coding sequence for aKLH 120.6 IgG2 HC monomer (SEQ ID NO:29). A pTT5 vector was cut with SalI and NotI, run out on a 1% agarose gel and the larger fragment cut out and gel purified by Qiagen's Gel Purification Kit. The Avimer and aKLH 120.6 IgG2 HC fragments were ligated to the pTT5 fragment and transformed into OneShot Top10 bacteria. DNAs 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-VK1SP-aKLH 120.6 IgG2 HC-C681 construct encoded aKLH 120.6 IgG2 HC-(G5)-C681 fusion polypeptide (SEQ ID NO:357).

[00832] The components of the C681-aKLH 120.6 kappa LC fusion included the monomers:

[00833] (a) aKLH 120.6 IgG2 HC (SEQ ID NO:29); and

[00834] (b) C681-aKLH 120.6 kappa LC fusion having the following amino acid sequence:

MDMRVPAQLLGLLLLWLRGARCSGGSCLPDQFRCGNGQCIPLDWVCDGVNDCPDD
SDEEGCPPRTCAPSQFQCGSGYCISQRWVCDGENDCEDGSDEANCAGSVPTCPSDEF
RCRNGRCIPRAWRCDGVNDCADNSDEEDCTEHTGGGGGDIQMTQSPSSLSASVGDR
VTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISS
LQPEDFATYYCLQHNSYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCL
LNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV
YACEVTHQGLSSPVTKSFNRGEC// (SEQ ID NO:359).

[00835] The desired C681-aKLH 120.6 kappa LC product was a full antibody with the Avimer fused to the N-terminus of both light chains. The ratio of C681-light chain:heavy chain was 1:1. The expected C681-aKLH 120.6 kappa LC fusion protein was isolated using ion exchange chromatography, as described herein.

[00836] The (VK-1 SP)-C681-(G5)-aKLH 120.6 kappa variable LC fusion was ordered from Blue Heron with flanking SalI BsiWI restriction sites as a synthetic gene encoding the following amino acid sequence:

MDMRVPAQLLGLLLLWLRGARCSGGSCLPDQFRCGNGQCIPLDWVCDGVNDCPDD
SDEEGCPPRTCAPSQFQCGSGYCISQRWVCDGENDCEDGSDEANCAGSVPTCPSDEF
RCRNGRCIPRAWRCDGVNDCADNSDEEDCTEHTGGGGGDIQMTQSPSSLSASVGDR
VTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISS
LQPEDFATYYCLQHNSYPLTFGGGTKVEIKRTVA// (SEQ ID NO:360)

[00837] The fragment was digested with SalI and BsiWI, run out on a 1% agarose gel and the corresponding fragment cut out and purified by Qiagen's Gel Purification Kit. At the same time, a pTT5-VK1SP-aKLH 120.6 kappa LC DNA template was digested and purified similarly, yielding a pTT5 vector backbone with the constant LC region. The Avimer fragment was ligated to the pTT5-kappa LC constant region and transformed into OneShot Top10 bacteria. DNAs 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-VK1SP-C681-aKLH 120.6 kappa LC construct encoded a C681-(G5)-aKLH 120.6 kappa LC fusion polypeptide (SEQ ID NO:359).

[00838] Method for Isolating Avimer-immunoglobin 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 Tris-buffered saline, 1 mM $CaCl_2$ (Teknova) and step elution with 100 mM acetic acid, 1 mM $CaCl_2$, pH 3.5 at a flow rate of 2.5 cm/min. Protein containing fractions were pooled, and the pH was adjusted to 8.0 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 anion exchange FPLC (Q Sepharose High Performance; GE Healthcare). Samples were loaded onto a column equilibrated with 100% buffer A (20 mM Tris, 1 mM, pH 8.0) and eluted with a gradient of 0 to 80% buffer B (20 mM Tris, 1 M NaCl, 1 mM $CaCl_2$, pH 8.0) over 30 column volumes at a flowrate of 1.5 cm/min. Peaks containing target species were pooled and formulated into 10 mM Tris, 150 mM NaCl, 1 mM $CaCl_2$, pH 8.0. Exemplary purifications of N-terminal HC and LC and C-terminal HC fusion proteins are shown in Figures 36-38. The non-reducing SDS-PAGE analysis (Figure 36) demonstrates that the fully assembled antibody can be formed and the reducing SDS-PAGE analysis demonstrates that the desired components are present. The size exclusion chromatogram (Figure 37) shows that the majority of the purified product is in the desired non-aggregated state. Finally, the mass spectral analysis (Figure 38) demonstrates that the desired fusion products are present. Taken together these examples demonstrate that the aKLH 120.6 antibody can accept fusions to Avimers forming the desired product.

[00839] **Example 12**

[00840] **BIAcore® Binding Assays of aDNP and aKLH Antibodies**

[00841] Materials. Purified anti-DNP antibodies from either hybridoma (3A1, 3C2, 3A4 and 3B1) or recombinant CHO (3A4-F-G2 and 3B1-G2) expression were tested. Anti-human IgG, Fcγ-specific antibody was from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). DNP-BSA (2,4-dinitrophenol conjugated to bovine serum albumin) was from

Biosearch Technologies, Inc. (Novato, CA). BIACore 2000, research grade sensor chip CM5, surfactant P-20 (polyoxyethylenesorbitan), HBS-EP (10mM HEPES, 0.15M NaCl, 3.4mM EDTA, 0.005% P-20, pH 7.4), amine coupling reagents, 10mM acetate pH 4.0 and 10mM glycine, pH 1.5 were from BIACore, Inc. (Piscataway, NJ). Phosphate-buffered saline (PBS, 1X, no calcium chloride, no magnesium chloride) was from Invitrogen (Carlsbad, CA). Bovine serum albumin (BSA, fraction V, IgG free) was from Sigma (St. Louis, MO).

[00842] Purified anti-KLH antibody (human IgG1, clone 120.6.1) expressed from hybridoma was tested. Multimeric high molecular weight keyhole limpet hemocyanin (KLH) was from Pierce (Rockford, IL). Anti-human IgG, Fcγ-specific antibody was from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). BIACore 2000, research grade sensor chip CM5, surfactant P-20 (polyoxyethylenesorbitan), HBS-EP (10mM HEPES, 0.15M NaCl, 3.4mM EDTA, 0.005% P-20, pH 7.4), amine coupling reagents, 10mM acetate, pH 4.5, and 10mM glycine, pH 1.5 were from BIACore, Inc. (Piscataway, NJ). Phosphate-buffered saline (PBS, 1X, no calcium chloride, no magnesium chloride) was from Invitrogen (Carlsbad, CA). Bovine serum albumin (BSA, fraction V, IgG free) was from Sigma (St. Louis, MO).

[00843] Methods. BIAcore® analyses were carried out as follows. Immobilization of anti-human IgG, Fcγ-specific antibody to the CM5 sensor chip surface was performed according to manufacturer's instructions, using a continuous flow of 10 mM HEPES, 0.15M NaCl, 3.4mM EDTA, 0.005% P-20, pH 7.4 (HBS-EP buffer). Briefly, carboxyl groups on the sensor chip surfaces were activated by injecting 60 μL of a mixture containing 0.2 M 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) and 0.05 M N-hydroxysuccinimide (NHS). Specific surfaces were obtained by injecting 180 μL of anti-human IgG, Fcγ-specific antibody diluted in 10 mM acetate buffer (for assay of aKLH antibodies: pH 4.5 at a concentration of 30 μg/mL; for assay of aDNP antibodies: pH 4.0 at a concentration of 60 μg/mL). Excess reactive groups on the surfaces were deactivated by injecting 60 μL of 1 M ethanolamine. Final immobilized levels were about 9,000 (for assay of aKLH antibodies) or about 10,000 (forassay of aDNP antibodies) resonance units (RU). A blank, mock-coupled reference surface was also prepared on the sensor chip. Antibodies and antigen were diluted in sample buffer consisting of PBS + 0.005% P-20 + 0.1 mg/mL BSA.

[00844] Anti-DNP antibodies were captured on individual flow cells, followed by injection of either sample buffer or DNP-BSA, ranging in concentration from 0.78 – 100 nM. Two different DNP-BSA samples were tested for affinity to the anti-DNP antibodies. The DNP-BSA samples differed in the number of DNP moieties coupled to each molecule of BSA, with one sample containing 3 DNP moieties per BSA and the other containing 31 DNP moieties per BSA. Only the DNP(31)-BSA (at concentrations from 0.39 – 50 nM) was tested for affinity to the recombinant anti-DNP antibodies. In each cycle, three individual antibodies were captured on flow cells 2, 3 and 4, with flow cell 1 left blank to serve as a reference surface. Following sample buffer or antigen injection, each surface was regenerated by two injections of 10 mM glycine, pH 1.5 to dissociate captured antibody from the immobilized anti-human Fc surfaces. BIAevaluation software was used to determine apparent kinetic parameters for binding of DNP-BSA to captured anti-DNP antibodies.

[00845] Anti-KLH antibody wase captured on individual flow cells, followed by injection of either sample buffer or KLH, ranging in concentration from 0.19 – 100 nM. To prepare dilutions of the multimeric high molecular weight KLH, an average molecular weight of 5,000,000 daltons was used. Following sample buffer or antigen injection, each surface was regenerated by two injections of 10 mM glycine, pH 1.5 to dissociate captured antibody from the immobilized anti-human Fc surfaces. BIAevaluation software was used to determine apparent kinetic parameters for binding of KLH to captured anti-KLH antibodies.

[00846] BIAcore® binding aassay results. Table 8A below summarizes the apparent association (k_a) and dissociation (k_d) rate constants, as well as equilibrium dissociation constants (K_D) obtained for the binding analysis of anti-DNP antibodies binding to DNP-BSA. The data in Table 8A demonstrate that the anti-DNP antibodies bind specifically to DNP, and that they bind more tightly to the higher density DNP(31)-BSA than to the lower density DNP(3)-BSA, as would be expected. Apparent binding affinities for DNP(31)-BSA are all single digit nanomolar or higher.

[00847] Table 8B below summarizes the apparent association (k_a) and dissociation (k_d) rate constants, as well as equilibrium dissociation constants (K_D) obtained for the binding analysis of anti-KLH 120.6.1 antibody binding to KLH. The data in Table 8B demonstrate that this hybridoma-produced anti-KLH antibody binds specifically to multimeric KLH, with an apparent sub-nanomolar binding affinity.

Table 8A. BIAcore® binding assays for aDNP antibodies. ND = not determined; *n*e(y) = *n* x $10^{(y)}$

	Binding to DNP(3)-BSA				Binding to DNP(31)-BSA		
Antibody	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)		k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)
3A1	1.2e3	6.5e-4	526		3.4e4	3.1e-4	9
3C2	9.2e4	5.5e-4	6		1.3e5	2.8e-4	2
3A4	2.0e5	4e-4	2		5.9e5	4.8e-4	0.8
3B1	1.4e5	3.6e-4	3		3.7e5	5.8e-5	0.2
3A4-F-G2 (recombinant	ND	ND	ND		2.6e5	5.5e-4	2
3B1-G2 (recombinant)	ND	ND	ND		4.2e5	3.1e-4	0.7

Table 8B. BIAcore® binding assays for aKLH antibodies. *n*e(y) = *n* x $10^{(y)}$

		Binding to Multimeric KLH		
Antibody		k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)
IgG1 (120.6.1)		1.2e5	2.5e-5	0.2

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 (about 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

CLAIMS

What is claimed is:

1. An isolated antigen binding protein, comprising an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region, wherein the heavy chain variable region comprises an amino acid sequence at least 95% identical to the sequence of SEQ ID NO:250, SEQ ID NO:252, SEQ ID NO:254, SEQ ID NO:256, SEQ ID NO:258, or SEQ ID NO:260.

2. The isolated antigen binding protein of Claim 1, wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO:232, SEQ ID NO:234, SEQ ID NO:236, SEQ ID NO:238, or SEQ ID NO:240.

3. The isolated antigen binding protein of Claim 1, wherein the heavy chain variable region comprises three complementarity determining regions designated CDRHl, CDRH2 and CDRH3, wherein:
(a) CDRHl comprises the amino acid sequence of SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, or SEQ ID NO:191;
(b) CDRH2 comprises the amino acid sequence of SEQ ID NO:192, SEQ ID NO:193, SEQ ID NO:194, or SEQ ID NO:195; and
(c) CDRH3 comprises the amino acid sequence of SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, or SEQ ID NO:201.

4. The isolated antigen binding protein of Claim 1, comprising an immunoglobulin light chain variable region, the light chain variable region comprising three CDRs designated CDRLl, CDRL2 and CDRL3, wherein:
(a) CDRL1 comprises the amino acid sequence of SEQ ID NO:202, SEQ

ID NO:203, SEQ ID NO:204, or SEQ ID NO:205;
(b) CDRL2 comprises the amino acid sequence of SEQ ID NO:206 or SEQ ID NO:207; and
(c) CDRL3 comprises the amino acid sequence of SEQ ID NO:208, SEQ ID NO:209, SEQ ID NO:210, SEQ ID NO:211, or SEQ ID NO:212.

5. The isolated antigen binding protein of Claim 1, wherein the heavy chain variable region comprises three complementarity determining regions designated CDRHl, CDRH2 and CDRH3, and the light chain variable region comprises three CDRs designated CDRLl, CDRL2 and CDRL3, wherein:
(a) CDRHl comprises the amino acid sequence of SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, or SEQ ID NO:191;
(b) CDRH2 comprises the amino acid sequence of SEQ ID NO:192, SEQ ID NO:193, SEQ ID NO:194, or SEQ ID NO:195;
(c) CDRH3 comprises the amino acid sequence of SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, or SEQ ID NO:201;
(d) CDRL1 comprises the amino acid sequence of SEQ ID NO:202, SEQ ID NO:203, SEQ ID NO:204, or SEQ ID NO:205;
(e) CDRL2 comprises the amino acid sequence of SEQ ID NO:206 or SEQ ID NO:207; and
(f) CDRL3 comprises the amino acid sequence of SEQ ID NO:208, SEQ ID NO:209, SEQ ID NO:210, SEQ ID NO:211, or SEQ ID NO:212.

6. The isolated antigen binding protein of Claim 1, wherein the isolated antigen binding protein comprises an antibody or antibody fragment.

7. The isolated antigen binding protein of Claim 6, comprising an IgG1, IgG2, IgG3 or IgG4.

8. The isolated antigen binding protein of Claim 6, comprising a monoclonal antibody.

9. The isolated antigen binding protein of Claim 8, comprising a chimeric or humanized antibody.

10. The isolated antigen binding protein of Claim 8, comprising a human antibody.

11. The isolated antigen binding protein of Claim 6, comprising:
(a) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO:77, SEQ ID NO:107, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:123, SEQ ID NO:129, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:181, SEQ ID NO:182, SEQ ID NO:183, SEQ ID NO:184, or SEQ ID NO:185, or comprising any one of the foregoing sequences from which one, two, three, four or five amino acid residues are lacking from the N-terminal or C-terminal, or both;

(b) an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO:105, SEQ ID NO:109, SEQ ID NO:121; SEQ ID NO:125, or SEQ ID NO:127, or comprising any one of the foregoing sequences from which one, two, three, four or five amino acid residues are lacking from the N-terminal or C-terminal, or both; or

(c) the immunoglobulin heavy chain of (a) and the immunoglobulin light chain of (b).

12. The isolated antigen binding protein of any of Claims 1-11, further comprising one to twenty-four pharmacologically active chemical moieties conjugated thereto.

13. The isolated antigen binding protein of Claim 12, wherein the pharmacologically active chemical moiety is a pharmacologically active polypeptide.

14. The isolated antigen binding protein of Claim 13, wherein the antigen binding peptide is recombinantly produced.

15. The isolated antigen binding protein of Claim 14, wherein the antigen binding protein comprises at least one immunoglobulin heavy chain and at least one immunoglobulin light chain, and wherein the pharmacologically active polypeptide is inserted in the primary amino acid sequence of the of the immunoglobulin heavy chain within an internal loop of the Fc domain of the immunoglobulin heavy chain.

16. The isolated antigen binding protein of Claim 13, wherein the antigen binding protein comprises at least one immunoglobulin heavy chain and at least one immunoglobulin light chain, and wherein the pharmacologically active polypeptide is conjugated at the N-terminal or C-terminal of the immunoglobulin heavy chain.

17. The isolated antigen binding protein of Claim 13, wherein the antigen binding protein comprises at least one immunoglobulin heavy chain and at least one immunoglobulin light chain, and wherein the pharmacologically active polypeptide is conjugated at the N-terminal or C-terminal of the immunoglobulin light chain.

18. The isolated antigen binding protein of Claim 13, wherein the pharmacologically active polypeptide is a toxin peptide, an IL-6 binding peptide, a CGRP peptide antagonist, a bradykinin B1 receptor peptide antagonist, a PTH agonist peptide, a PTH antagonist peptide, an ang-1

binding peptide, an ang-2 binding peptide, a myostatin binding peptide, an EPO-mimetic peptide, a TPO-mimetic peptide, a NGF binding peptide, a BAFF antagonist peptide, a GLP-1 or peptide mimetic thereof, or a GLP-2 or peptide mimetic thereof.

19. The isolated antigen binding protein of Claim 18, wherein the toxin peptide is ShK or a ShK peptide analog.

20. A pharmaceutical composition comprising the antigen binding protein of any of Claims 1-19; and a pharmaceutically acceptable diluent, excipient or carrier.

21. An isolated nucleic acid that encodes the antigen binding protein of any of Claims 1-4.

22. An isolated nucleic acid that encodes the antigen binding protein of Claim 5.

23. An isolated nucleic acid that encodes the antigen binding protein of Claim 11.

24. An isolated nucleic acid that encodes the antigen binding protein of any of Claims 14-19.

25. A vector comprising the isolated nucleic acid of any of Claims 21-24.

26. The vector of Claim 25, comprising an expression vector.

27. An isolated host cell, comprising the expression vector of Claim 26.

28. A method, comprising:

(a) culturing the host cell of claim 27 in a culture medium under conditions permitting expression of the antigen binding protein encoded by the expression vector; and
(b) recovering the antigen binding protein from the culture medium.

29. A hybridoma, wherein the hybridoma produces the antigen binding protein of Claim 11.

30. A method, comprising:
(a) culturing the hybridoma of claim 29 in a culture medium under conditions permitting expression of the antigen binding protein by the hybridoma; and
(b) recovering the antigen binding protein from the culture medium.

31. An isolated antigen binding protein, comprising an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region, wherein the heavy chain variable region comprises an amino acid sequence at least 95 % identical to the sequence of SEQ ID NO:262, SEQ ID NO:264, or SEQ ID NO:266.

32. The isolated antigen binding protein of Claim 31, wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO:242, SEQ ID NO:244, SEQ ID NO:246, or SEQ ID NO:248.

33. The isolated antigen binding protein of Claim 31, wherein the heavy chain variable region comprises three complementarity determining regions designated CDRHl, CDRH2 and CDRH3, wherein:
(a) CDRHl comprises the amino acid sequence of SEQ ID NO:213, SEQ ID NO:214, or SEQ ID NO:215;
(b) CDRH2 comprises the amino acid sequence of SEQ ID NO:216, SEQ ID NO:217, or SEQ ID NO:218; and

(c) CDRH3 comprises the amino acid sequence of SEQ ID NO:219, SEQ ID NO:220, or SEQ ID NO:221.

34. The isolated antigen binding protein of Claim 31, comprising an immunoglobulin light chain variable region, the light chain variable region comprising three CDRs designated CDRLl, CDRL2 and CDRL3, wherein:
(a) CDRL1 comprises the amino acid sequence of SEQ ID NO:204, SEQ ID NO:222, SEQ ID NO:223, or SEQ ID NO:224;
(b) CDRL2 comprises the amino acid sequence of SEQ ID NO:206, SEQ ID NO:225, or SEQ ID NO:226; and
(c) CDRL3 comprises the amino acid sequence of SEQ ID NO:227, SEQ ID NO:228, SEQ ID NO:229, or SEQ ID NO:230.

35. The isolated antigen binding protein of Claim 31, wherein the heavy chain variable region comprises three complementarity determining regions designated CDRHl, CDRH2 and CDRH3, and the light chain variable region comprises three CDRs designated CDRLl, CDRL2 and CDRL3, wherein:
(a) CDRHl comprises the amino acid sequence of SEQ ID NO:213, SEQ ID NO:214, or SEQ ID NO:215;
(b) CDRH2 comprises the amino acid sequence of SEQ ID NO:216, SEQ ID NO:217, or SEQ ID NO:218; and
(c) CDRH3 comprises the amino acid sequence of SEQ ID NO:219, SEQ ID NO:220, or SEQ ID NO:221;
(d) CDRL1 comprises the amino acid sequence of SEQ ID NO:204, SEQ ID NO:222, SEQ ID NO:223, or SEQ ID NO:224;
(e) CDRL2 comprises the amino acid sequence of SEQ ID NO:206, SEQ ID NO:225, or SEQ ID NO:226; and
(f) CDRL3 comprises the amino acid sequence of SEQ ID NO:227, SEQ ID NO:228, SEQ ID NO:229, or SEQ ID NO:230.

36. The isolated antigen binding protein of Claim 31, wherein the isolated antigen binding protein comprises an antibody or antibody fragment.

37. The isolated antigen binding protein of Claim 36, comprising an IgG1, IgG2, IgG3 or IgG4.

38. The isolated antigen binding protein of Claim 36, comprising a monoclonal antibody.

39. The isolated antigen binding protein of Claim 38, comprising a chimeric or humanized antibody.

40. The isolated antigen binding protein of Claim 38, comprising a human antibody.

41. The isolated antigen binding protein of Claim 36, comprising:
(a) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO:46, SEQ ID NO:133, SEQ ID NO:139, SEQ ID NO:143, SEQ ID NO:186, or SEQ ID NO:187, SEQ ID NO:366, or SEQ ID NO:367, or comprising any one of the foregoing sequences from which one, two, three, four or five amino acid residues are lacking from the N-terminal or C-terminal, or both;

(b) an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO:28, SEQ ID NO:131, SEQ ID NO:135, SEQ ID NO:137; or SEQ ID NO:141, or comprising any one of the foregoing sequences from which one, two, three, four or five amino acid residues are lacking from the N-terminal or C-terminal, or both; or

(c) the immunoglobulin heavy chain of (a) and the immunoglobulin light chain of (b).

42. The isolated antigen binding protein of any of Claims 31-41, further comprising one to twenty-four pharmacologically active chemical moieties conjugated thereto.

43. The isolated antigen binding protein of Claim 42, wherein the pharmacologically active chemical moiety is a pharmacologically active polypeptide.

44. The isolated antigen binding protein of Claim 43, wherein the antigen binding peptide is recombinantly produced.

45. The isolated antigen binding protein of Claim 44, wherein the antigen binding protein comprises at least one immunoglobulin heavy chain and at least one immunoglobulin light chain, and wherein the pharmacologically active polypeptide is inserted in the primary amino acid sequence of the of the immunoglobulin heavy chain within an internal loop of the Fc domain of the immunoglobulin heavy chain.

46. The isolated antigen binding protein of Claim 43, wherein the antigen binding protein comprises at least one immunoglobulin heavy chain and at least one immunoglobulin light chain, and wherein the pharmacologically active polypeptide is conjugated at the N-terminal or C-terminal of the immunoglobulin heavy chain.

47. The isolated antigen binding protein of Claim 43, wherein the antigen binding protein comprises at least one immunoglobulin heavy chain and at least one immunoglobulin light chain, and wherein the pharmacologically active polypeptide is conjugated at the N-terminal or C-terminal of the immunoglobulin light chain.

48. The isolated antigen binding protein of Claim 43, wherein the pharmacologically active polypeptide is a toxin peptide, an IL-6 binding peptide, a CGRP peptide antagonist, a bradykinin B1 receptor peptide antagonist, a PTH agonist peptide, a PTH antagonist peptide, an ang-1 binding peptide, an ang-2 binding peptide, a myostatin binding peptide, an EPO-mimetic peptide, a TPO-mimetic peptide, a NGF binding peptide, a BAFF antagonist peptide, a GLP-1 or peptide mimetic thereof, or a GLP-2 or peptide mimetic thereof.

49. The isolated antigen binding protein of Claim 48, wherein the toxin peptide is ShK or a ShK peptide analog.

50. A pharmaceutical composition comprising the antigen binding protein of any of Claims 31-49; and a pharmaceutically acceptable diluent, excipient or carrier.

51. An isolated nucleic acid that encodes the antigen binding protein of any of Claims 31-34.

52. An isolated nucleic acid that encodes the antigen binding protein of Claim 35.

53. An isolated nucleic acid that encodes the antigen binding protein of Claim 41.

54. An isolated nucleic acid that encodes the antigen binding protein of any of Claims 44-49.

55. A vector comprising the isolated nucleic acid of any of Claims 51-54.

56. The vector of Claim 55, comprising an expression vector.

57. An isolated host cell, comprising the expression vector of Claim 56.

58. A method, comprising:
(a) culturing the host cell of Claim 57 in a culture medium under conditions permitting expression of the antigen binding protein encoded by the expression vector; and
(b) recovering the antigen binding protein from the culture medium.

59. A hybridoma, wherein the hybridoma produces the antigen binding protein of Claim 41.

60. A method, comprising:
(a) culturing the hybridoma of claim 59 in a culture medium under conditions permitting expression of the antigen binding protein by the hybridoma; and
(b) recovering the antigen binding protein from the culture medium.

FIG. 1A

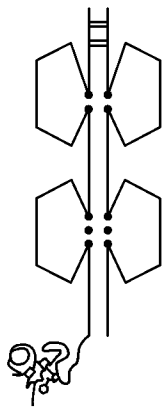


FIG. 1B

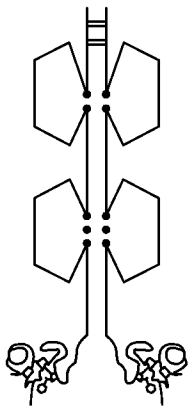


FIG. 1C

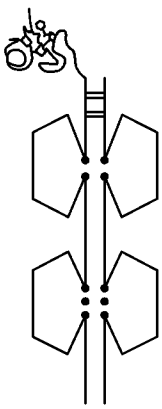


FIG. 1D

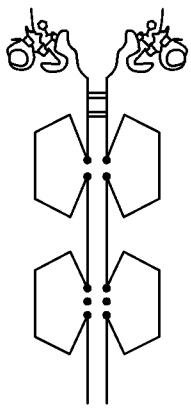


FIG. 1E

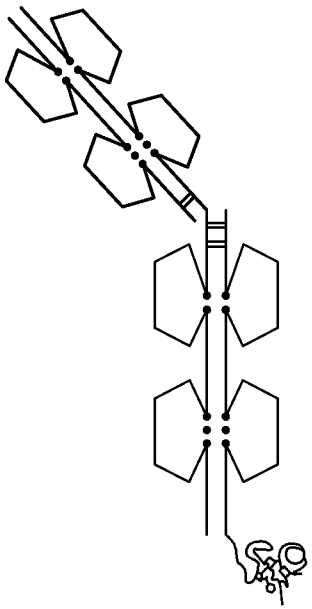


FIG. 1F

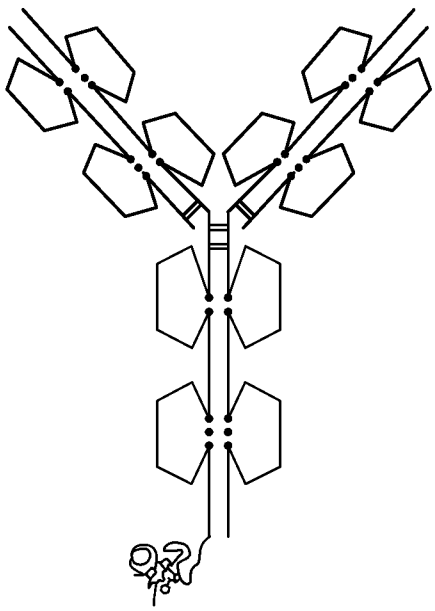


FIG. 1G

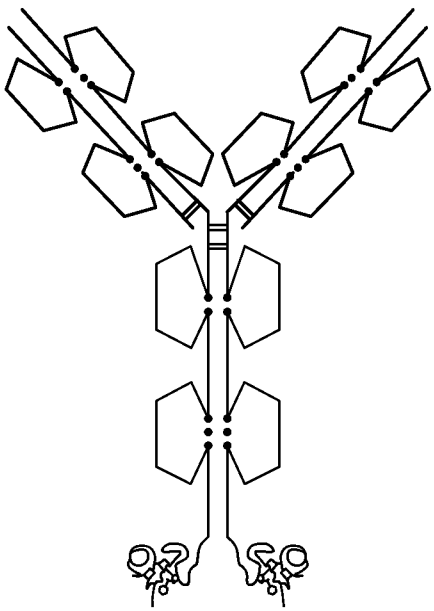


FIG. 1H

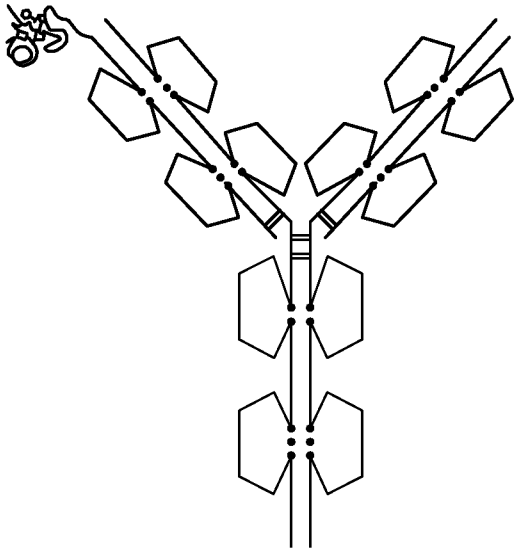


FIG. 1I

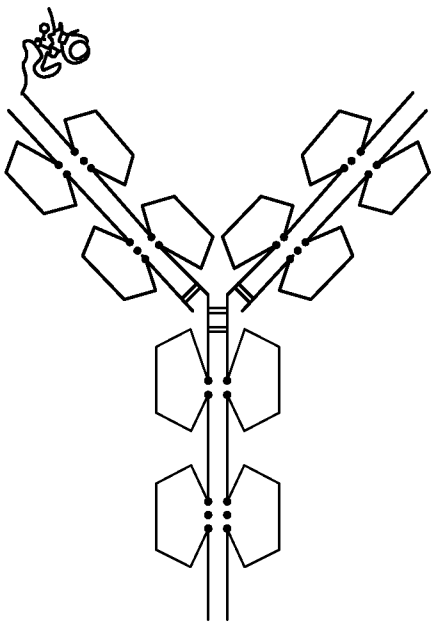


FIG. 1J

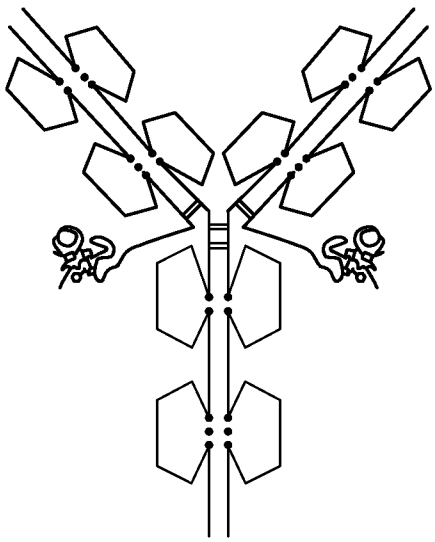


FIG. 1K

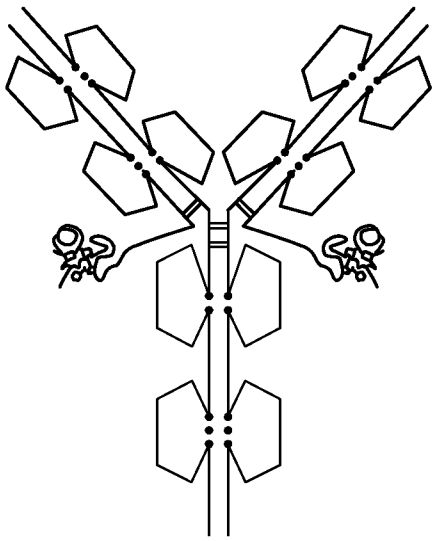


FIG. 1L

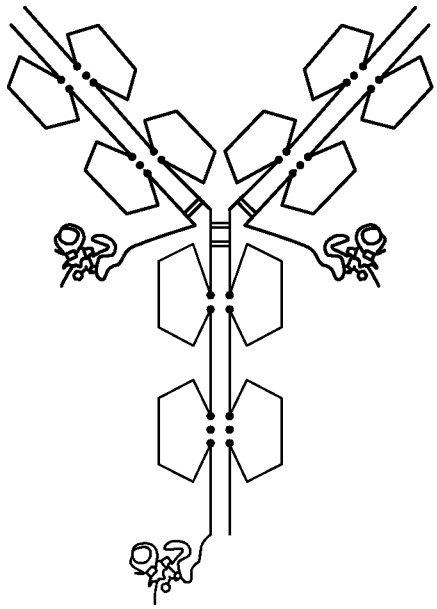


FIG. 1M

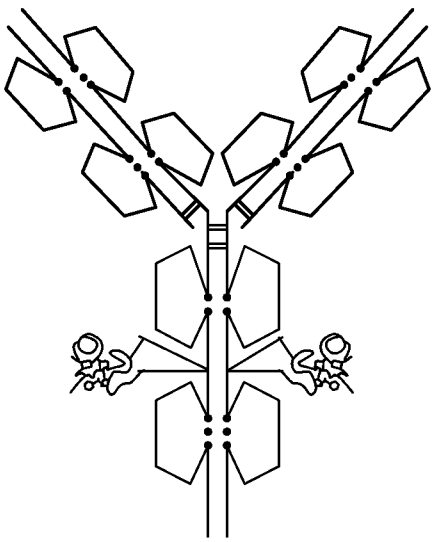


FIG. 1N

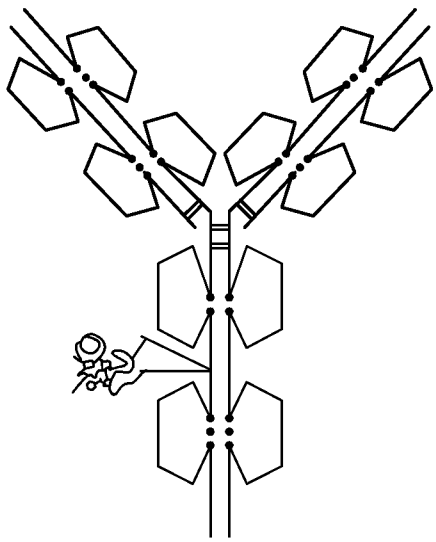


FIG. 2A

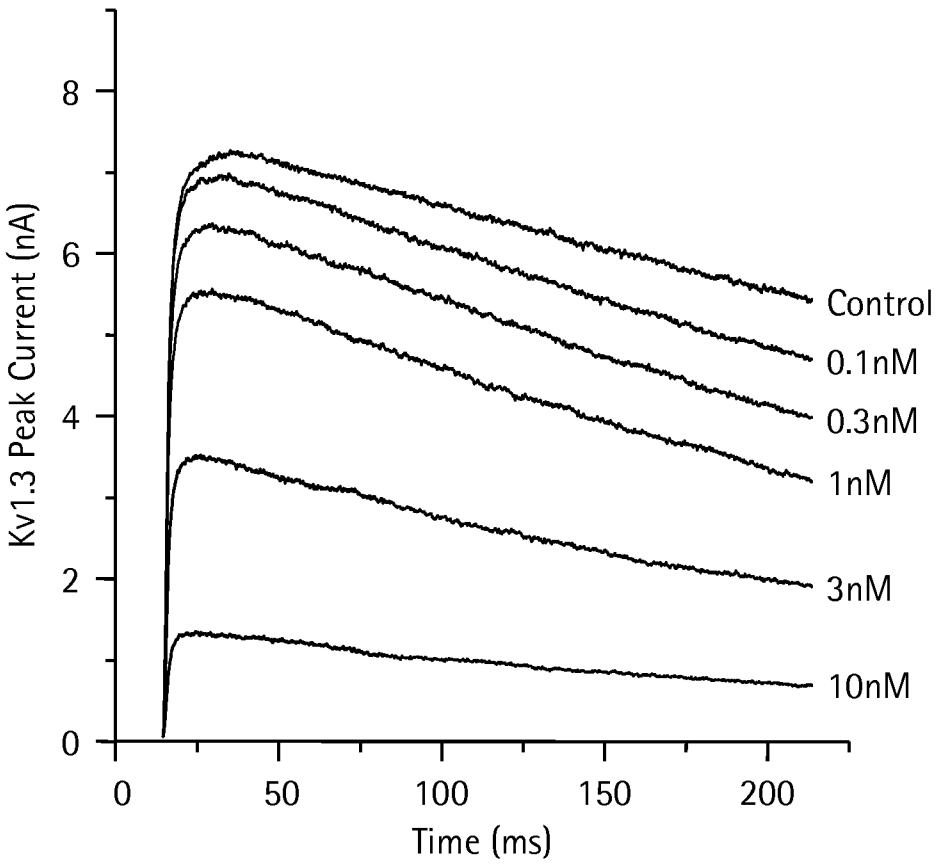

Kv1.3 Peak Current (nA)
0
2
4
6
8
0
50
100
150
200
Time (ms)
Control
0.1nM
0.3nM
1nM
3nM
10nM

FIG. 2B

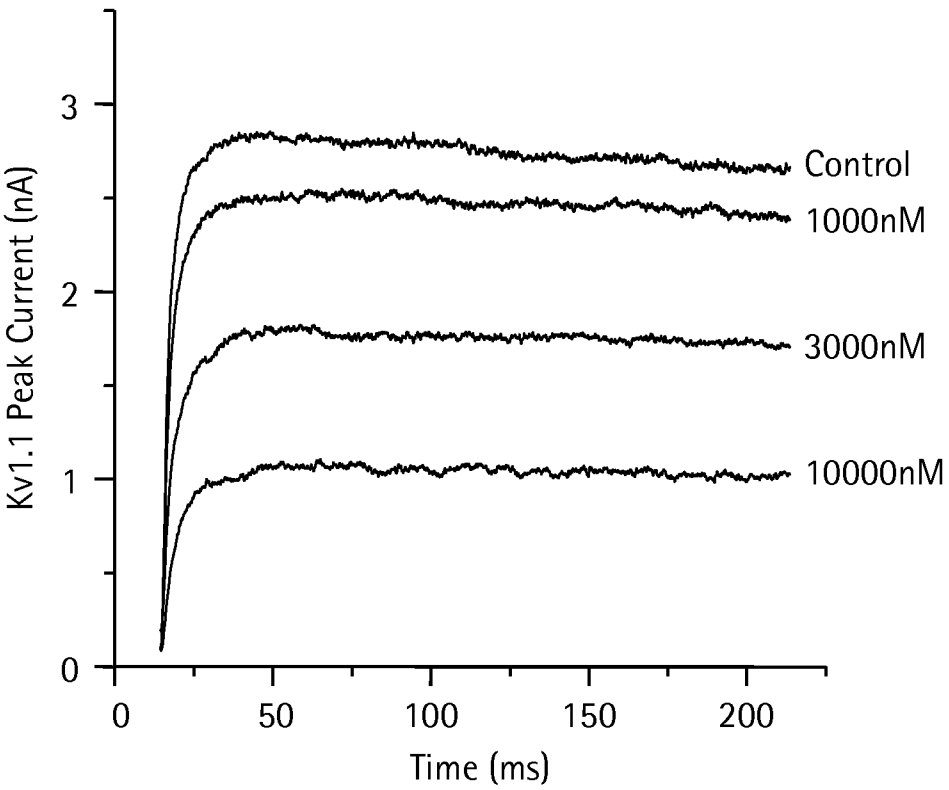

Kv1.1 Peak Current (nA)
0
1
2
3
0
50
100
150
200
Time (ms)
Control
1000nM
3000nM
10000nM

FIG. 3A

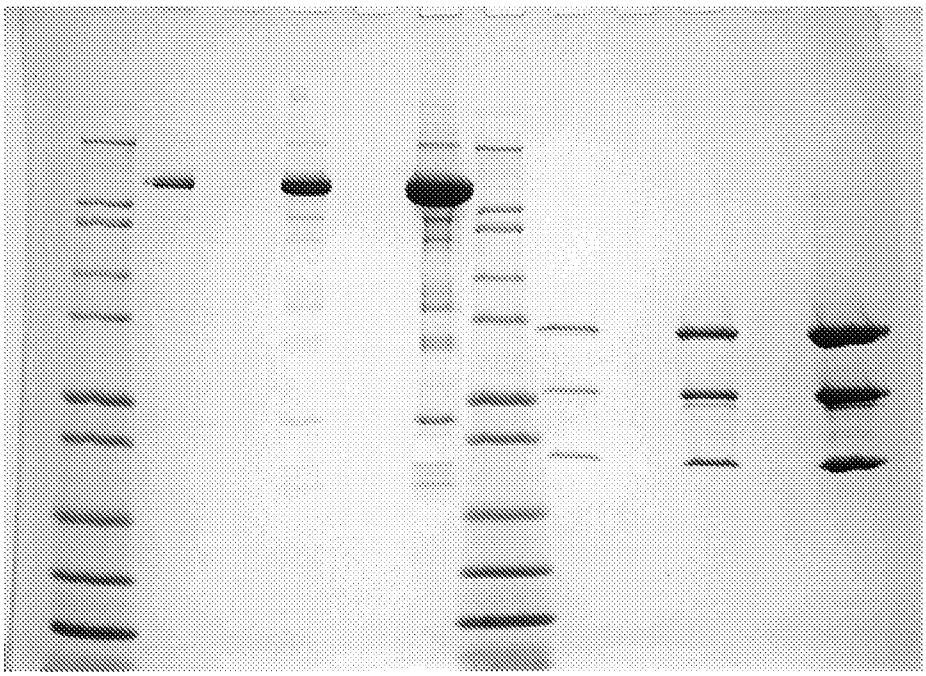


FIG. 3B

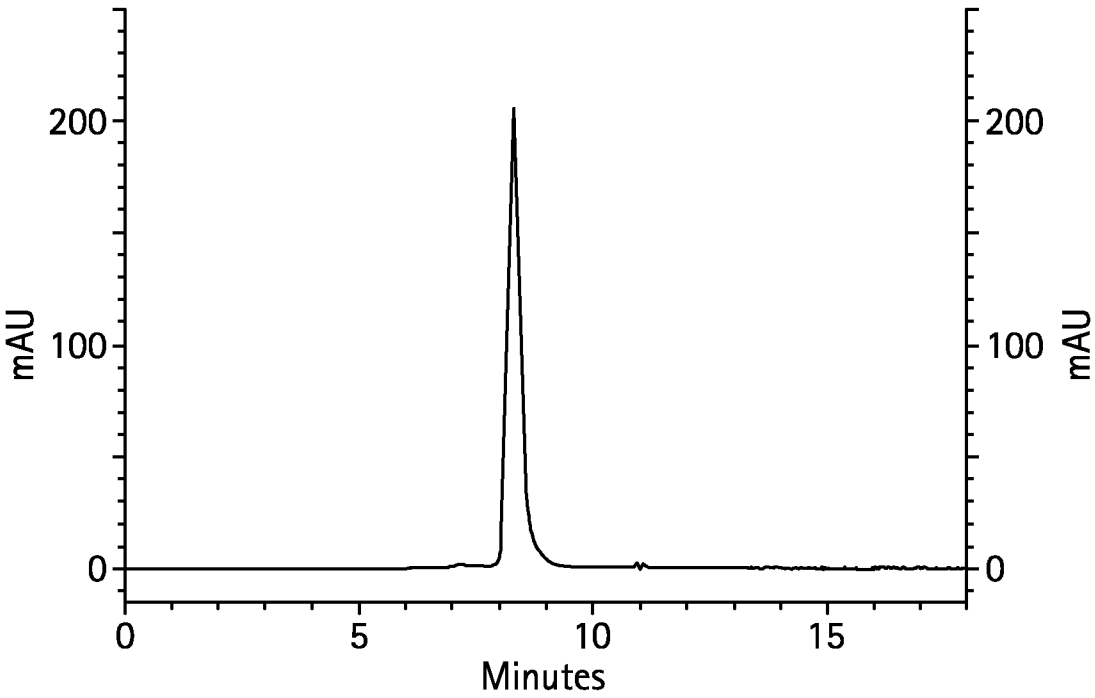


FIG. 3C

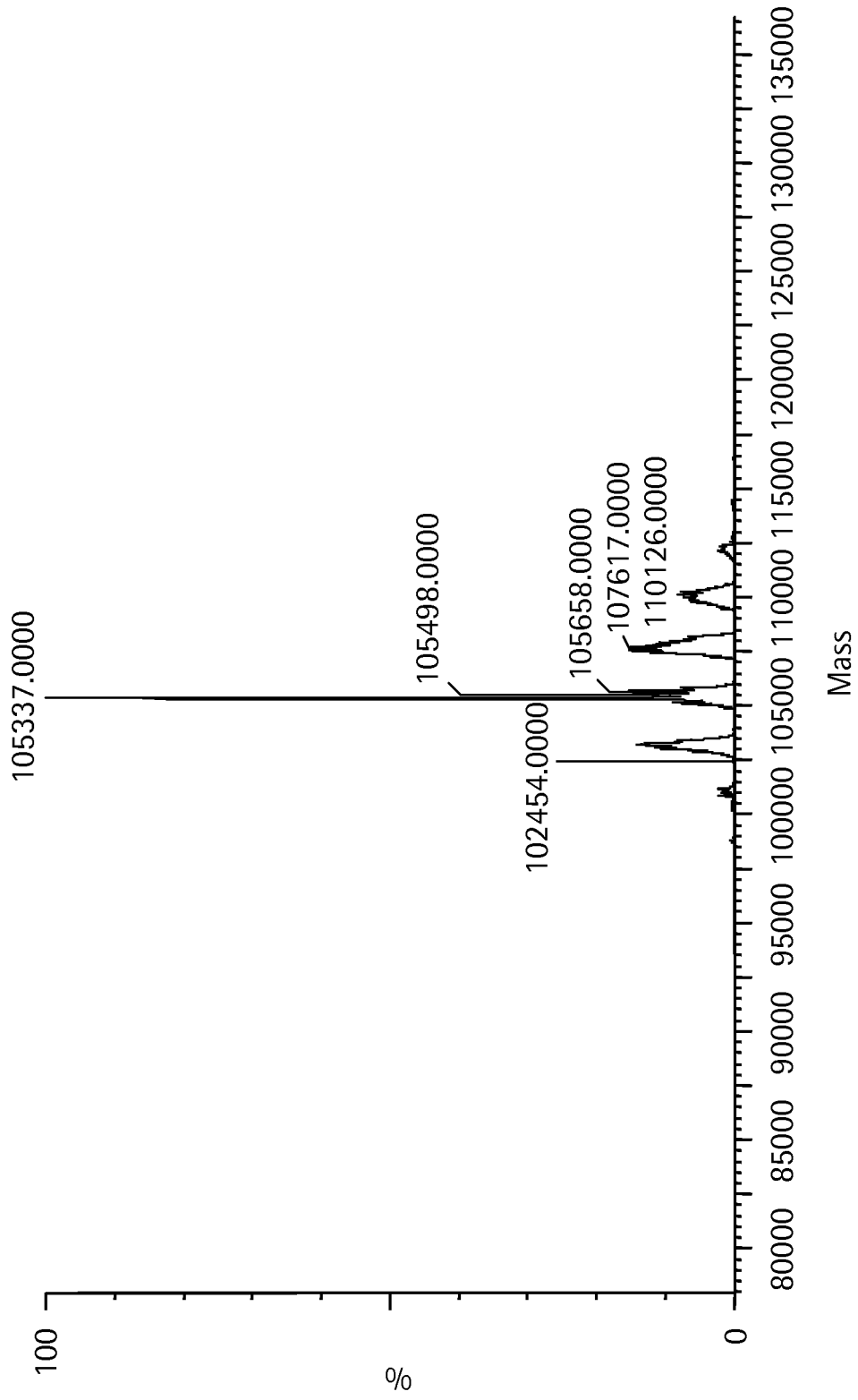

105337.0000
105498.0000
105658.0000
107617.0000
110126.0000
102454.0000
100
%
0
80000 85000 90000 95000 100000 105000 110000 115000 120000 125000 130000 135000
Mass

FIG. 4A

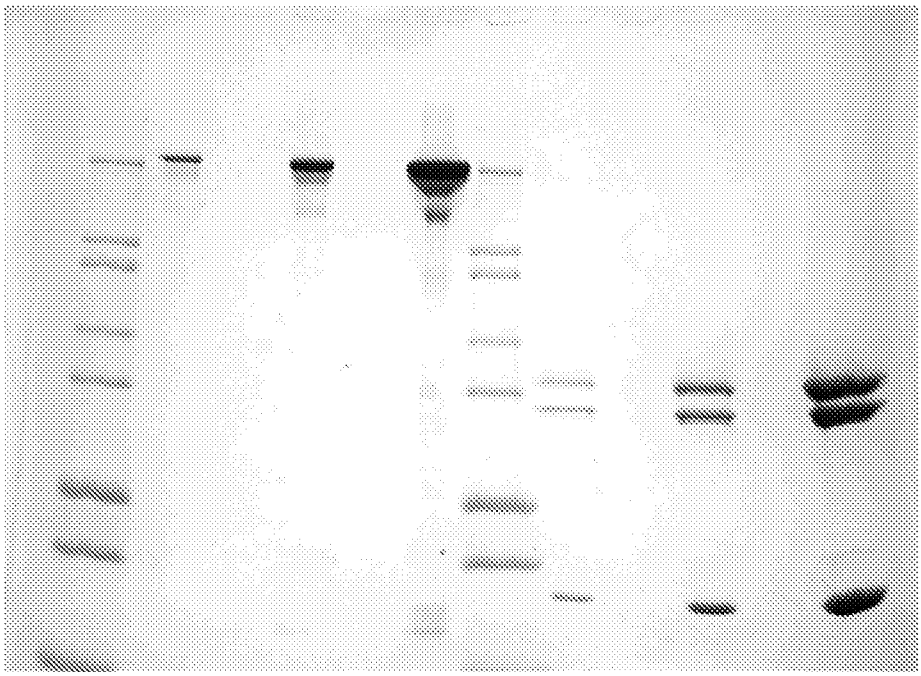


FIG. 4B

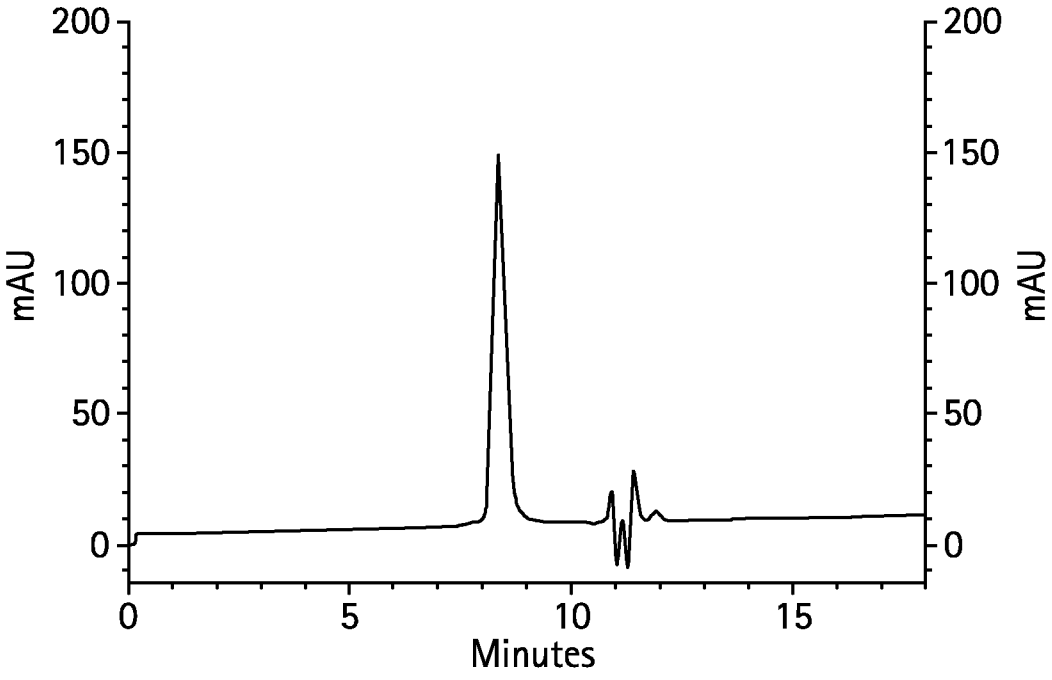


FIG. 4C

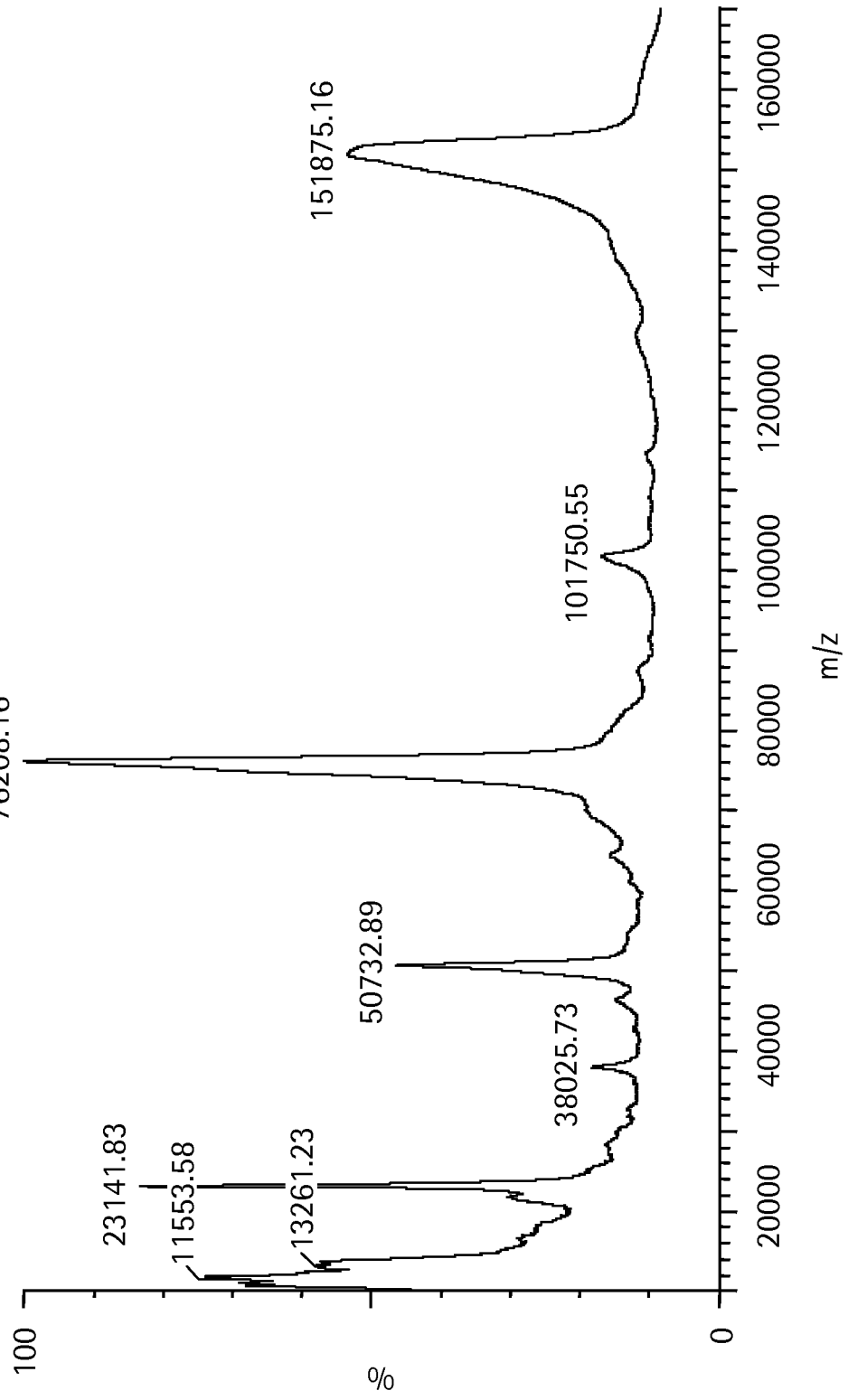

151875.16
101750.55
76208.16
50732.89
38025.73
23141.83
11553.58
13261.23
100
%
0
20000
40000
60000
80000
100000
120000
140000
160000
m/z

FIG. 5A

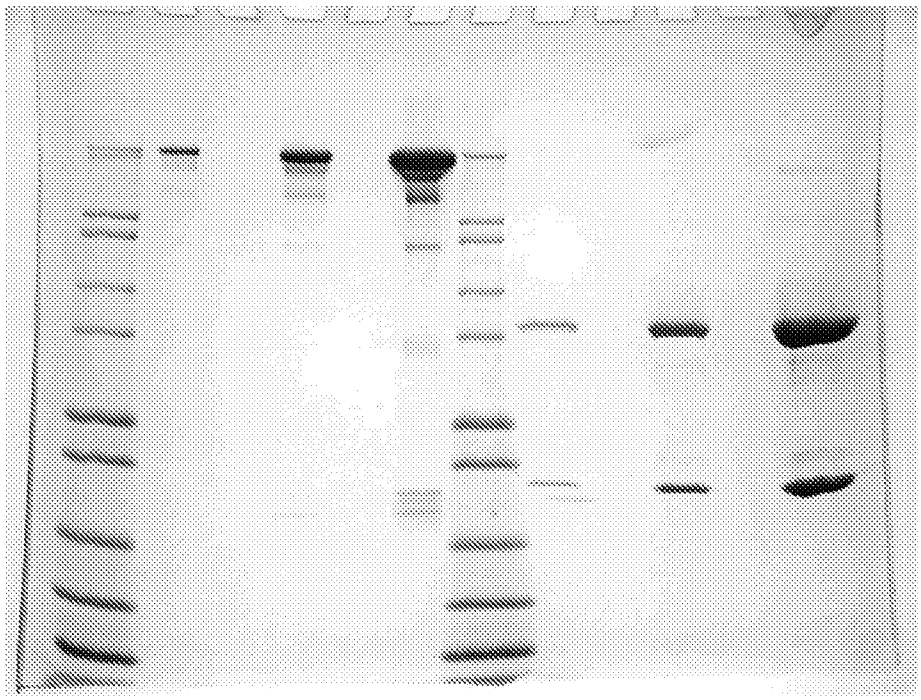


FIG. 5B

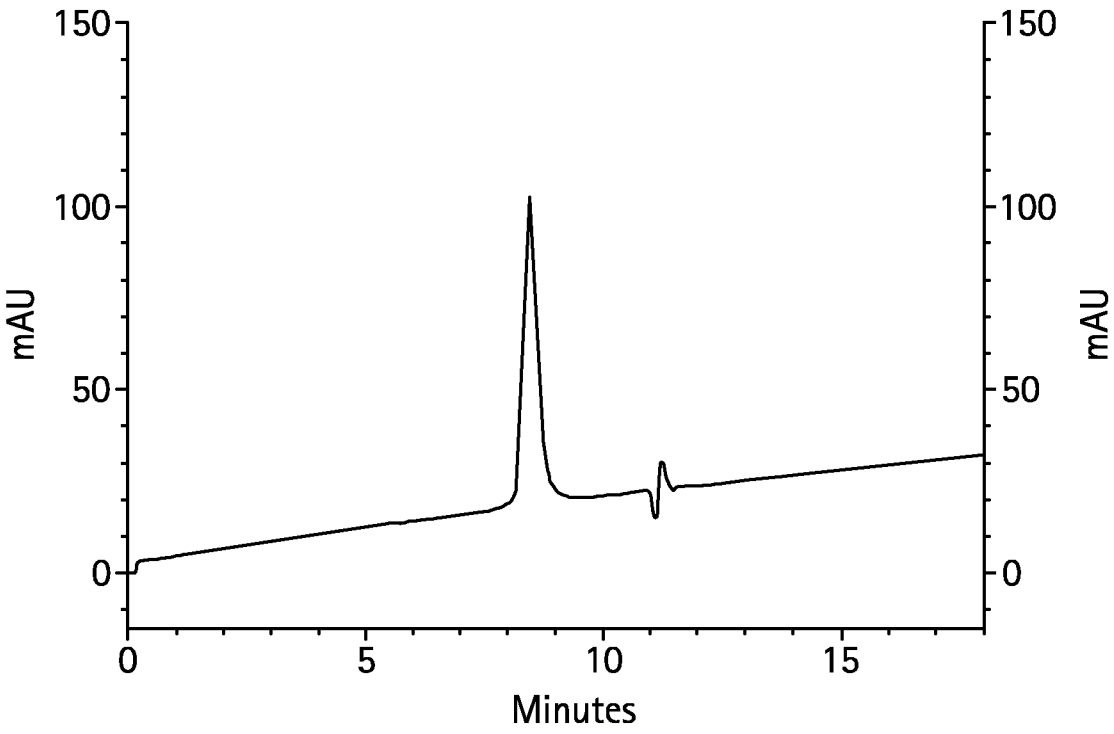


FIG. 5C

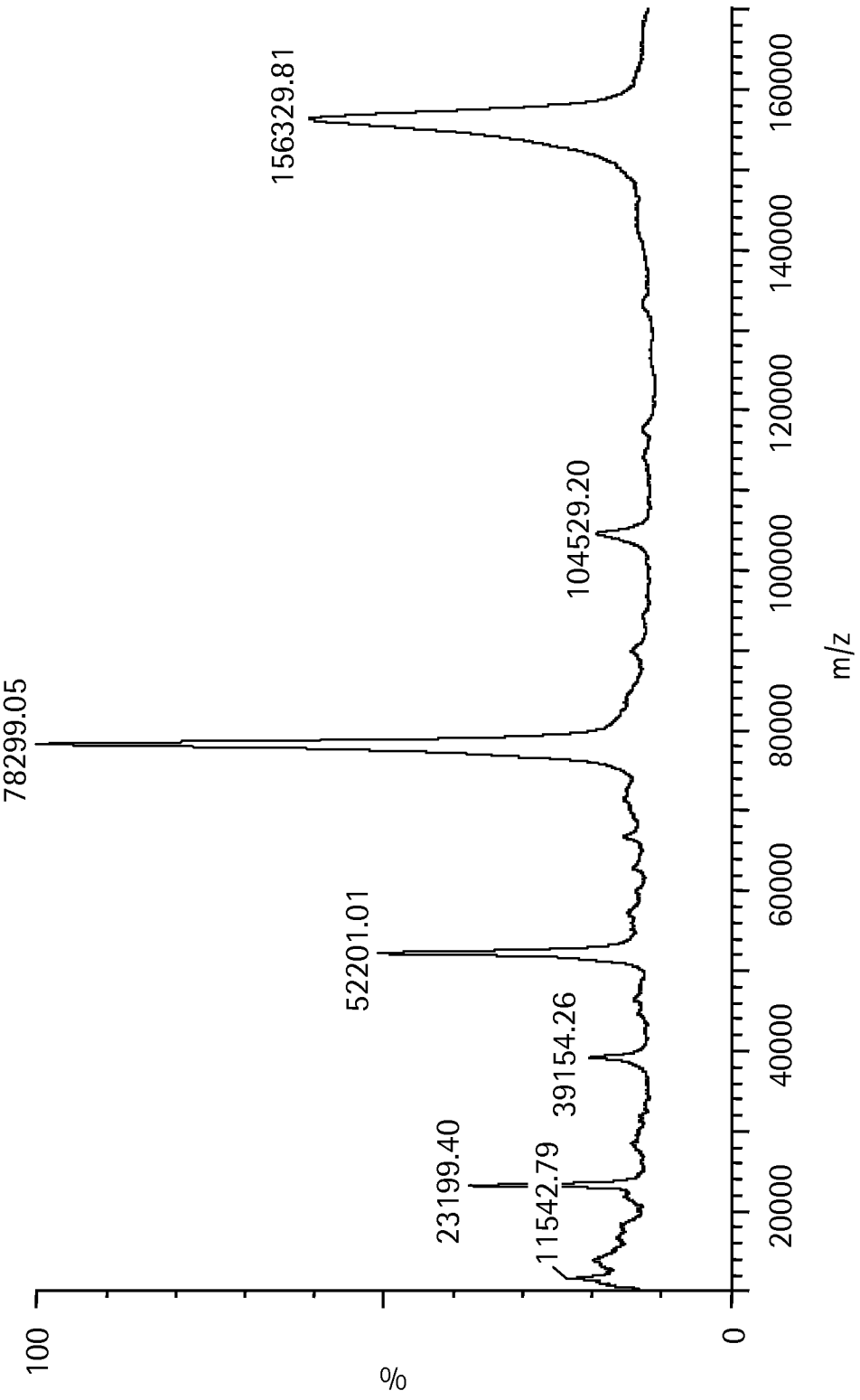

156329.81
104529.20
78299.05
52201.01
39154.26
23199.40
11542.79
100
%
0
20000
40000
60000
80000
100000
120000
140000
160000
m/z

FIG. 6A

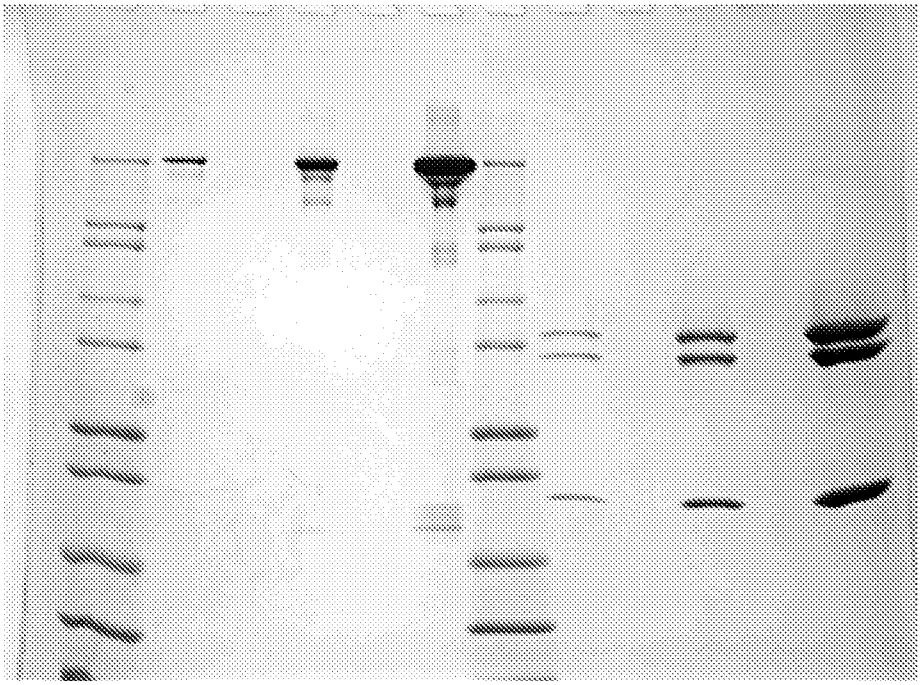


FIG. 6B

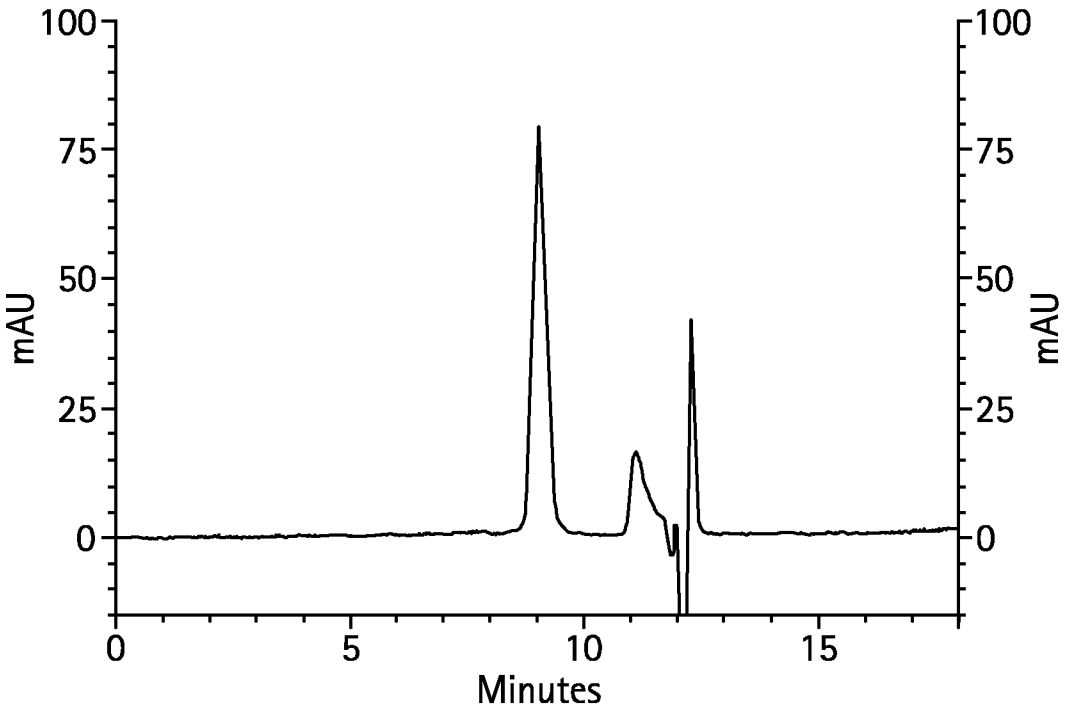


FIG. 6C

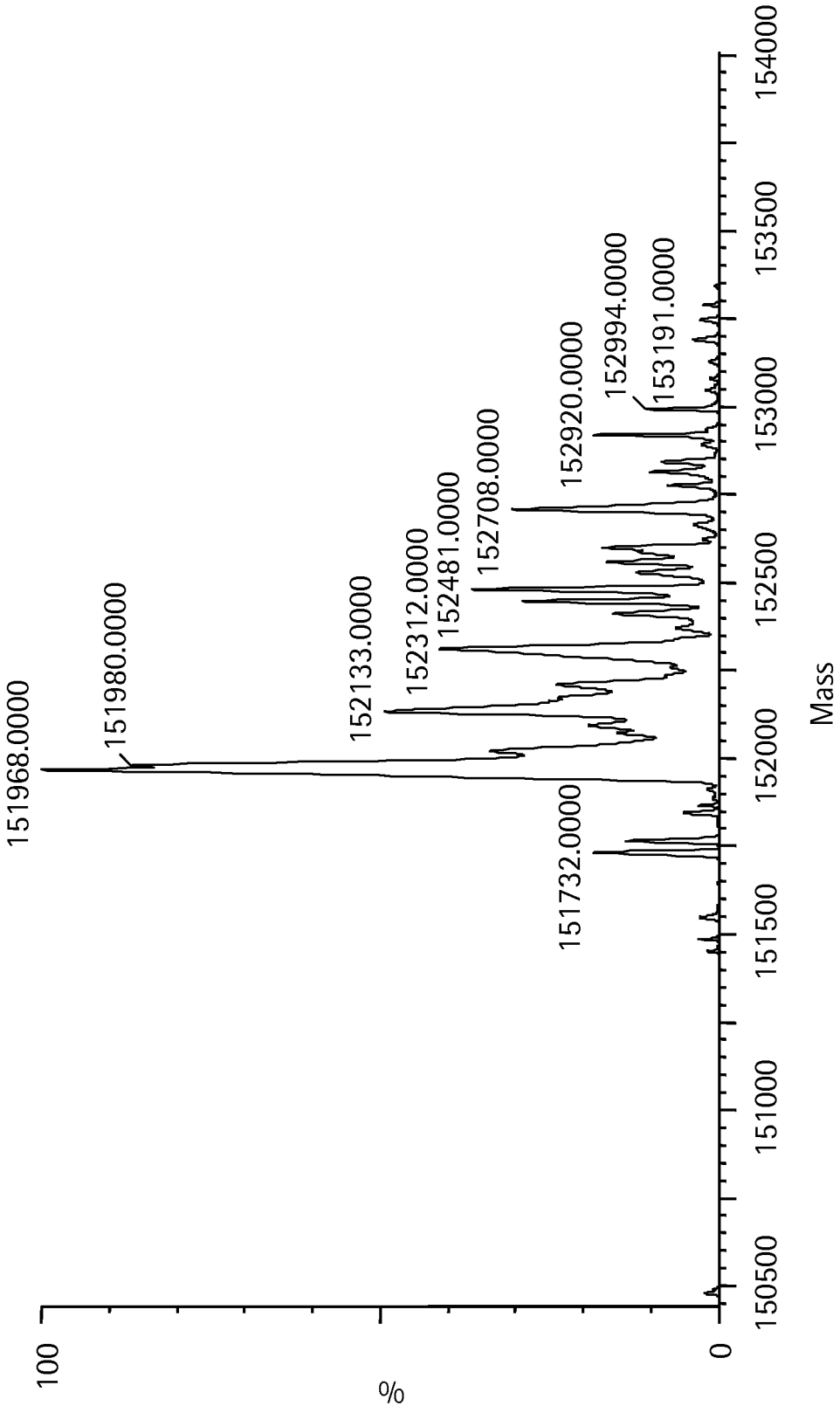

151968.0000
151980.0000
152133.0000
152312.0000
152481.0000
152708.0000
152920.0000
152994.0000
153191.0000
151732.0000
100
%
0
150500
151000
151500
152000
152500
153000
153500
154000
Mass

FIG. 7

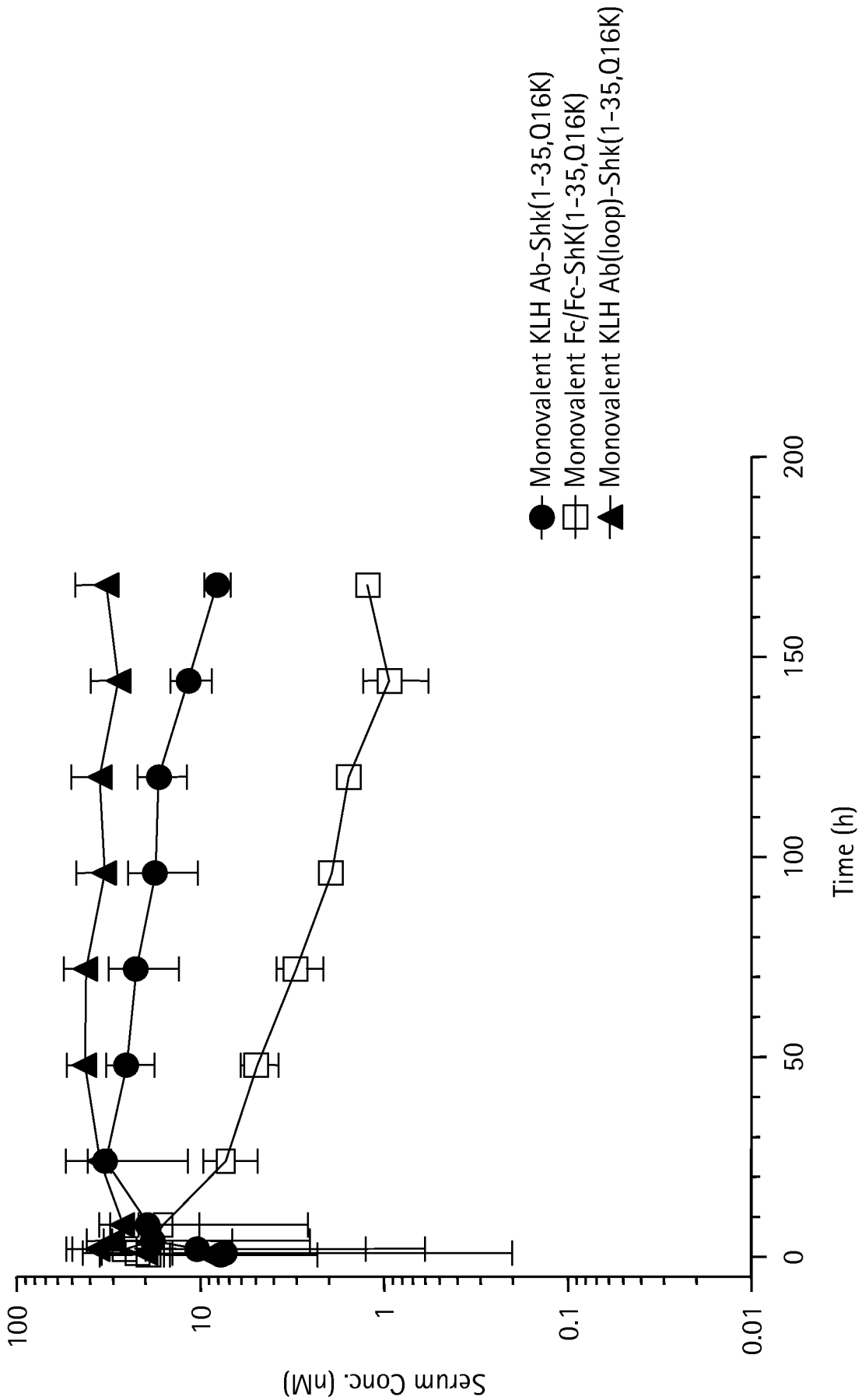

Monovalent KLH Ab-Shk(1-35,Q16K)
Monovalent Fc/Fc-ShK(1-35,Q16K)
Monovalent KLH Ab(loop)-Shk(1-35,Q16K)
Serum Conc. (nM)
100
10
1
0.1
0.01
0
50
100
150
200
Time (h)

FIG. 8

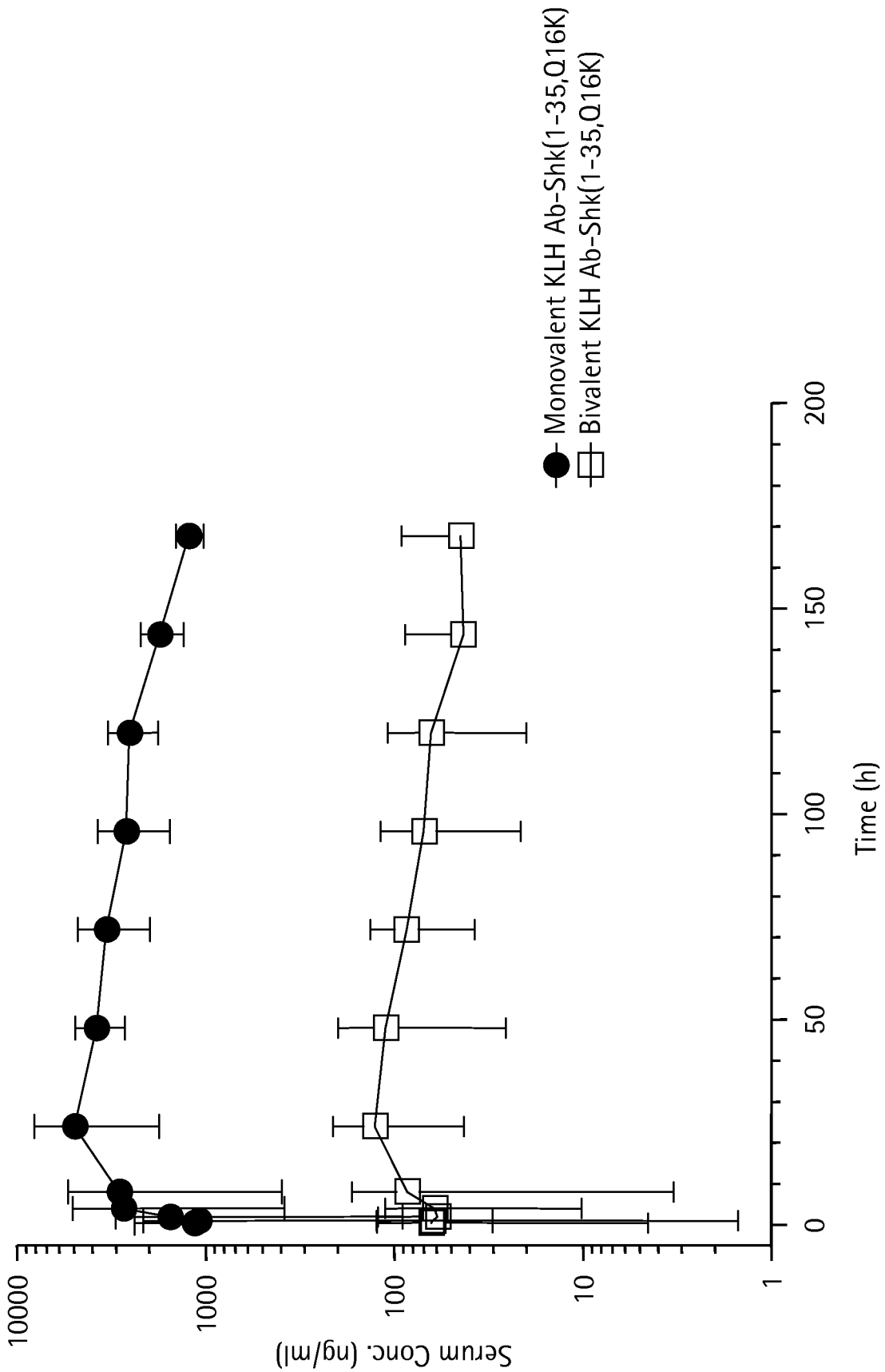

Monovalent KLH Ab-Shk(1-35,Q16K)
Bivalent KLH Ab-Shk(1-35,Q16K)
Serum Conc. (ng/ml)
10000
1000
100
10
1
0
50
100
150
200
Time (h)

FIG. 9

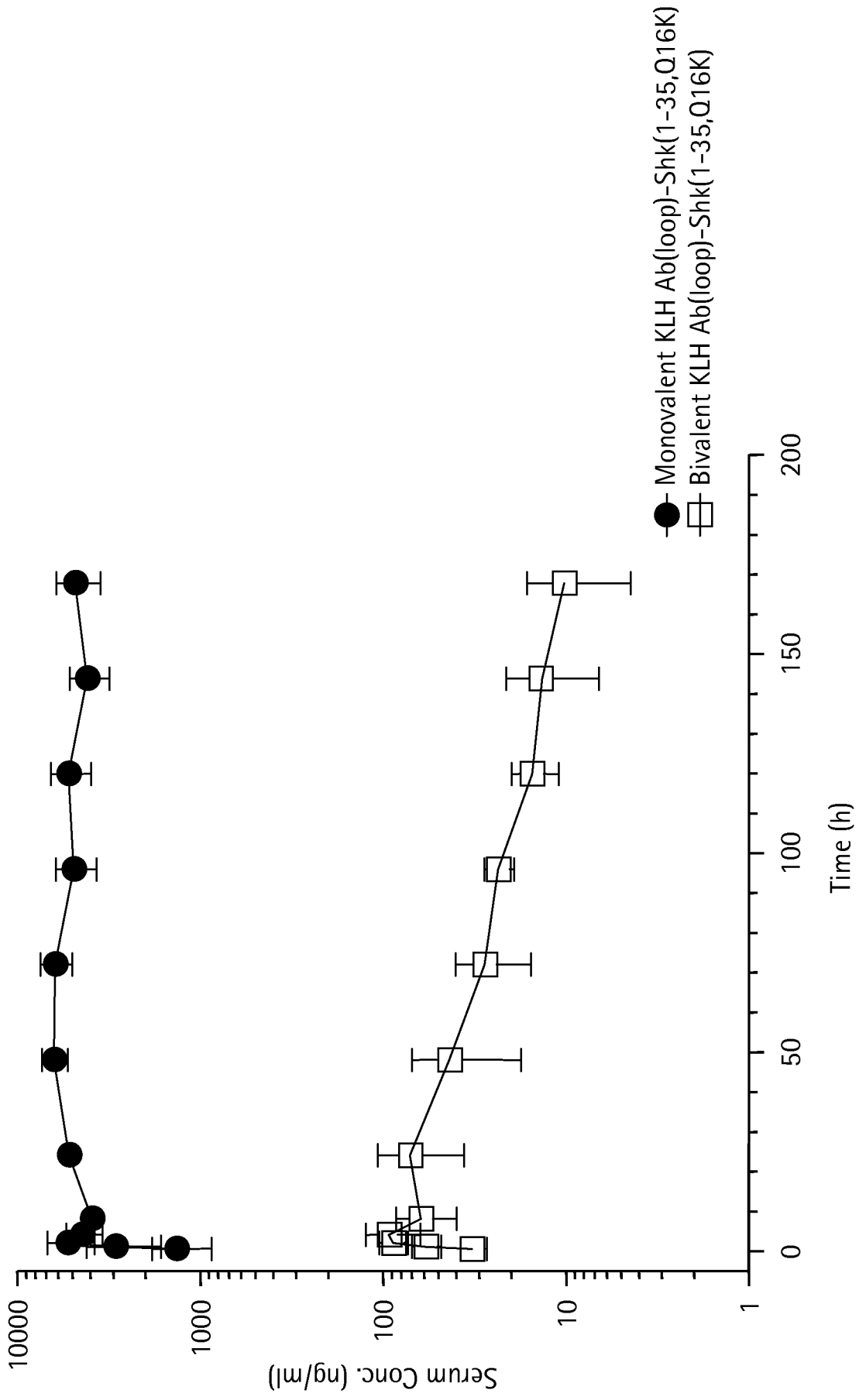

Monovalent KLH Ab(loop)-Shk(1-35,Q16K)
Bivalent KLH Ab(loop)-Shk(1-35,Q16K)
Serum Conc. (ng/ml)
10000
1000
100
10
1
0
50
100
150
200
Time (h)

FIG. 10

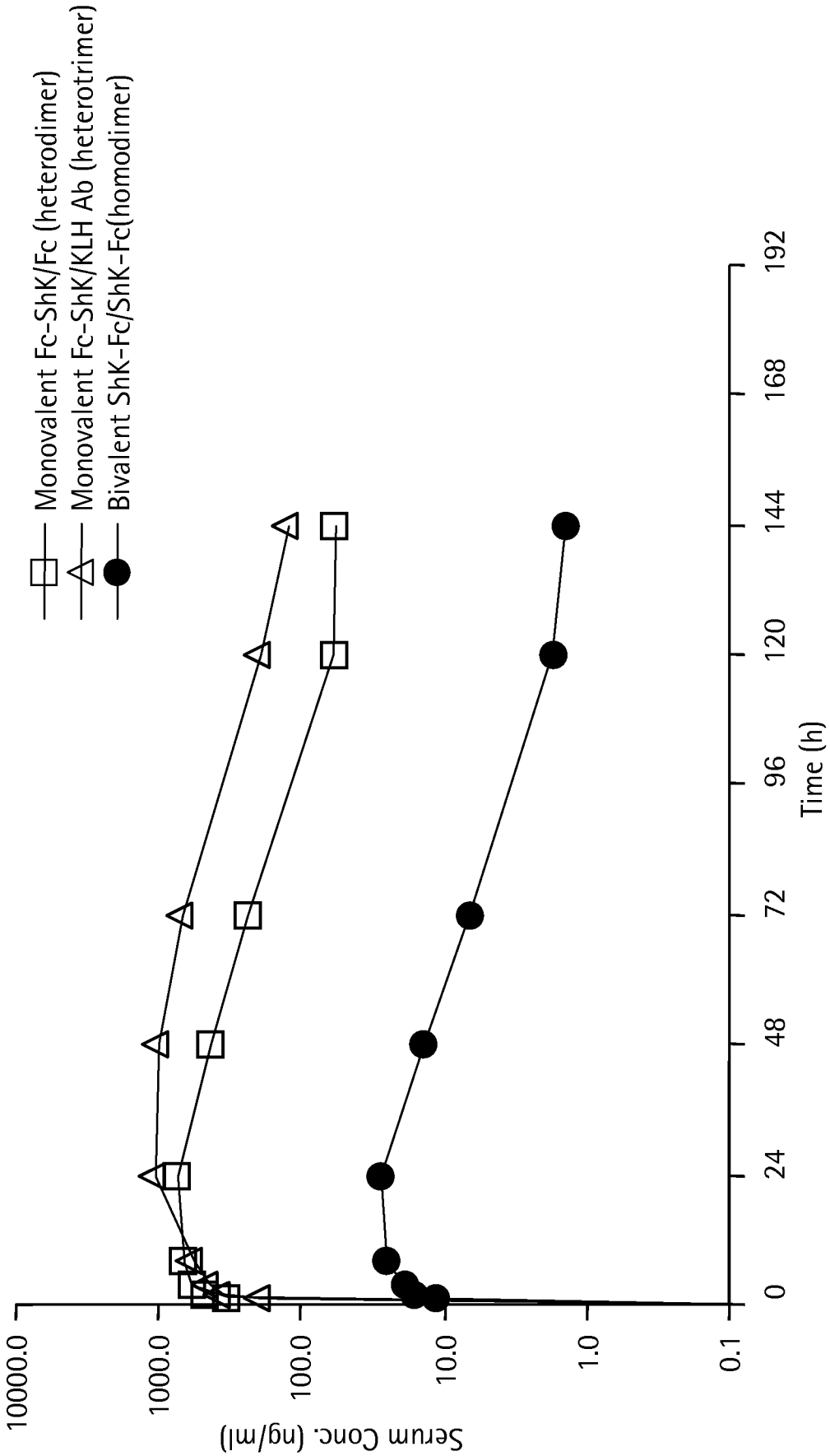

Monovalent Fc-ShK/Fc (heterodimer)
Monovalent Fc-ShK/KLH Ab (heterotrimer)
Bivalent ShK-Fc/ShK-Fc(homodimer)
Serum Conc. (ng/ml)
10000.0
1000.0
100.0
10.0
1.0
0.1
0
24
48
72
96
120
144
168
192
Time (h)

FIG. 11

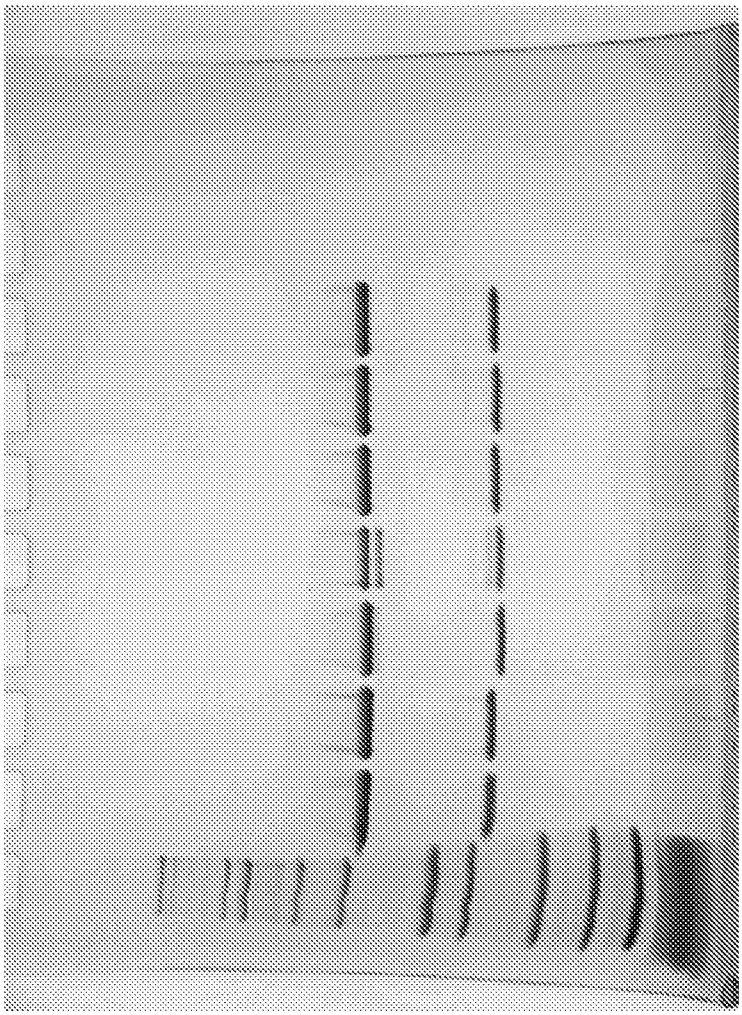


FIG. 12B

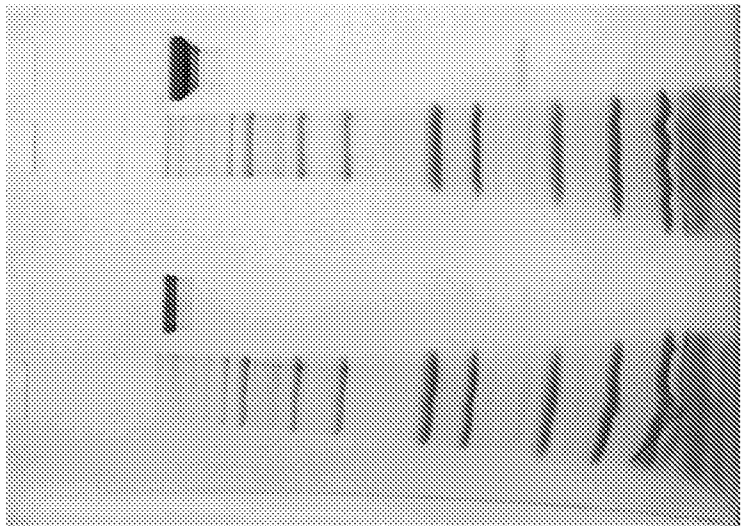


FIG. 12A

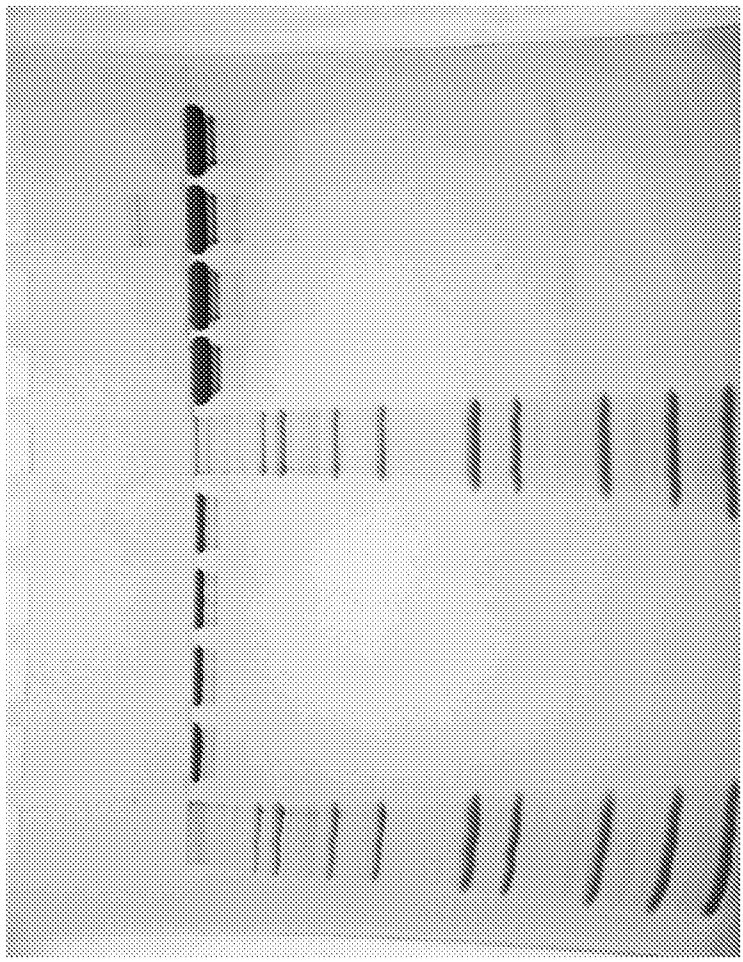


FIG. 13B

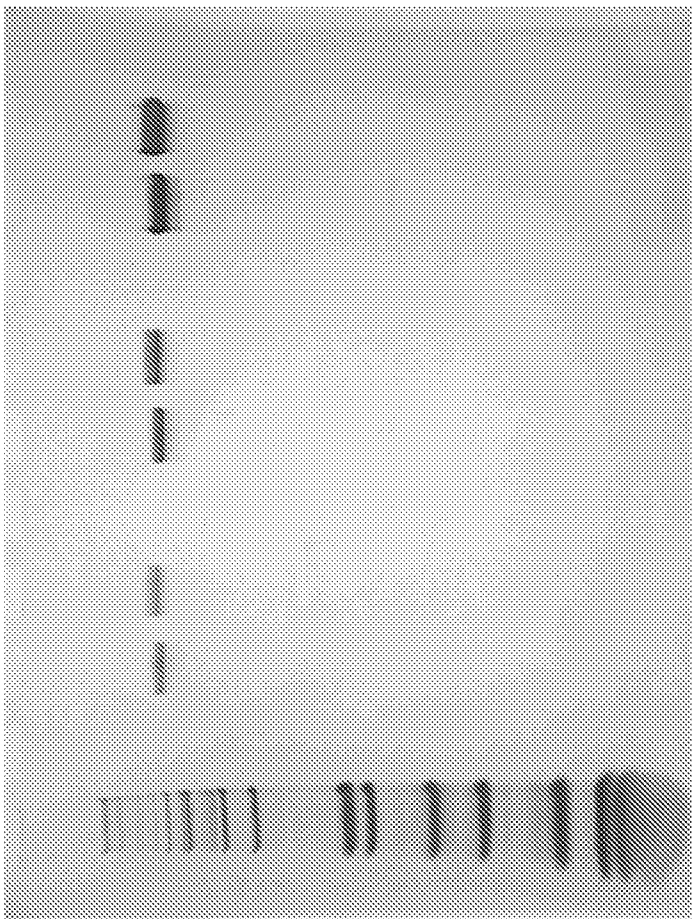


FIG. 13A

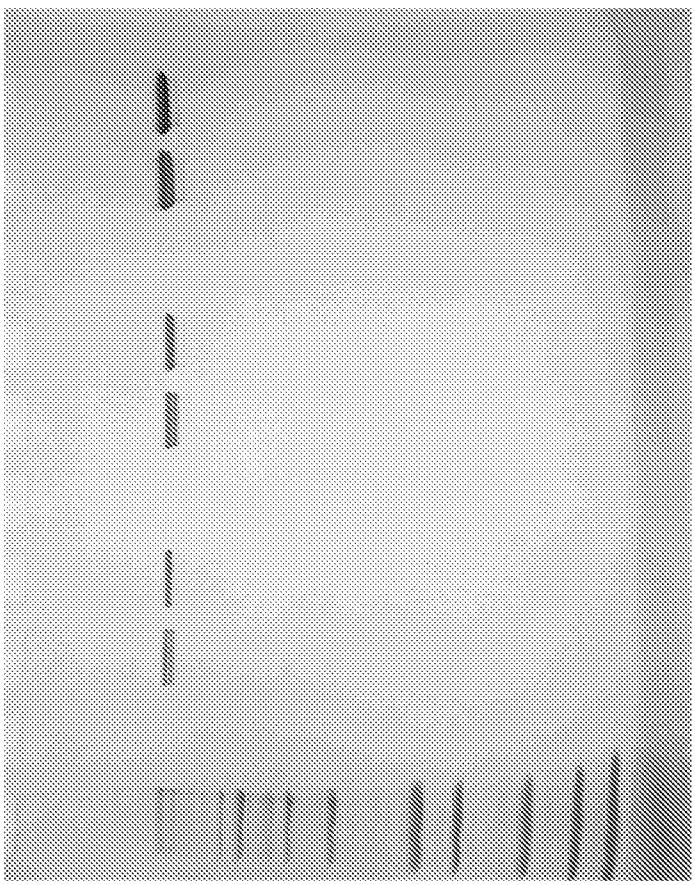


FIG. 14B

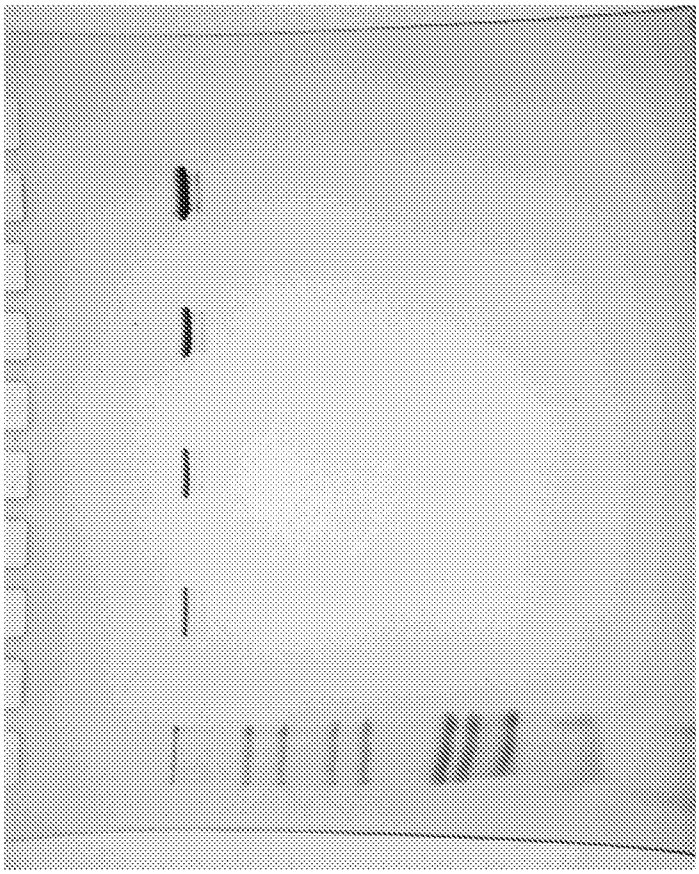


FIG. 14A

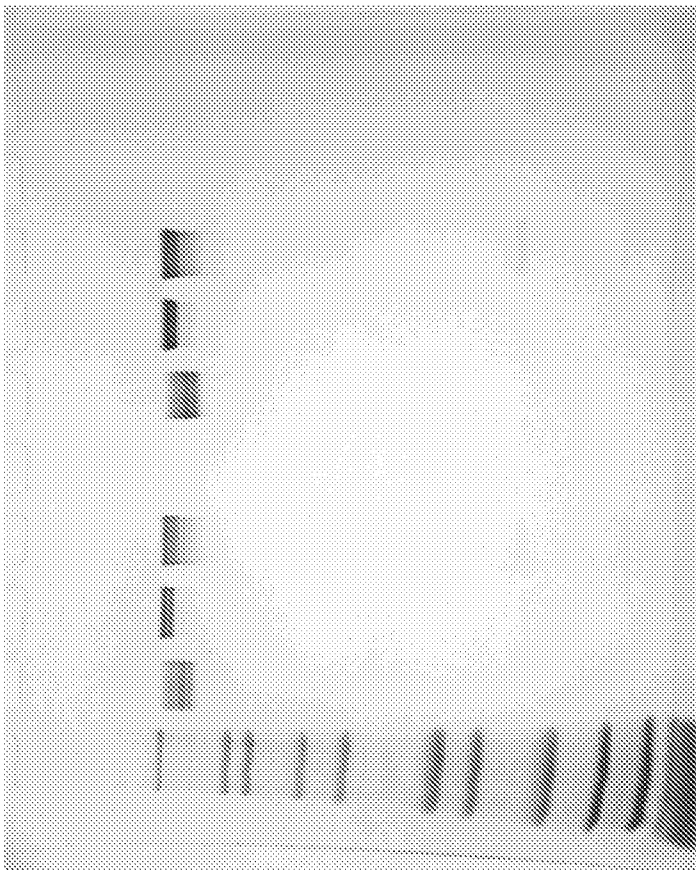


FIG. 15

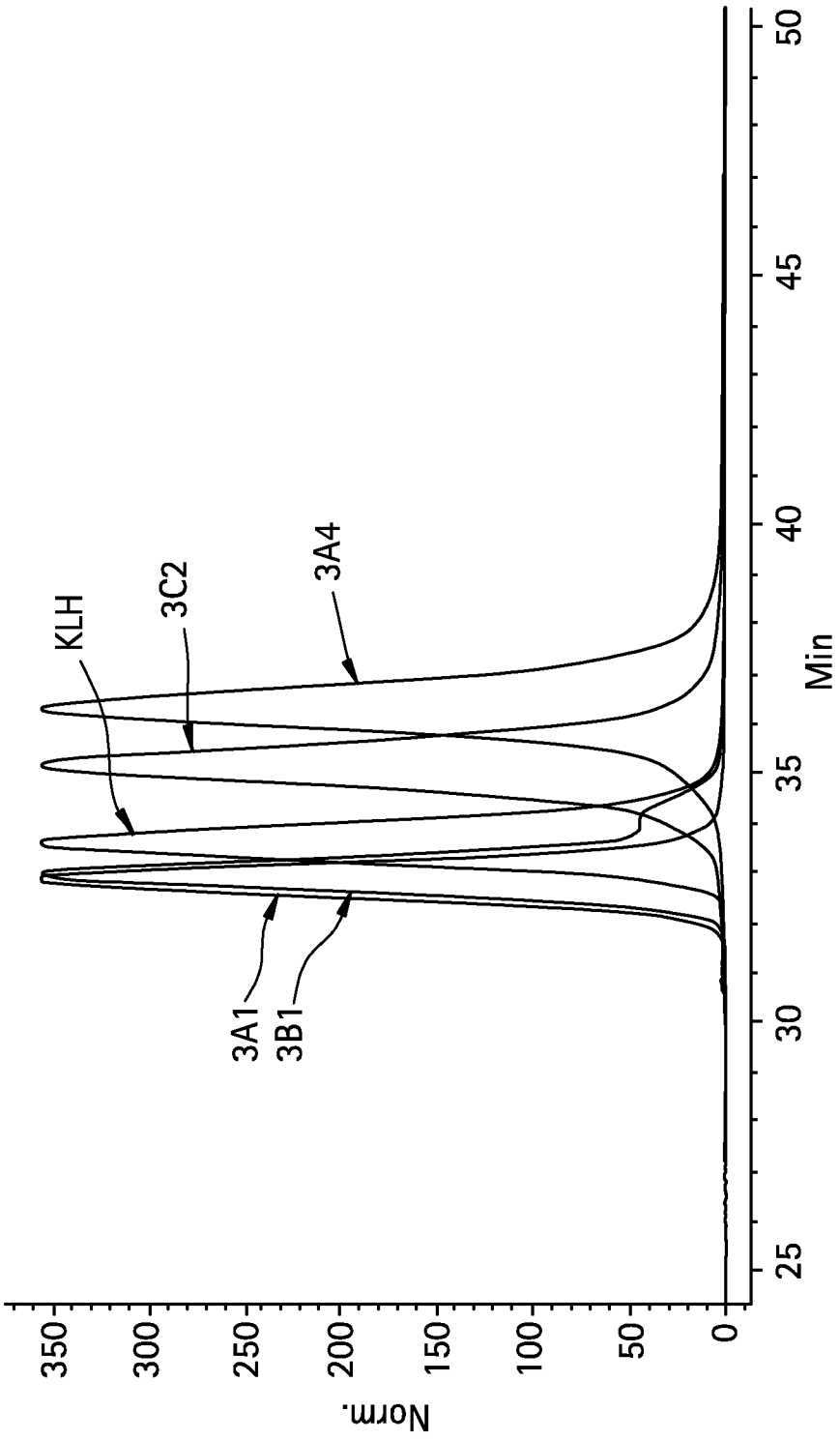

KLH
3C2
3A4
3A1
3B1
Norm.
0
50
100
150
200
250
300
350
25
30
35
40
45
50
Min

FIG. 16

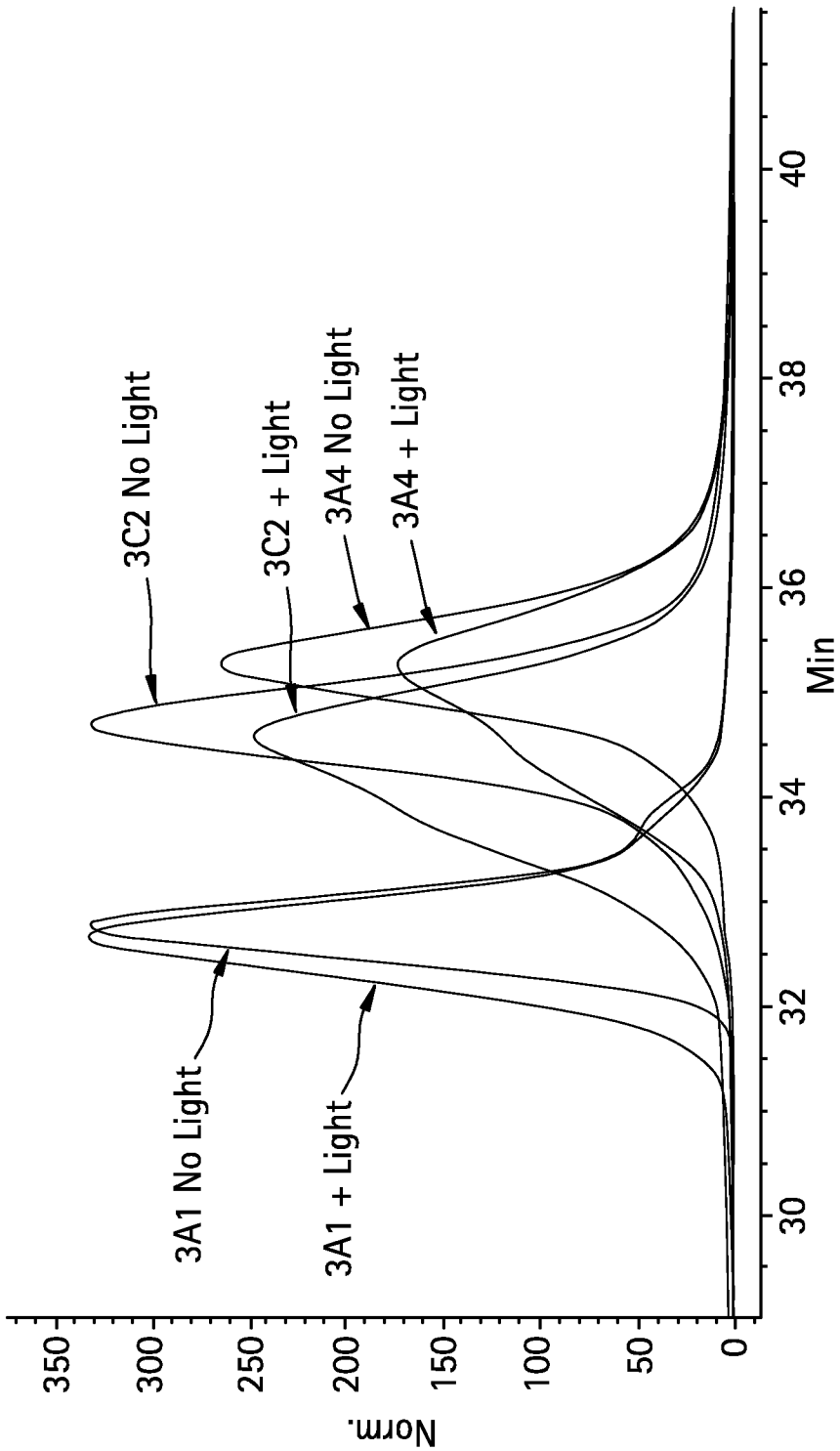

3C2 No Light
3C2 + Light
3A4 No Light
3A4 + Light
3A1 No Light
3A1 + Light
Norm.
Min
0
50
100
150
200
250
300
350
30
32
34
36
38
40

FIG. 17A

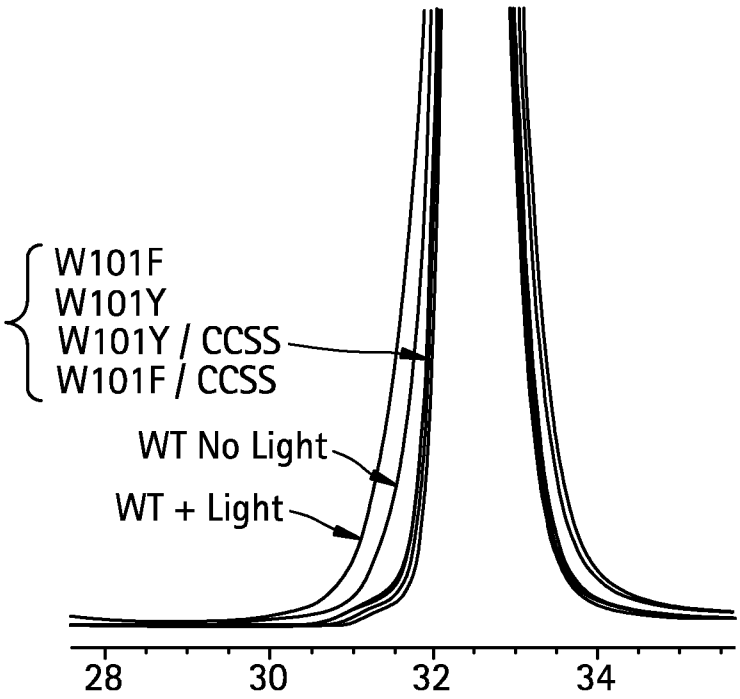


FIG. 17B

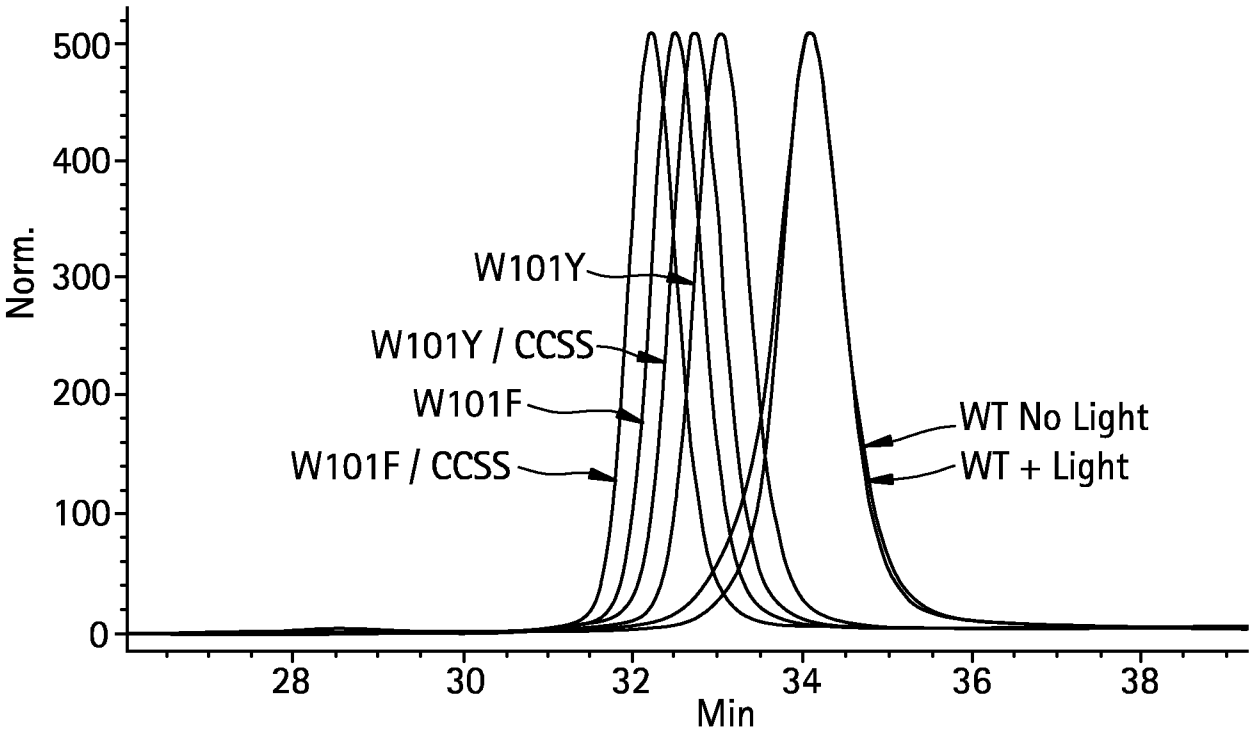


FIG. 18

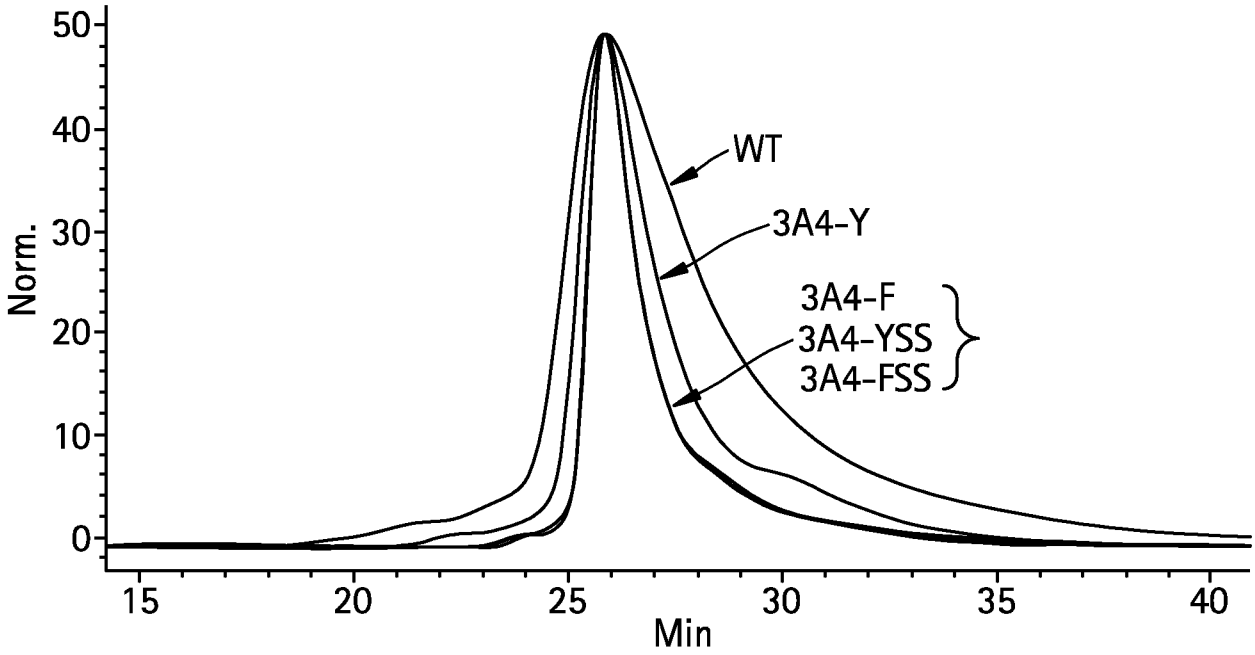

50
40
30
20
10
0
Norm.
15
20
25
30
35
40
Min
WT
3A4-Y
3A4-F
3A4-YSS
3A4-FSS

FIG. 19A

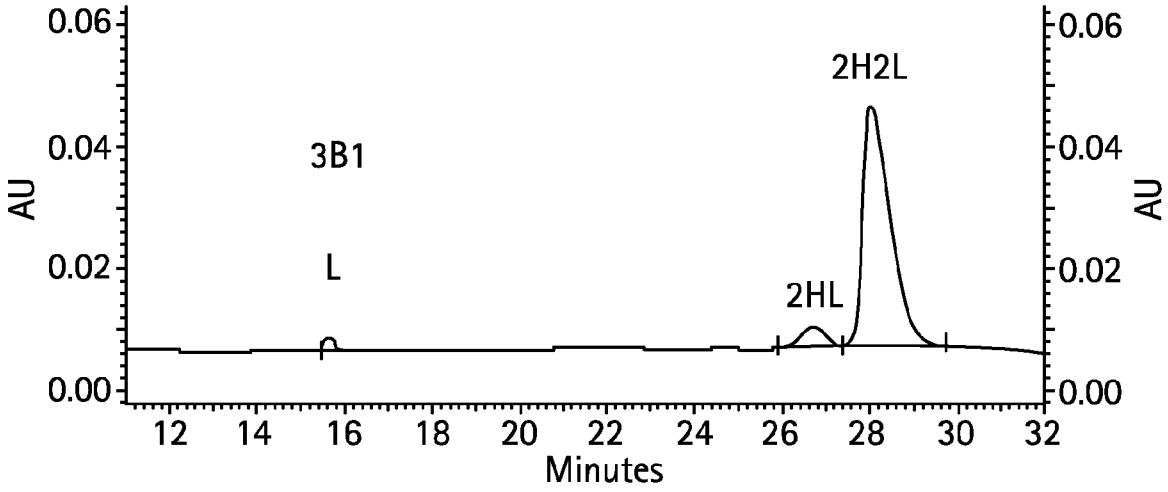


FIG. 19B

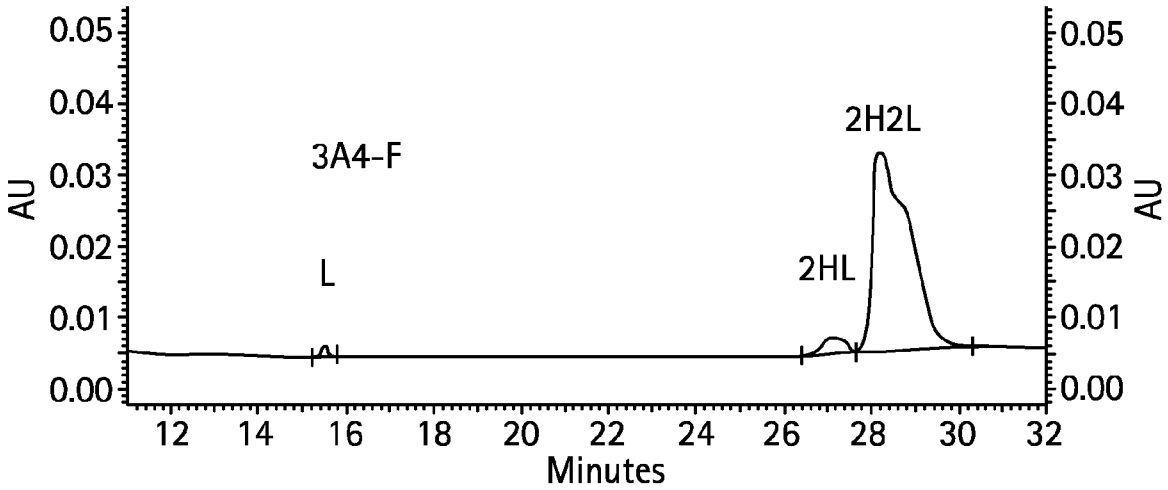


FIG. 19C

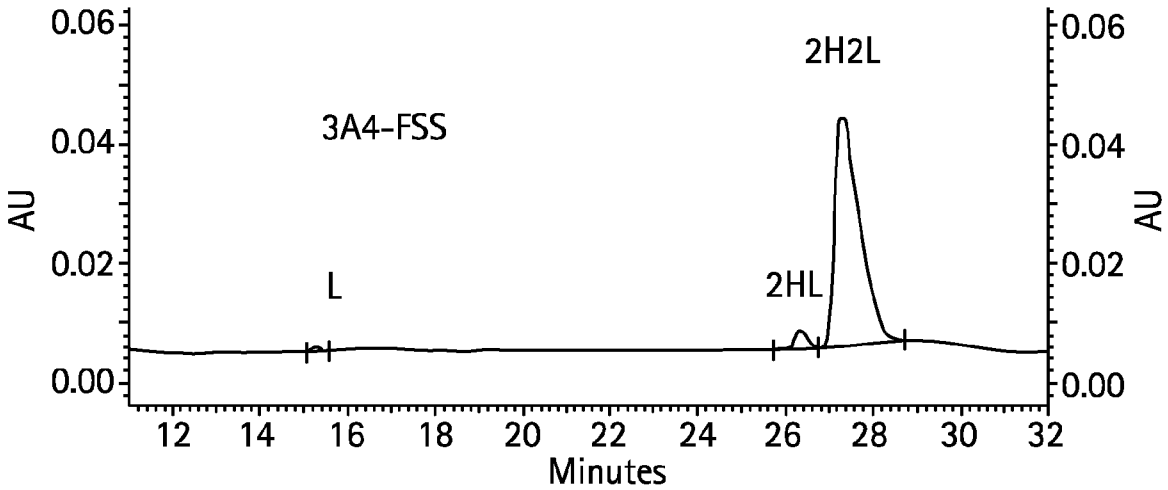


FIG. 20A

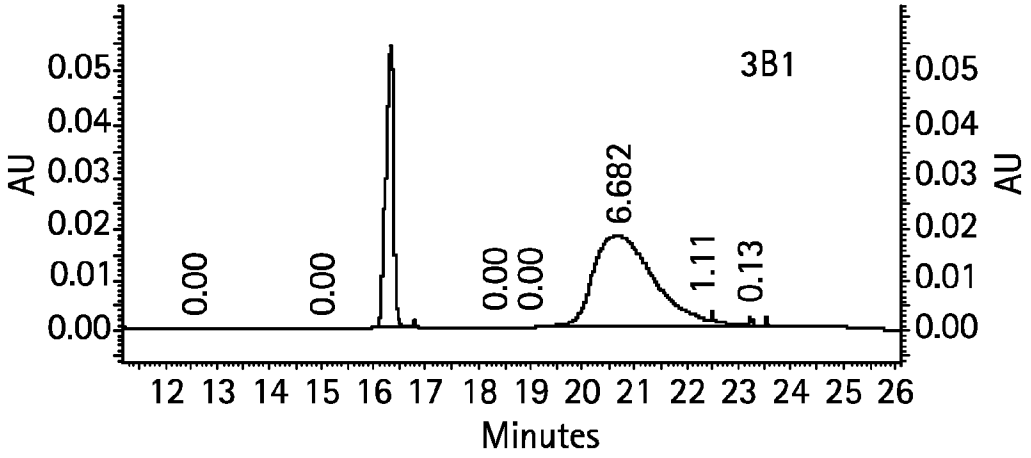


FIG. 20B

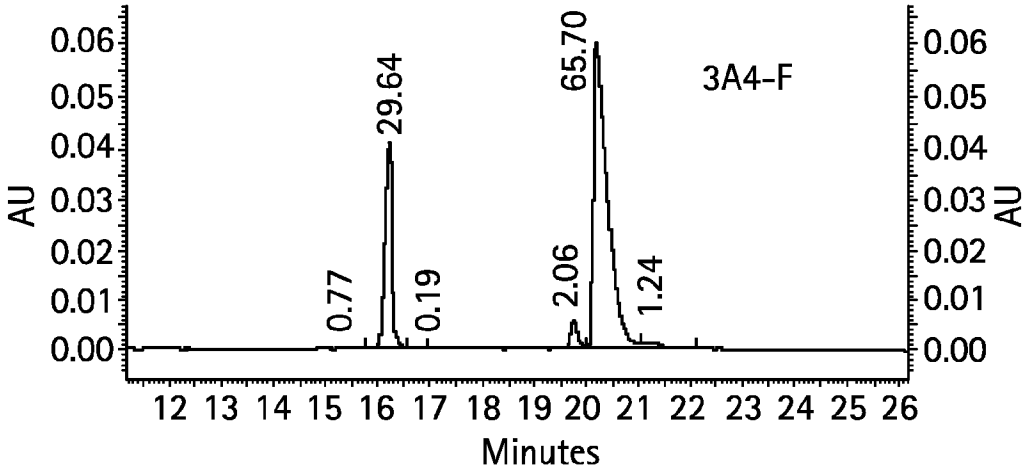


FIG. 20C

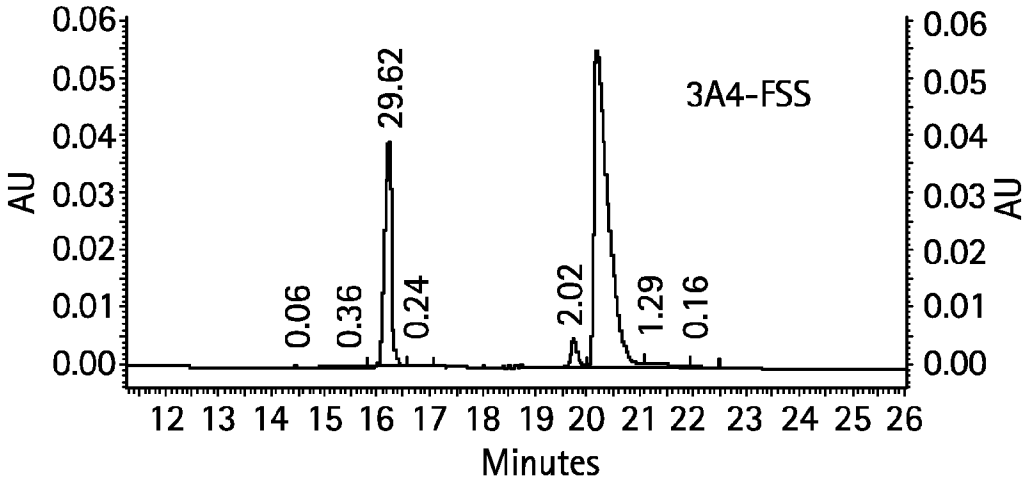


FIG. 21

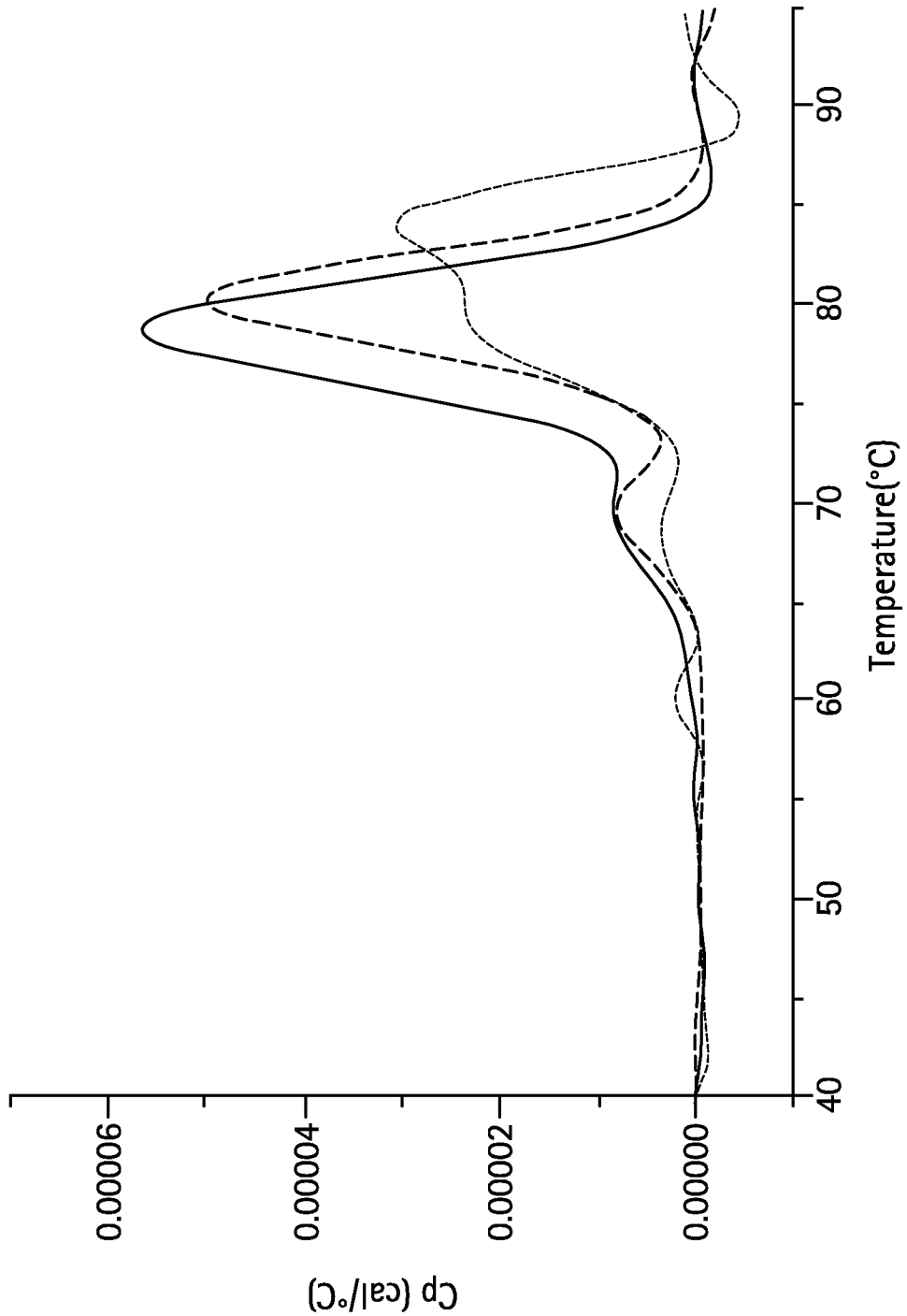

Cp (cal/°C)
0.00006
0.00004
0.00002
0.00000
40
50
60
70
80
90
Temperature(°C)

FIG. 22

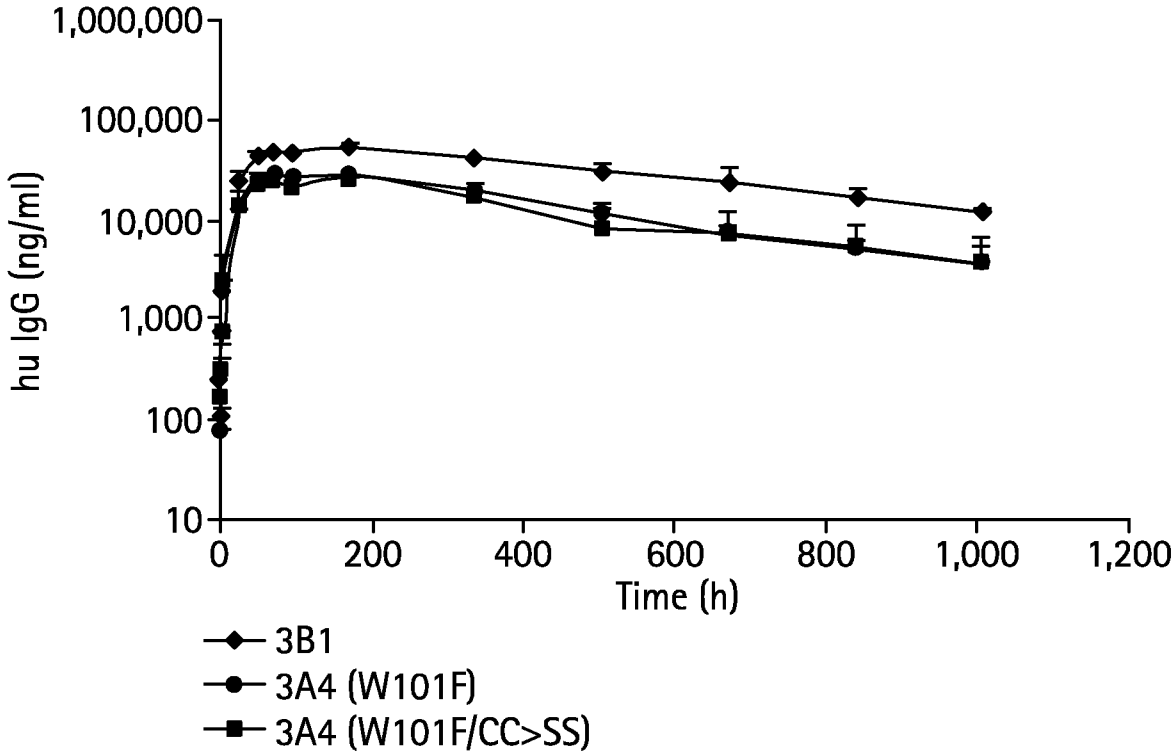

1,000,000
100,000
10,000
1,000
100
10
hu IgG (ng/ml)
0
200
400
600
800
1,000
1,200
Time (h)
3B1
3A4 (W101F)
3A4 (W101F/CC>SS)

FIG. 23

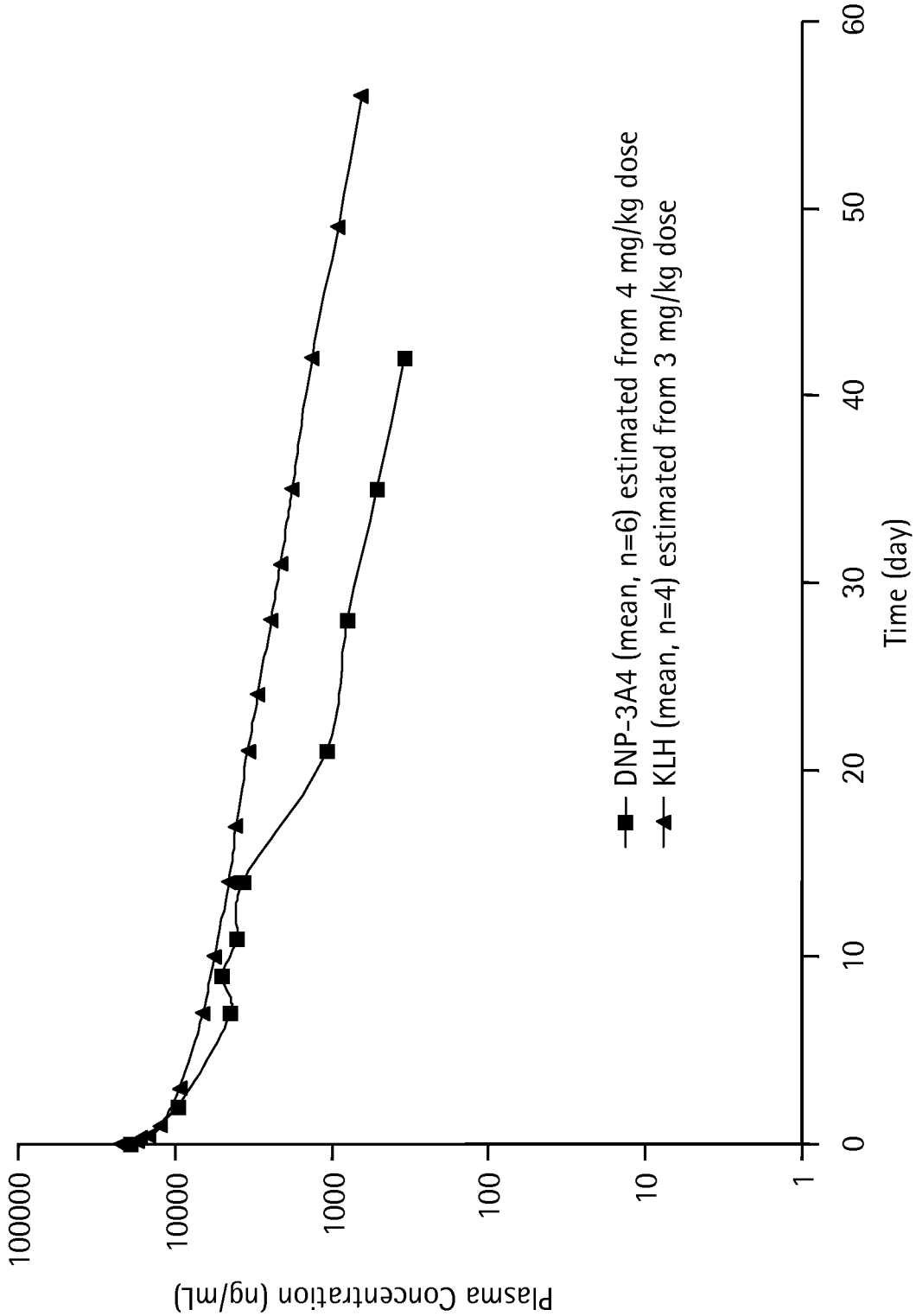

Plasma Concentration (ng/mL)
100000
10000
1000
100
10
1
0
10
20
30
40
50
60
Time (day)
DNP-3A4 (mean, n=6) estimated from 4 mg/kg dose
KLH (mean, n=4) estimated from 3 mg/kg dose

FIG. 24

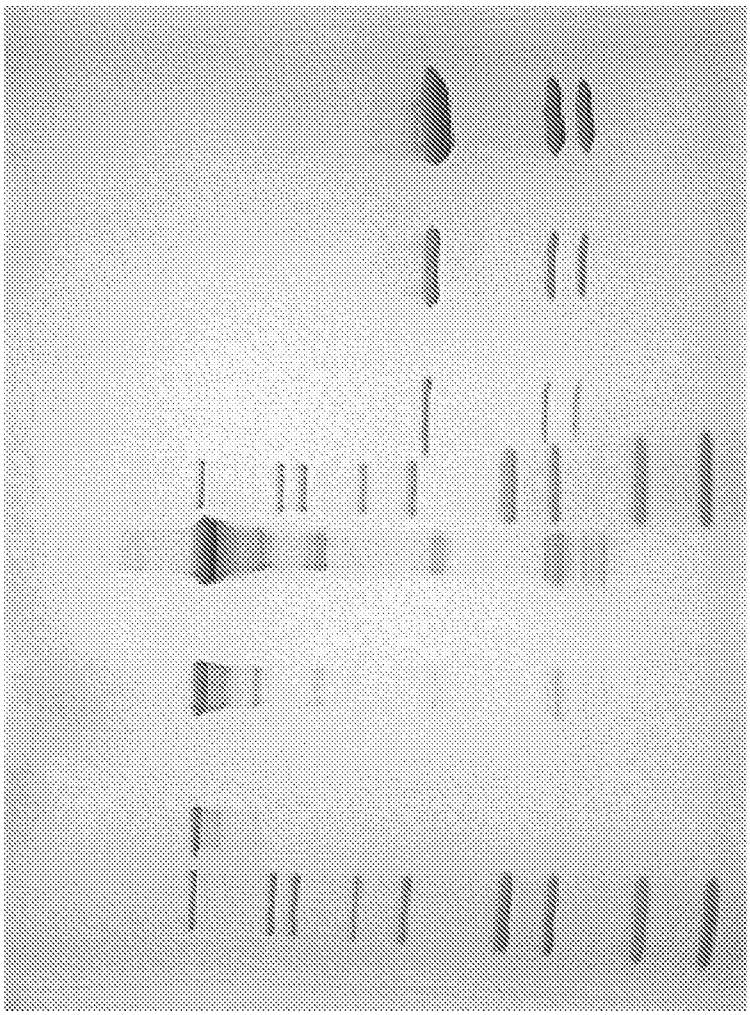


FIG. 25

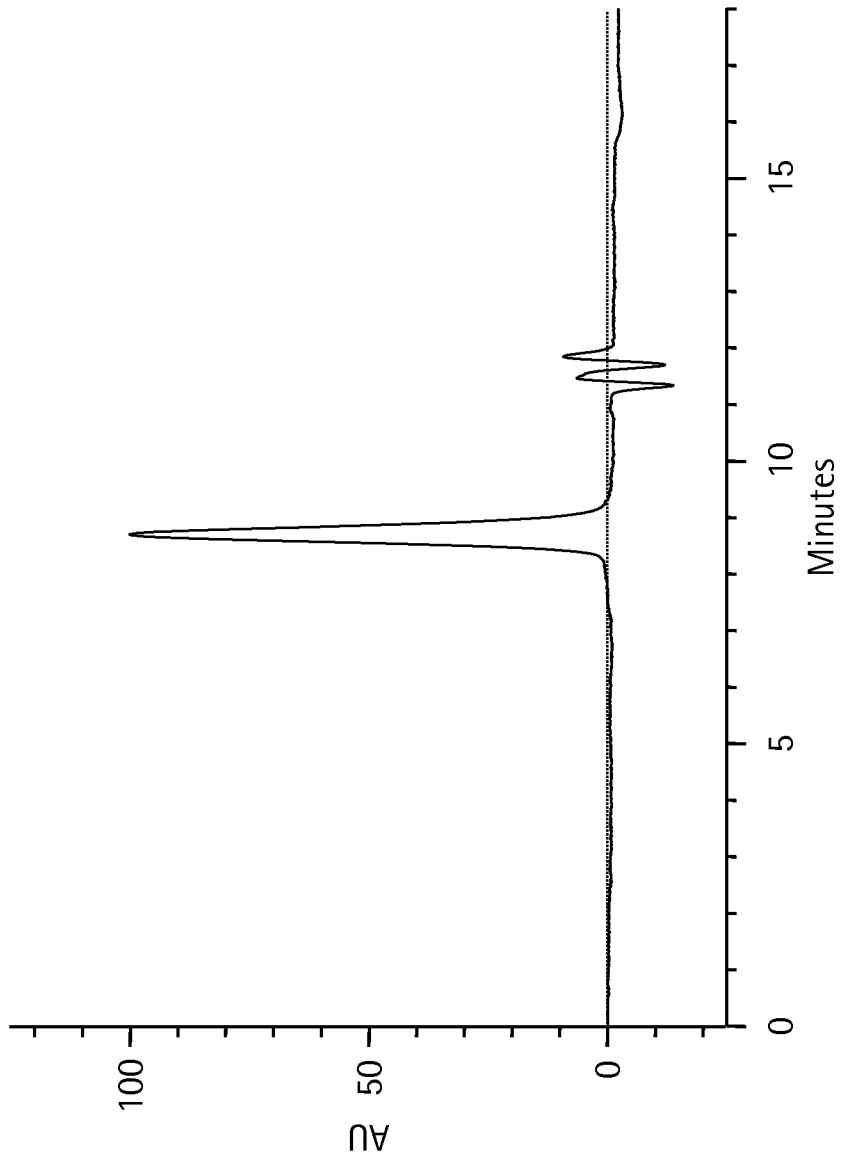


FIG. 26A

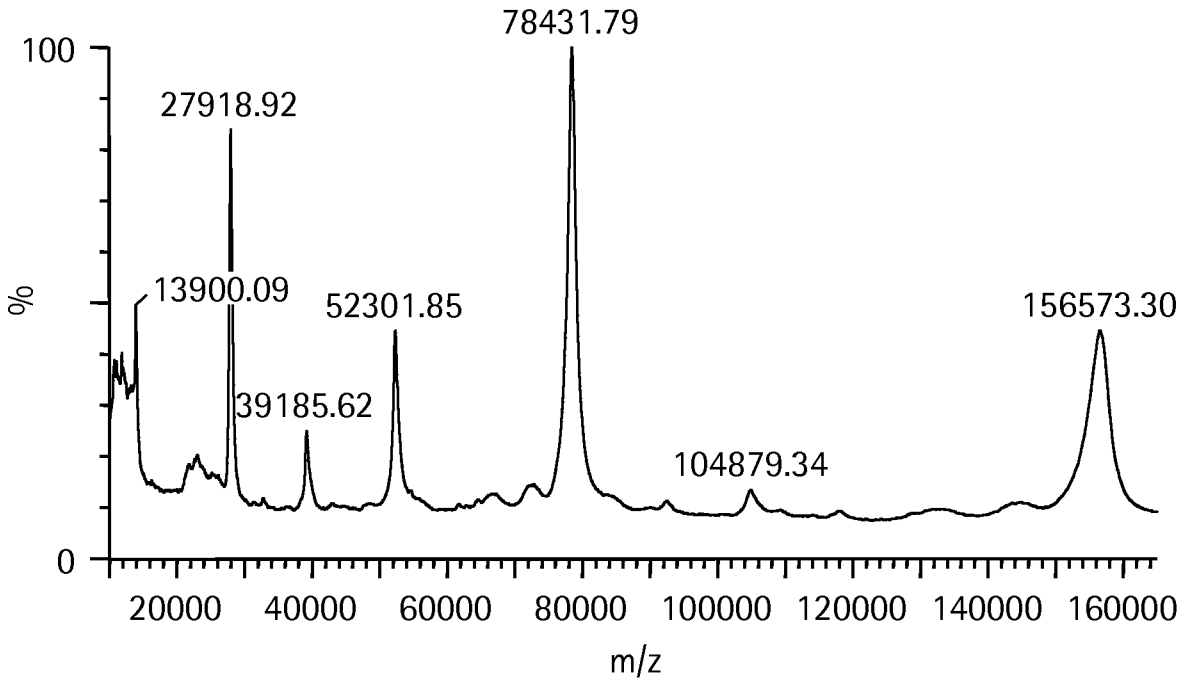


FIG. 26B

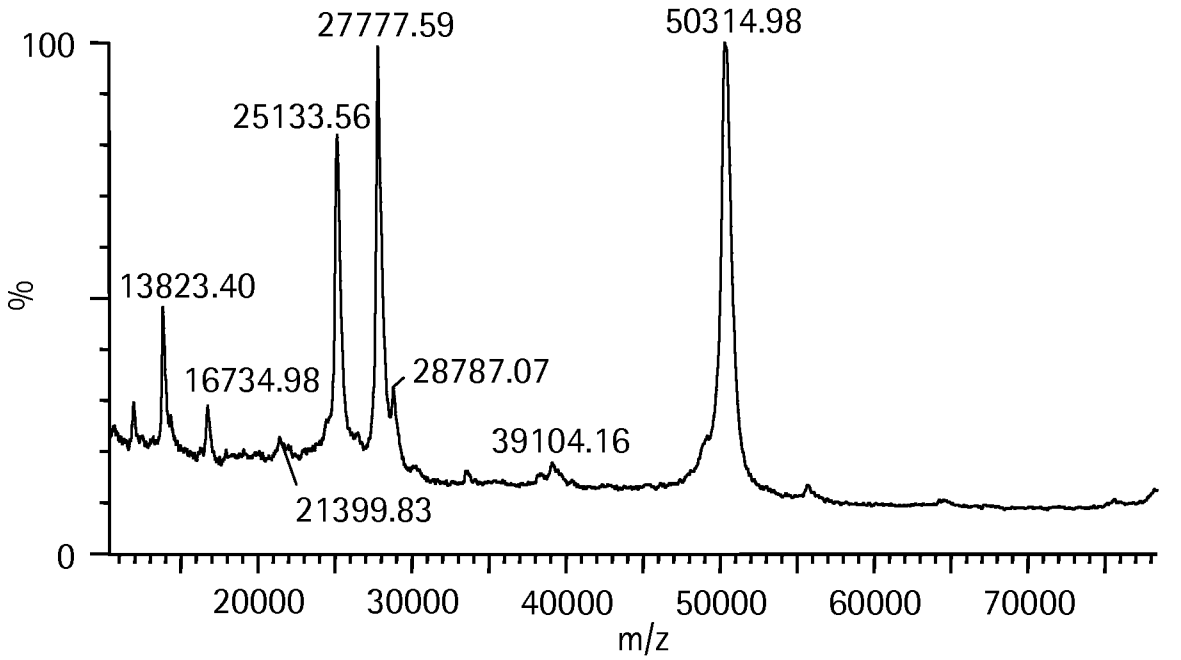


FIG. 27

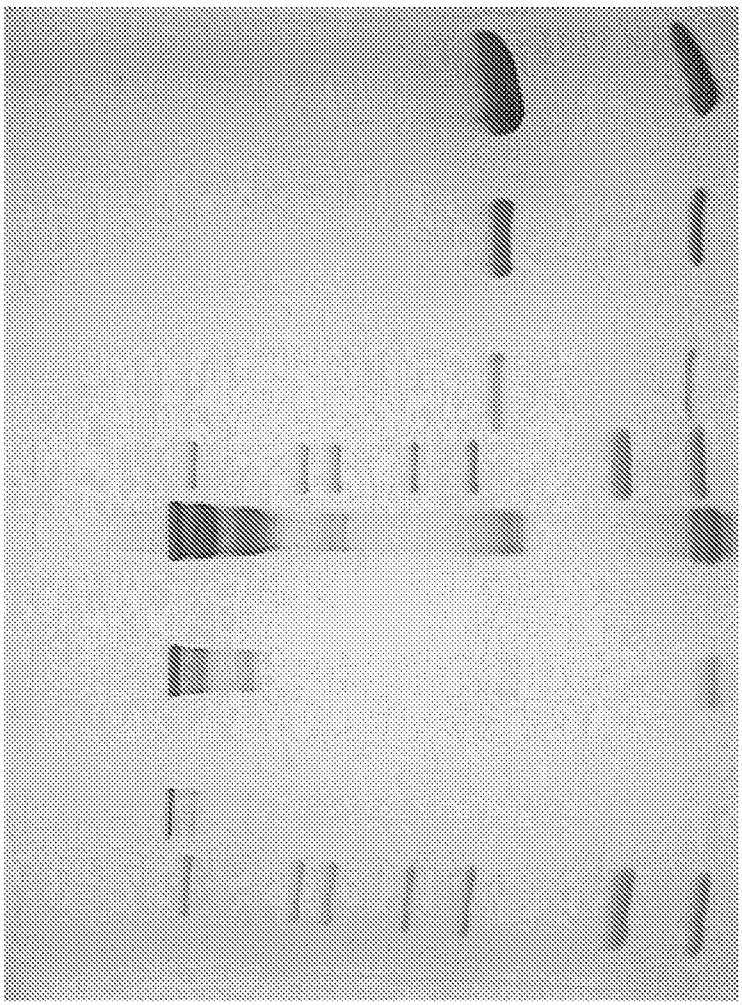


FIG. 28

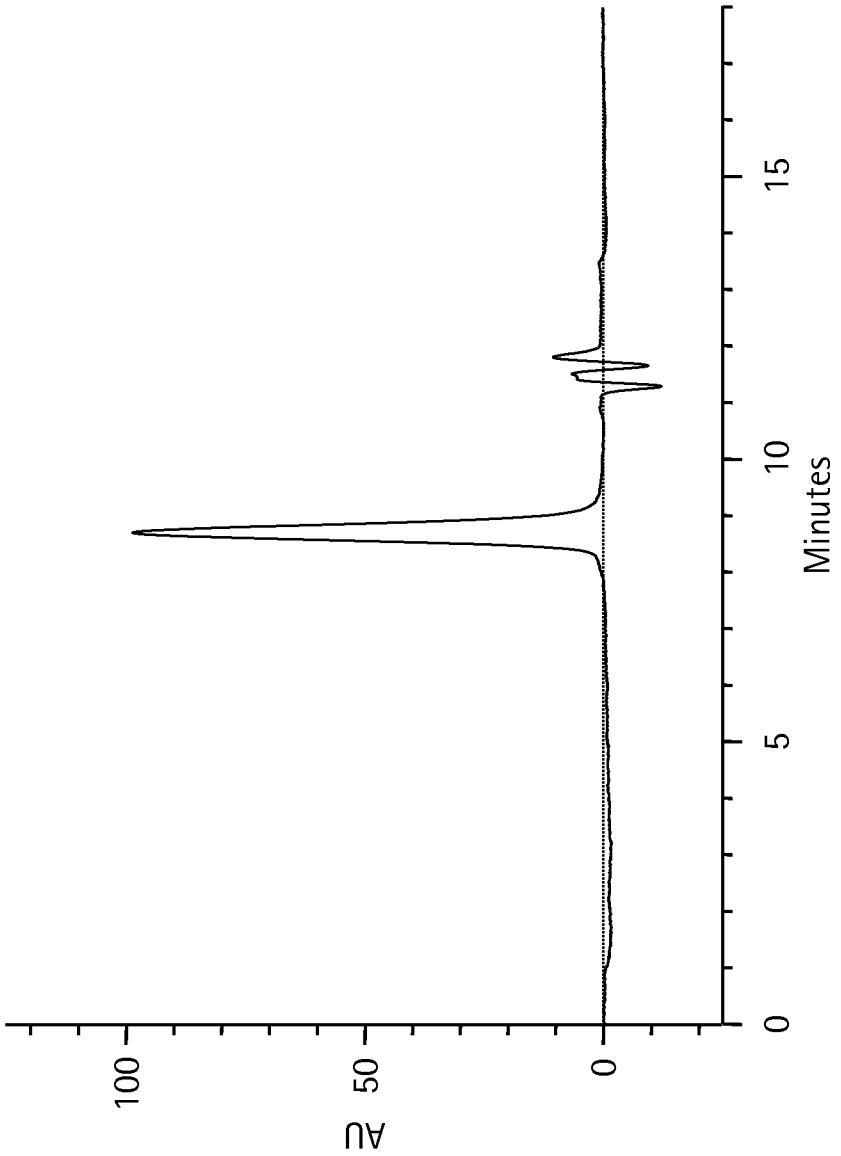


FIG. 29A

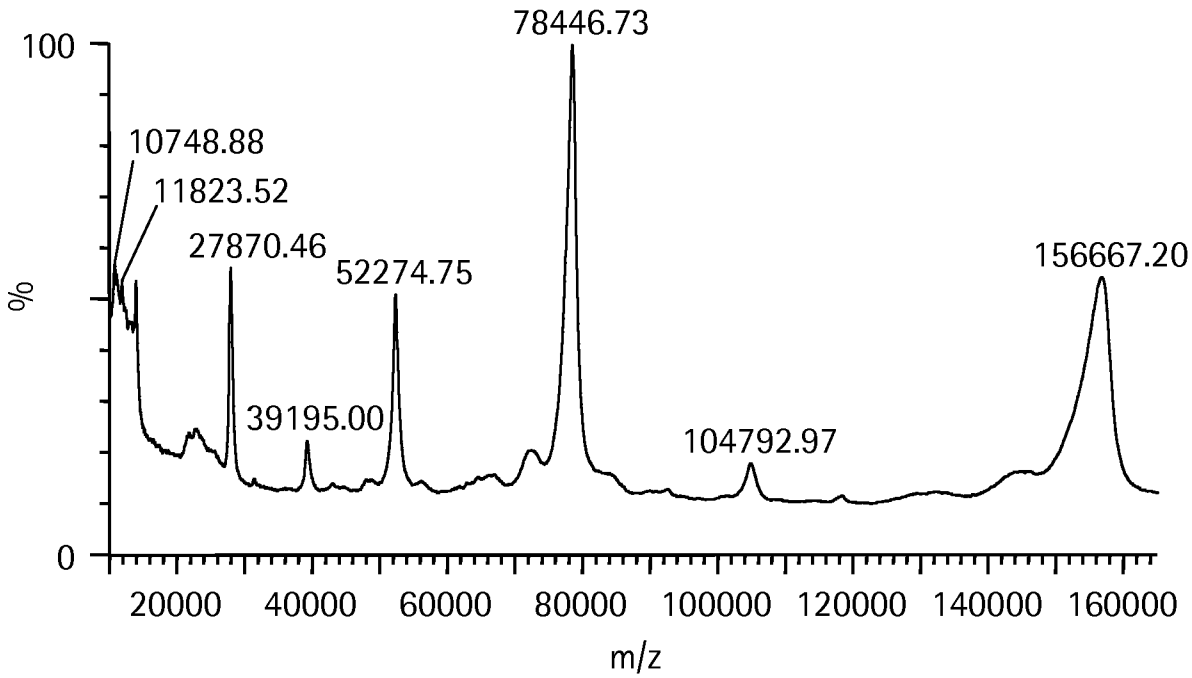


FIG. 29B

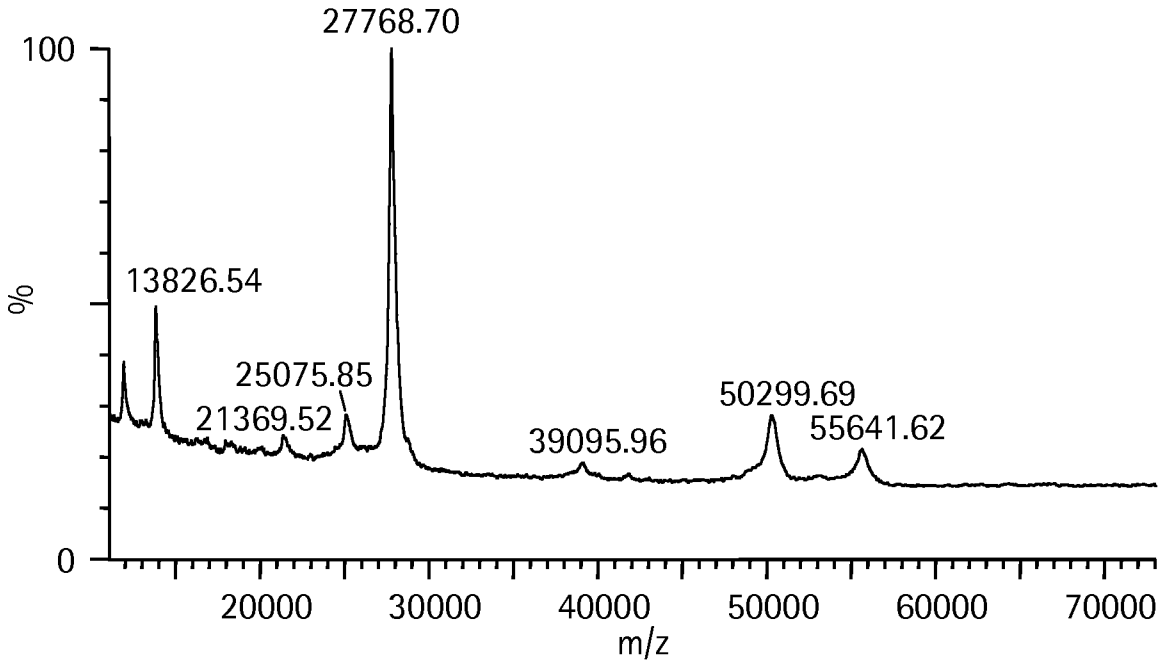


FIG. 30

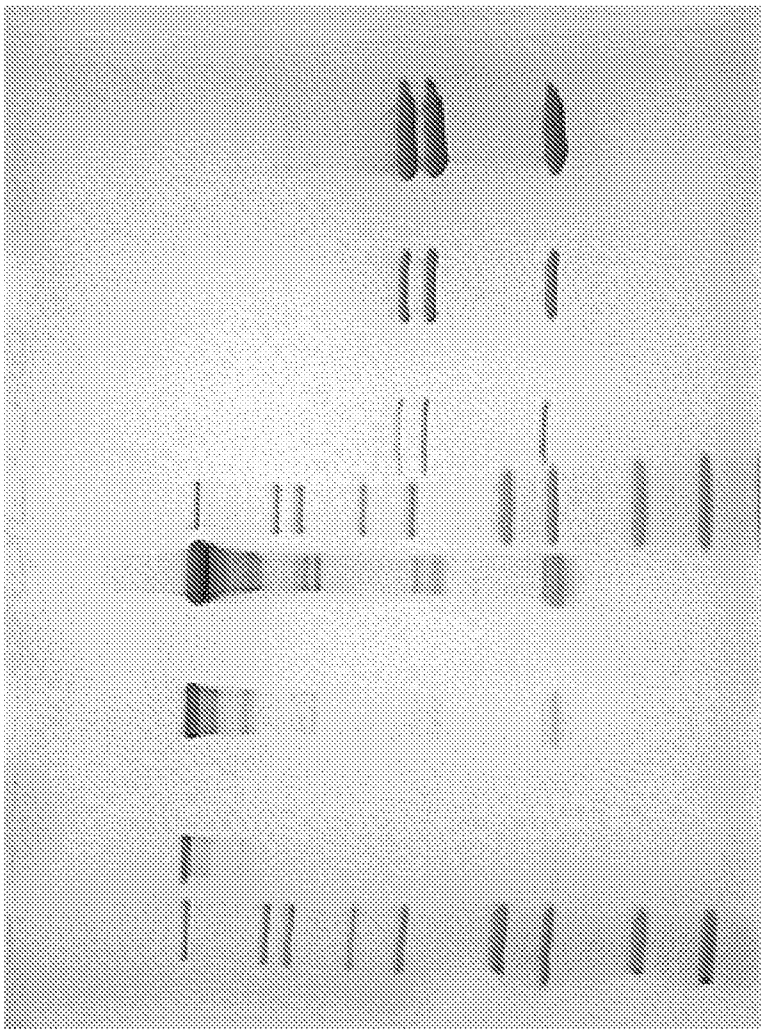


FIG. 31

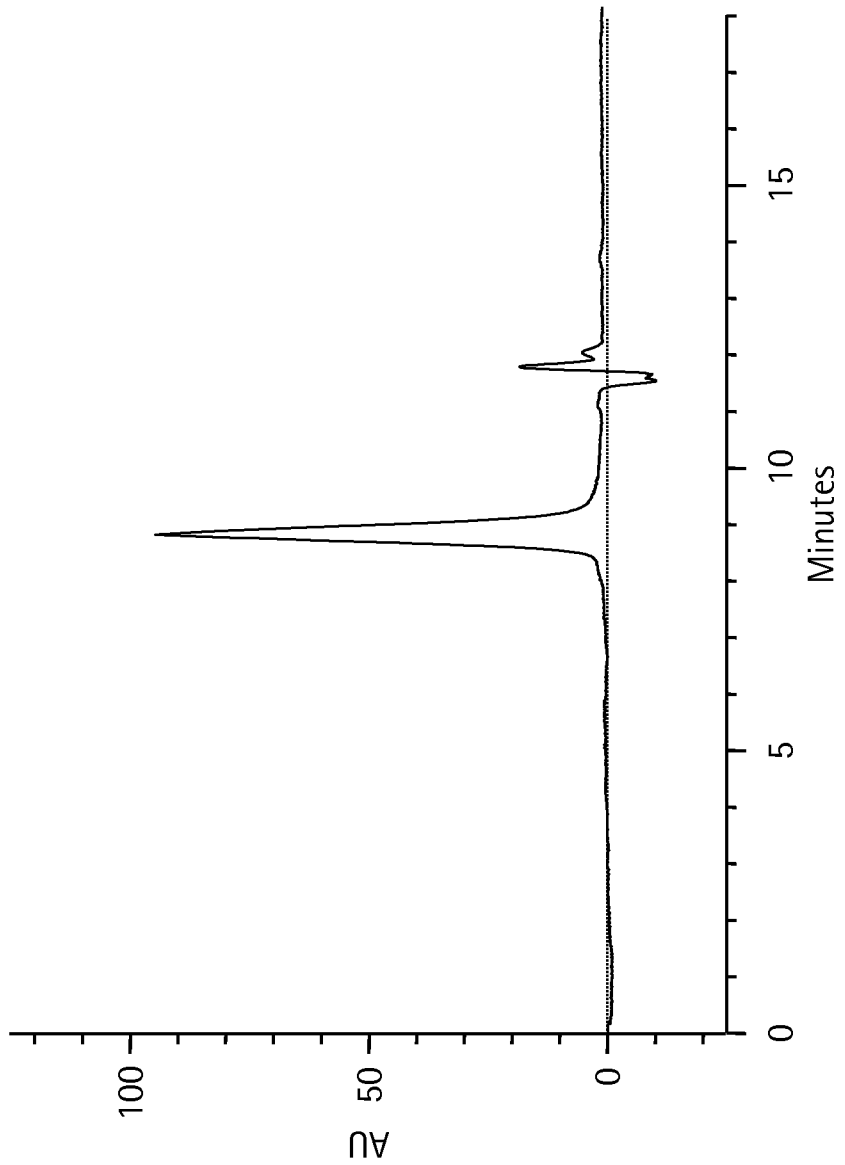


FIG. 32A

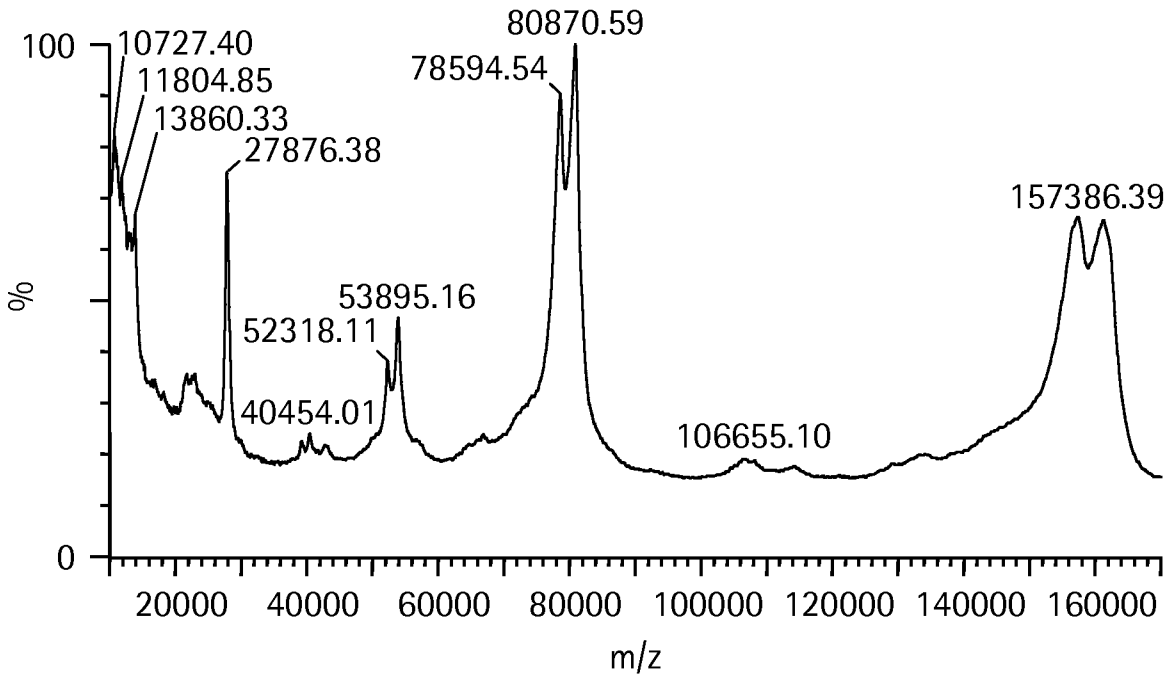


FIG. 32B

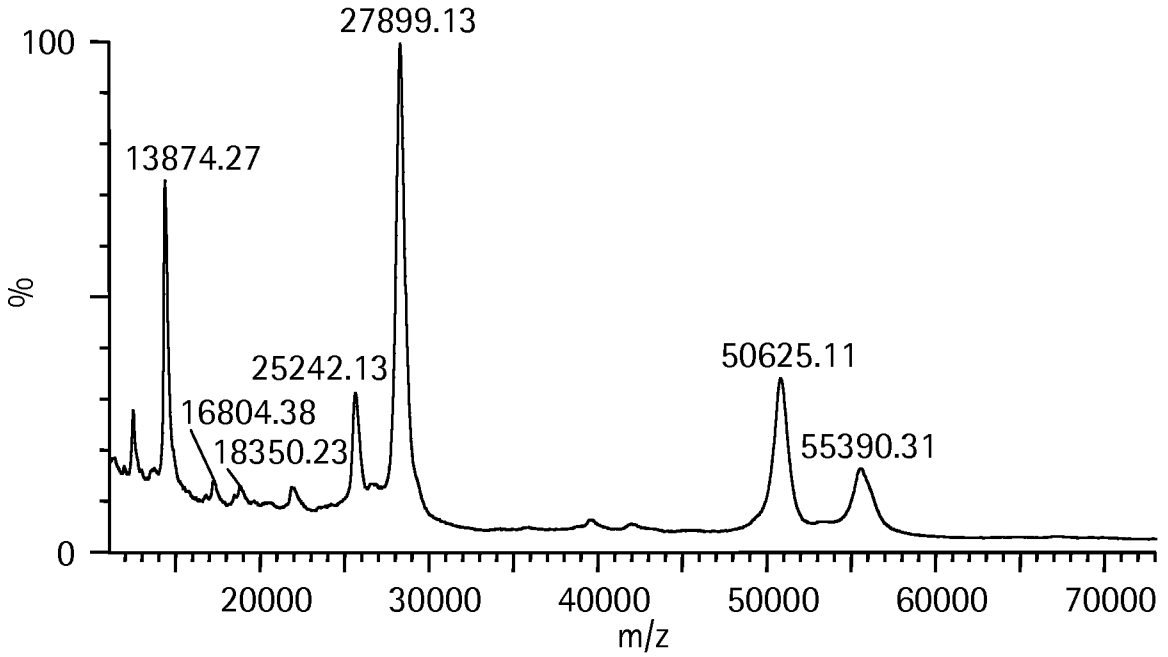


FIG. 33

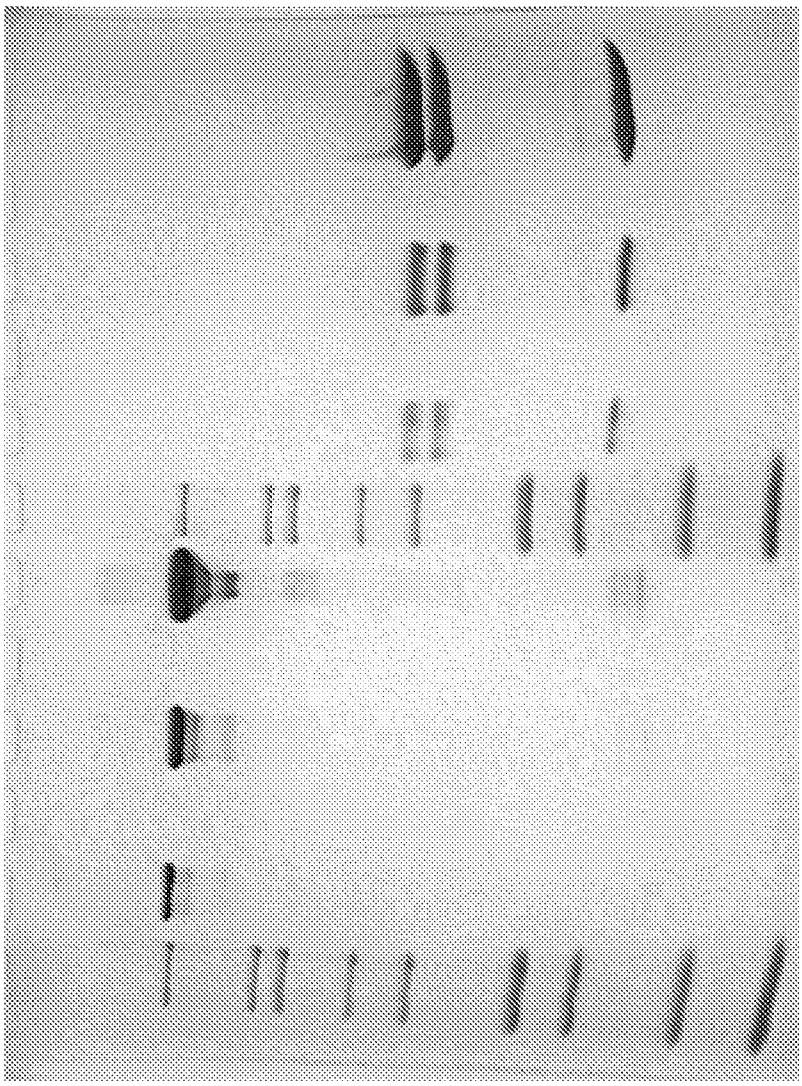


FIG. 34

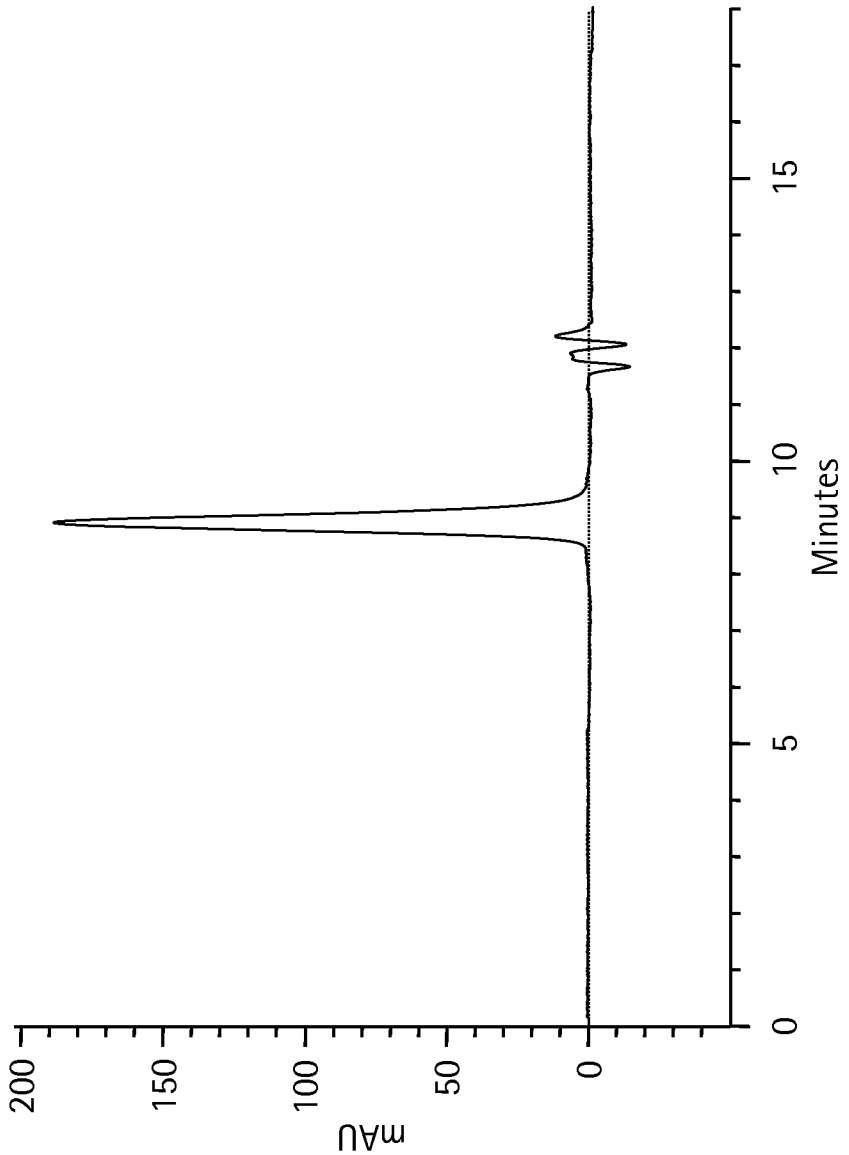


FIG. 35

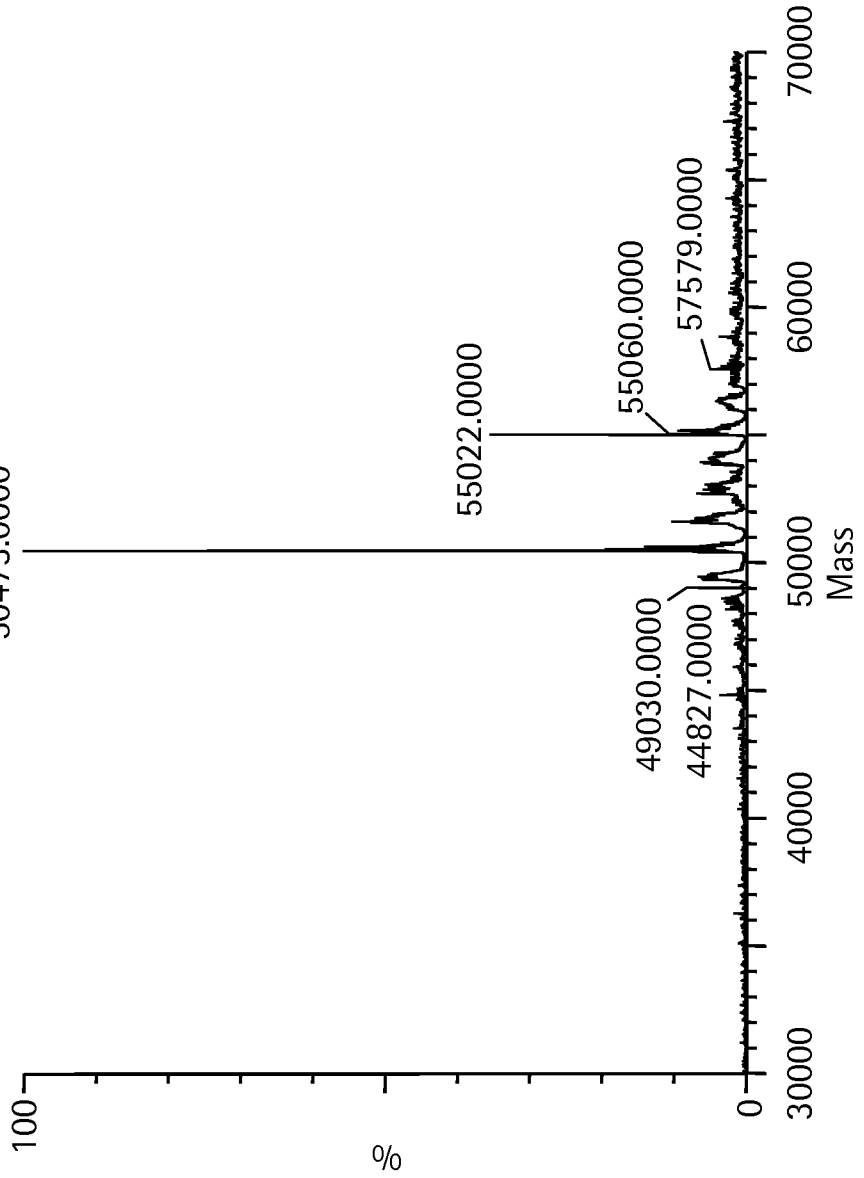

50473.0000
55022.0000
55060.0000
57579.0000
49030.0000
44827.0000
100
%
0
30000
40000
50000
60000
70000
Mass

FIG. 36

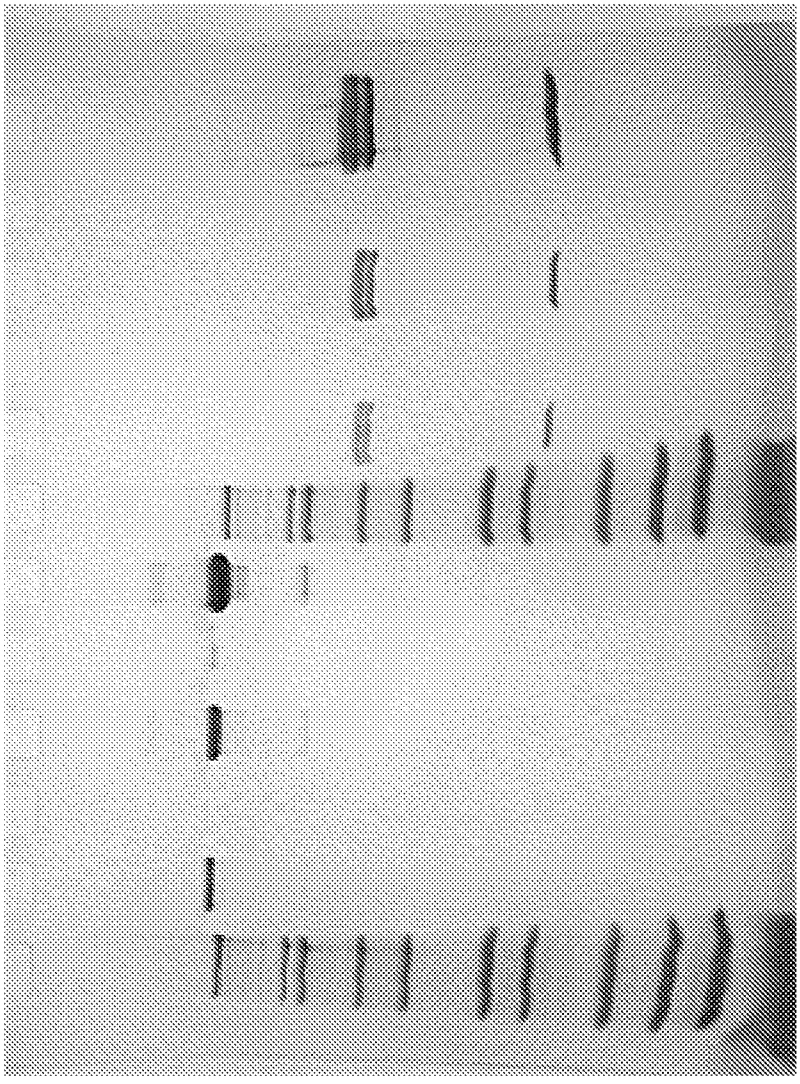


FIG. 37

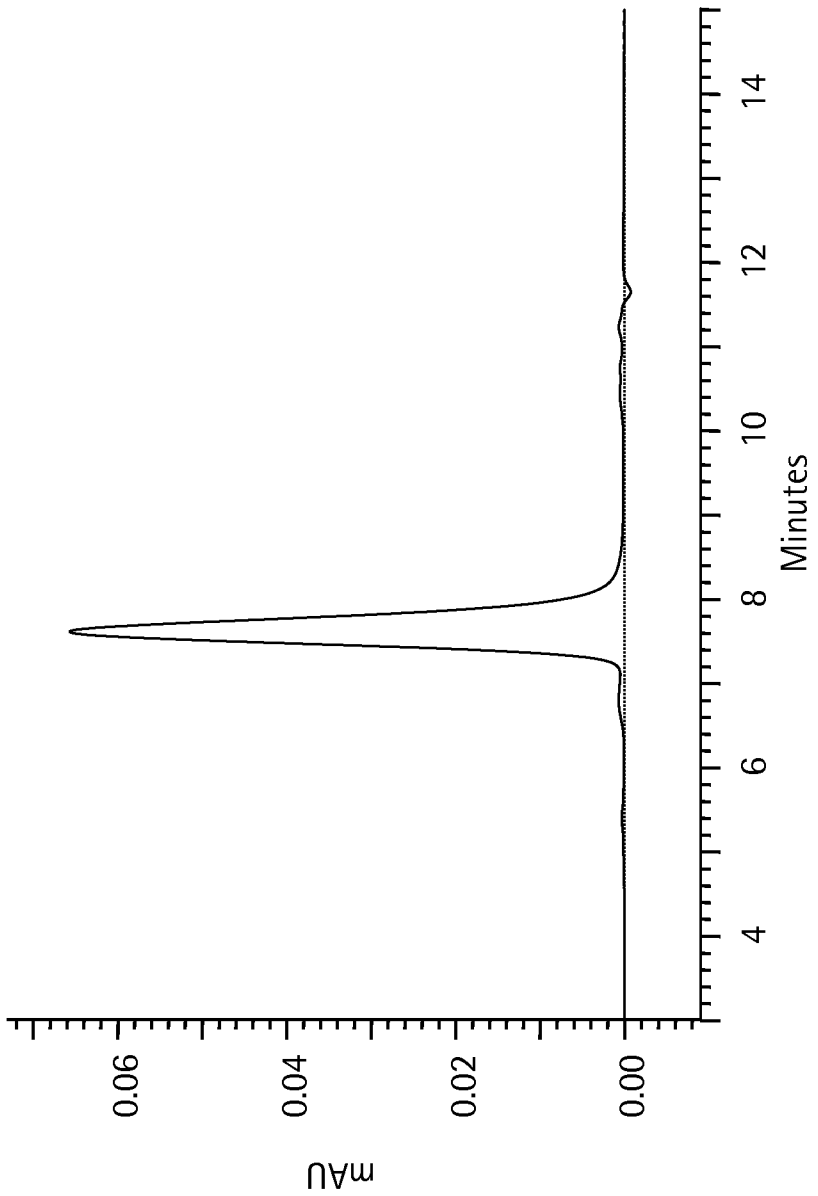


FIG. 38A

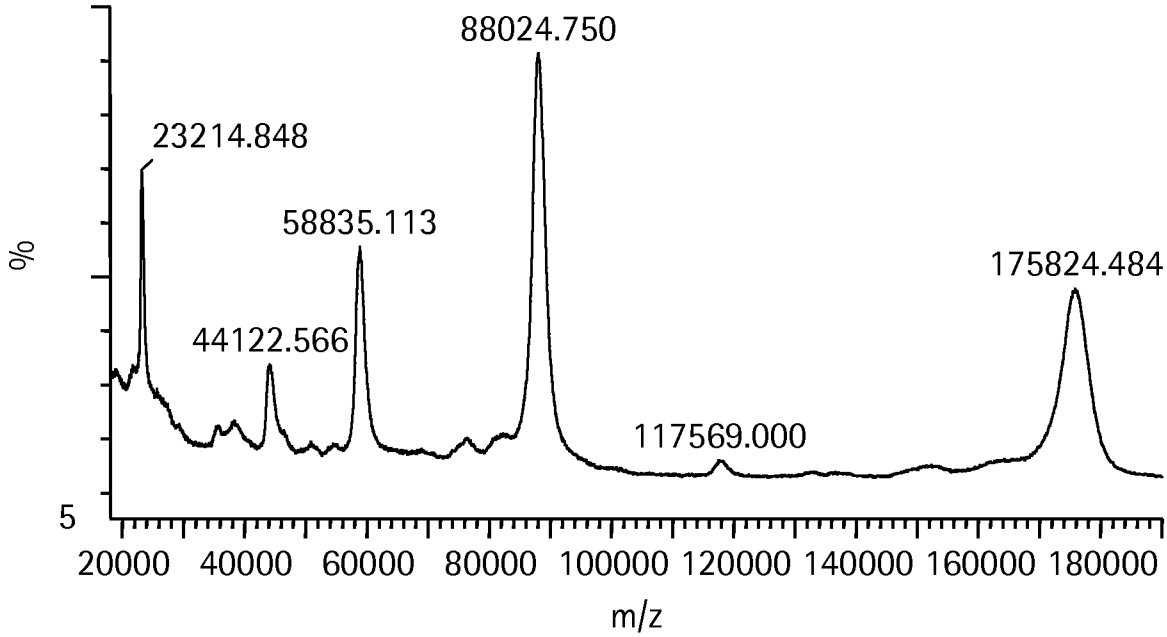


FIG. 38B

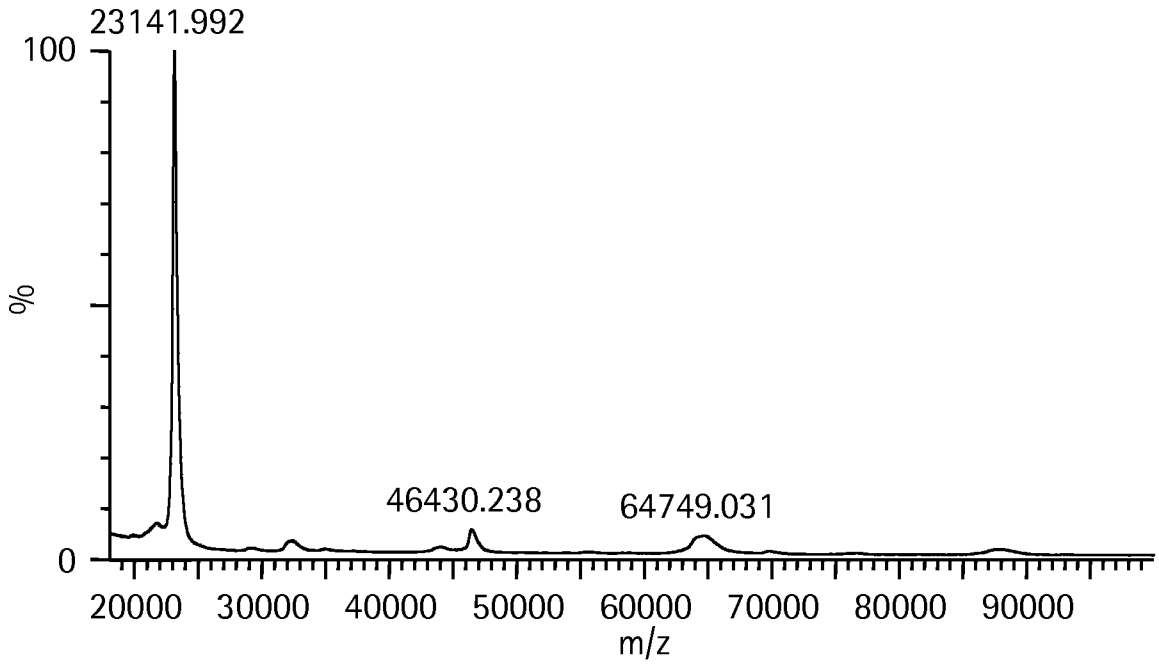


FIG. 39

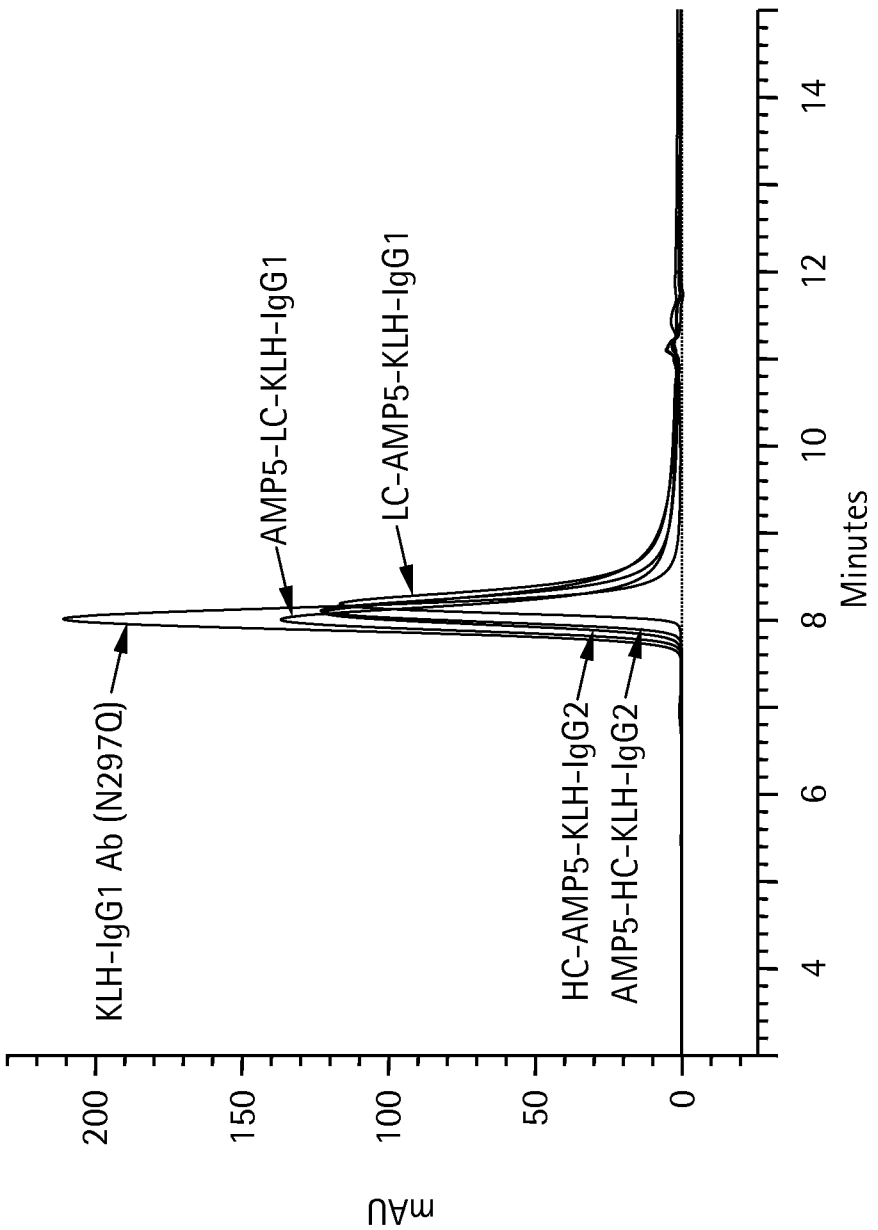

KLH-IgG1 Ab (N297Q)
AMP5-LC-KLH-IgG1
LC-AMP5-KLH-IgG1
HC-AMP5-KLH-IgG2
AMP5-HC-KLH-IgG2
mAU
200
150
100
50
0
4
6
8
10
12
14
Minutes

FIG. 40A

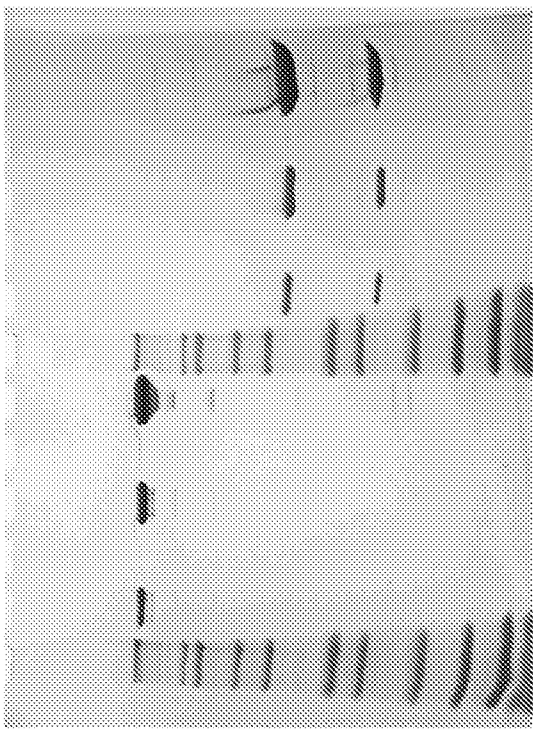


FIG. 40B

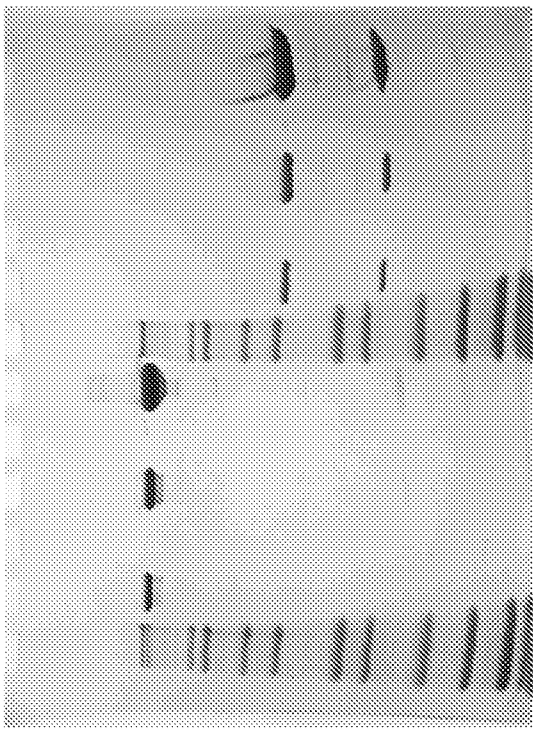


FIG. 40C

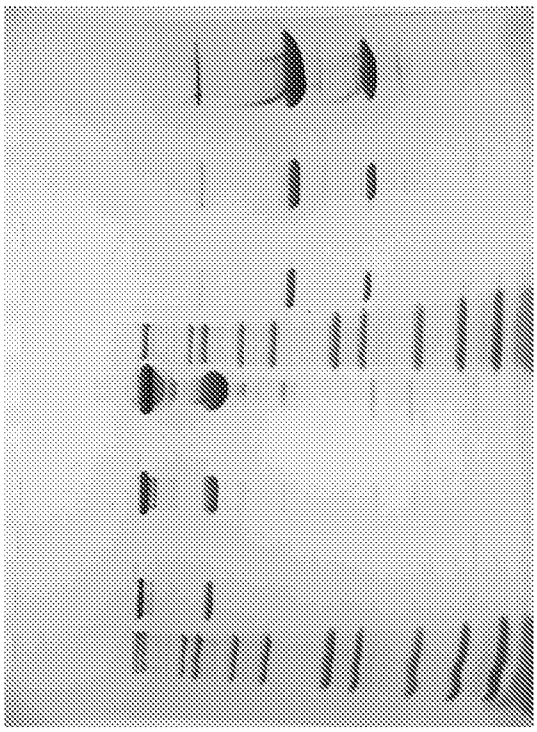


FIG. 40E

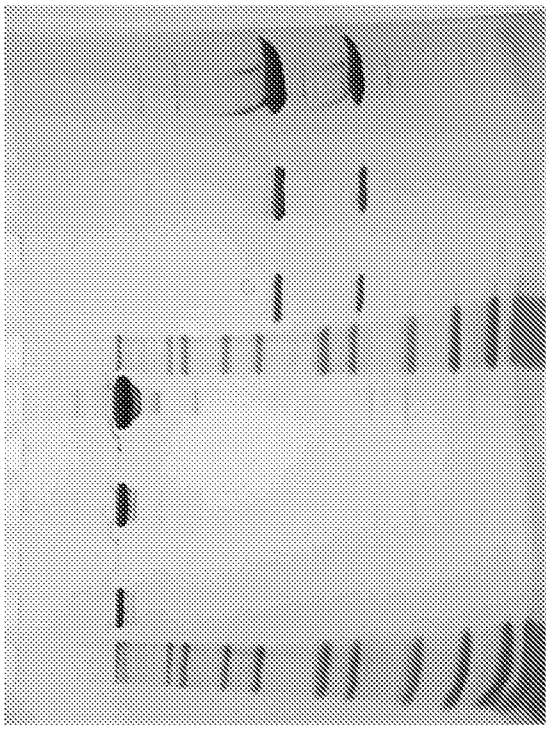


FIG. 40D

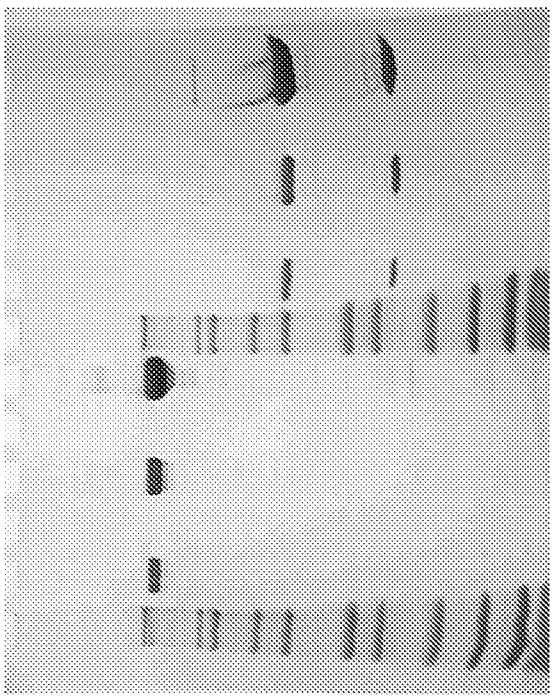


FIG. 41A

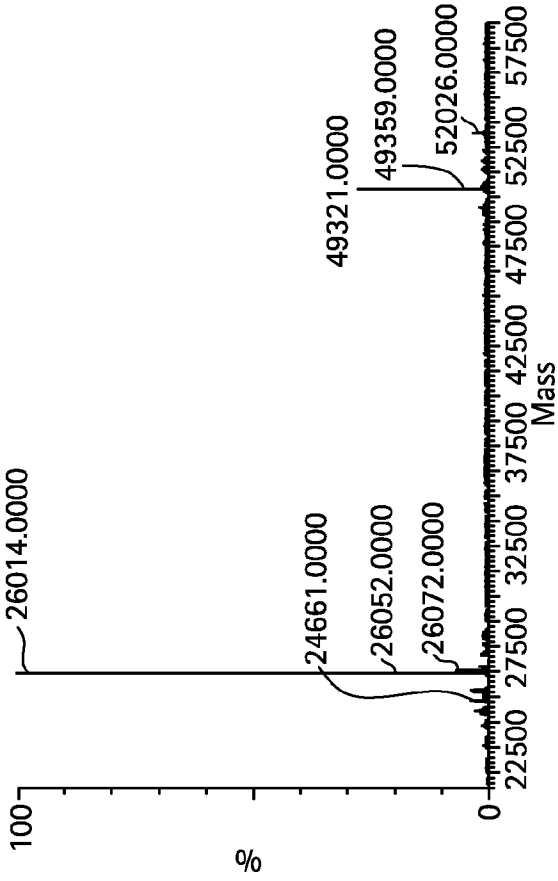


FIG. 41B

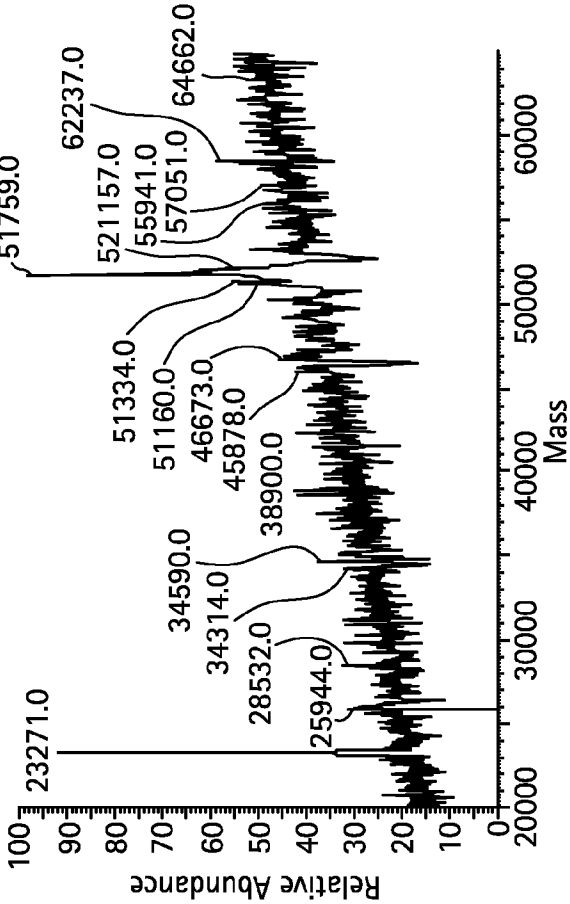


FIG. 41C

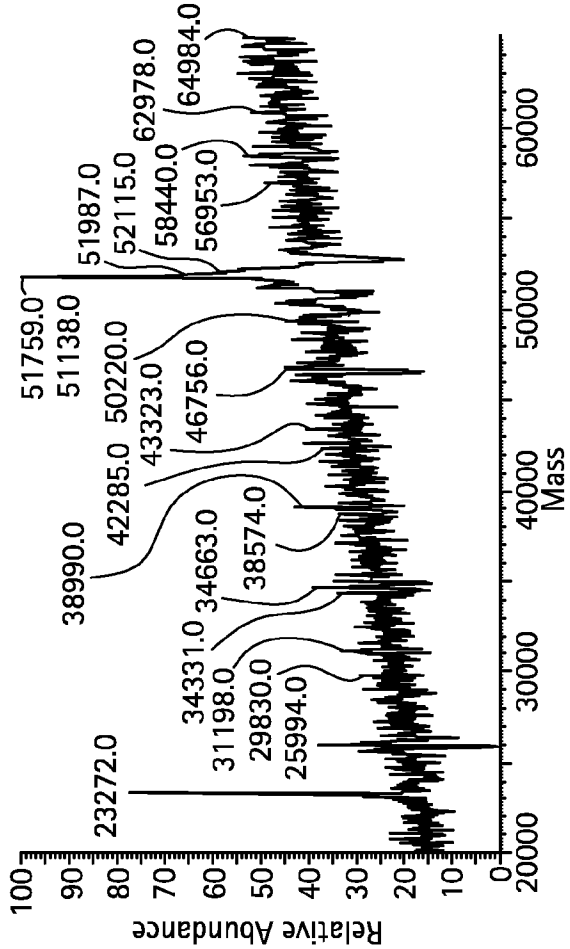


FIG. 41D

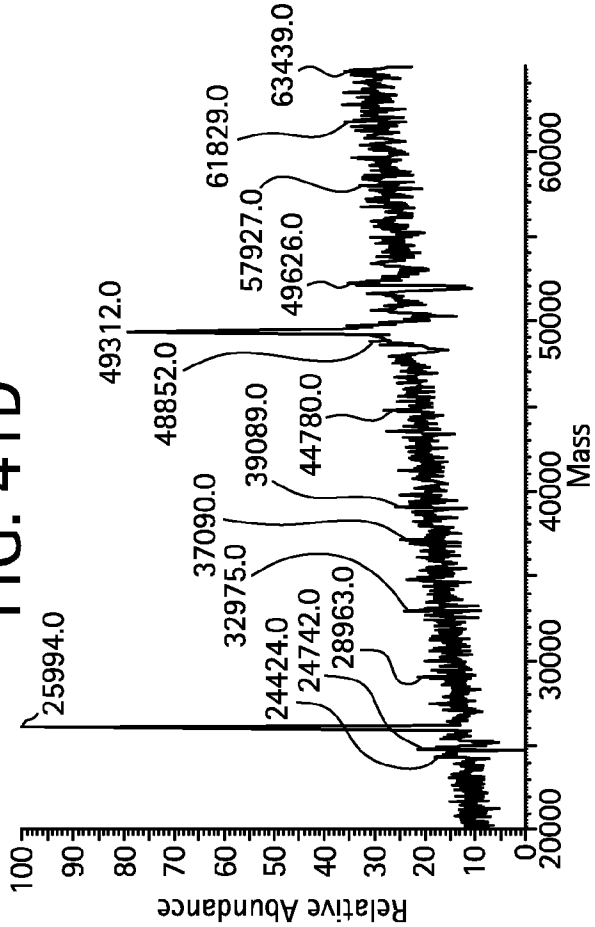
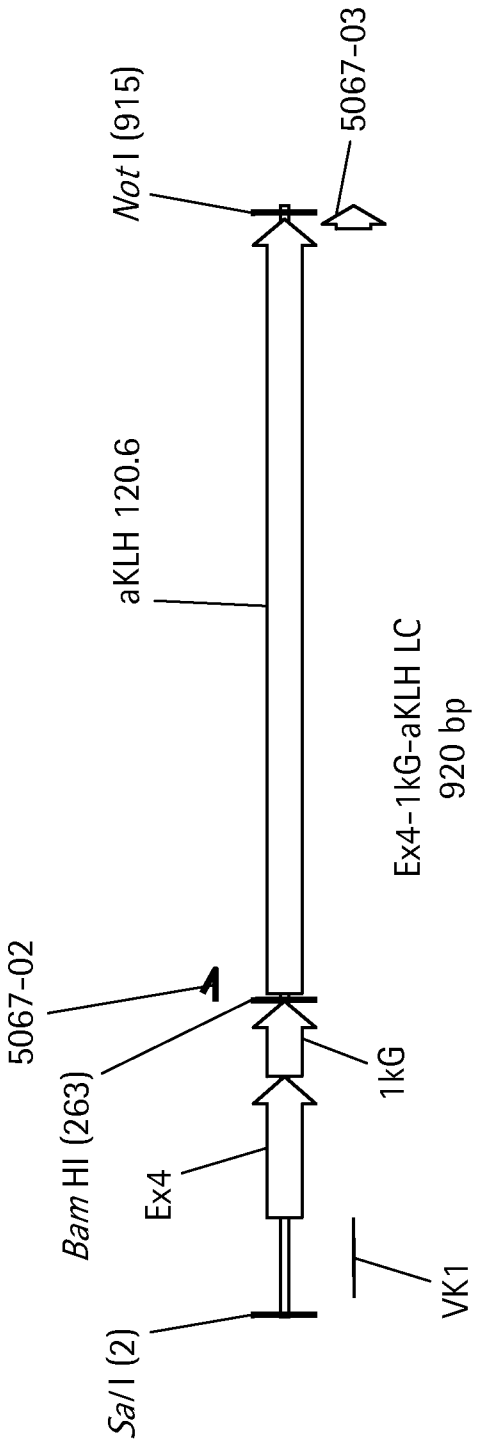


FIG. 42


NotI (915)
5067-03
aKLH 120.6
Ex4-1kG-aKLH LC
920 bp
5067-02
BamHI (263)
Ex4
1kG
SalI (2)
VK1

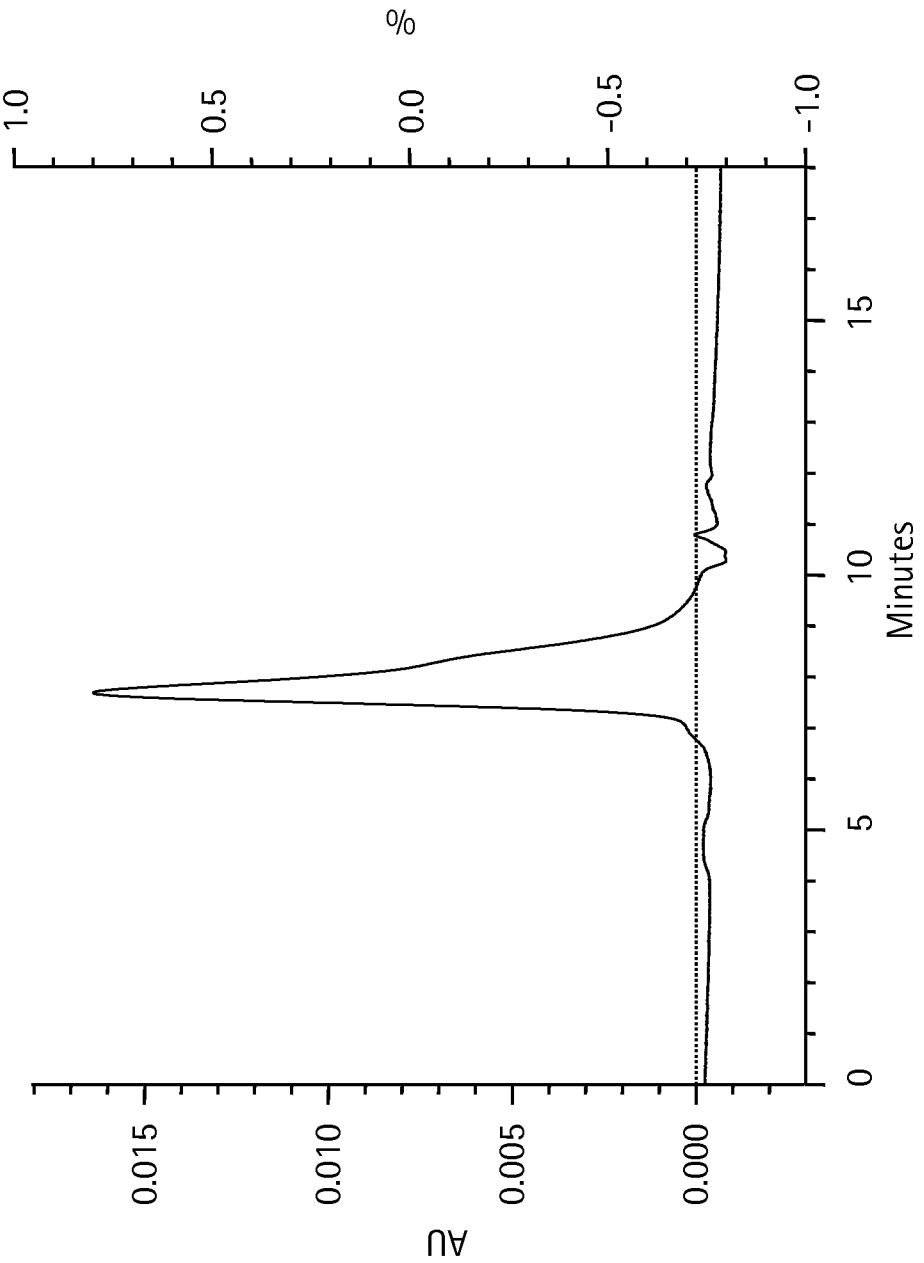


FIG. 43

FIG. 44

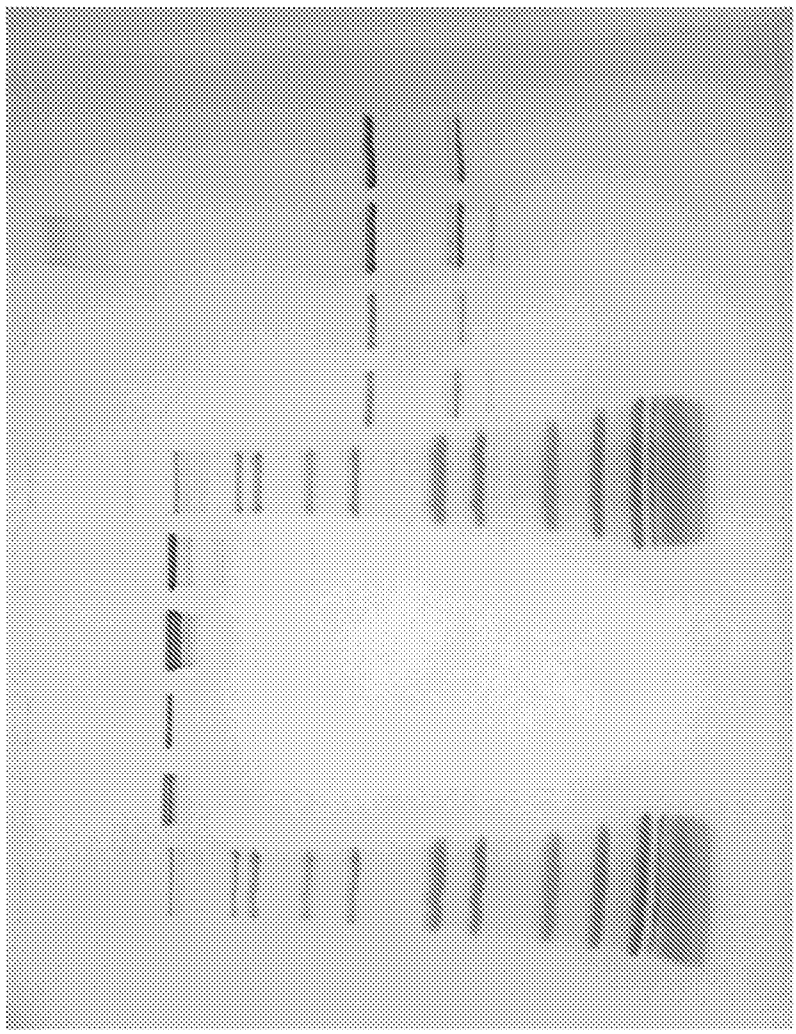


FIG. 45