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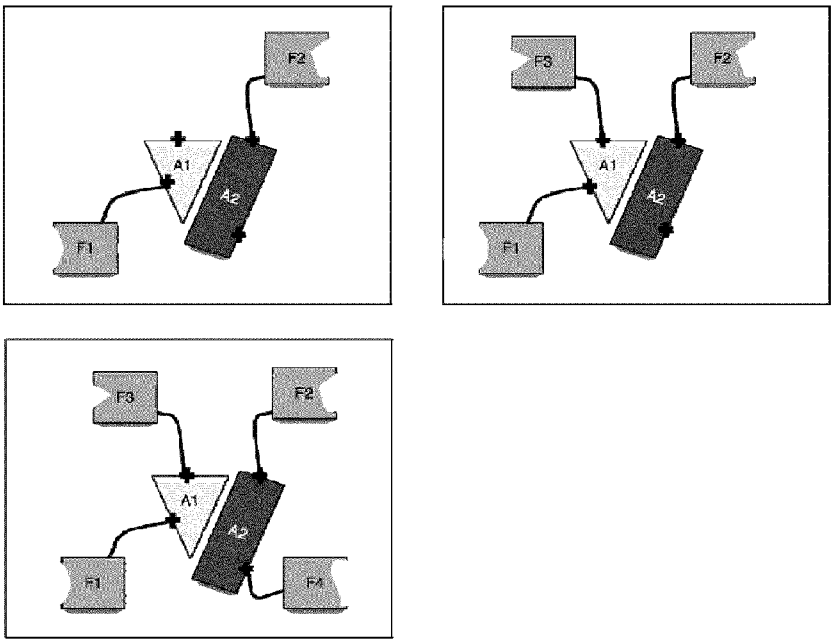
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[Continued on next page]

(54) Title: MULTIVALENT HETEROMULTIMER SCAFFOLD DESIGN AND CONSTRUCTS

Figure 4



(57) Abstract: Provided herein are multifunctional heteromer proteins. In specific embodiments is a heteromultimer that comprises: at least two monomeric proteins, wherein each monomeric protein comprises at least one cargo polypeptide, attached to a transporter polypeptide, such that said monomeric proteins associate to form the heteromultimer. These therapeutically novel molecules comprise monomers that function as scaffolds for the conjugation or fusion of therapeutic molecular entities resulting in the creation of bispecific or multivalent molecular species.

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MULTIVALENT HETEROMULTIMER SCAFFOLD DESIGN AND CONSTRUCTS

[0001] Field of Invention

[0002] The field of the invention is the rational design of a scaffold for custom development of biotherapeutics.

[0003] Description of Related Art

[0004] In the realm of therapeutic proteins, antibodies with their multivalent target binding features are excellent scaffolds for the design of drug candidates. Advancing these features further, designed bispecific antibodies and other fused multispecific therapeutics exhibit dual or multiple target specificities and an opportunity to create drugs with novel modes of action. The development of such multivalent and multispecific therapeutic proteins with favorable pharmacokinetics and functional activity has been a challenge.

[0005] Human serum albumin (HSA, or HA), a protein of 585 amino acids in its mature form is responsible for a significant proportion of the osmotic pressure of serum and also functions as a carrier of endogenous and exogenous ligands. The role of albumin as a carrier molecule and its stable nature are desirable properties for use as a carrier and transporter of polypeptides in vivo.

[0006] Human serum albumin possesses many desirable characteristics. HSA is found throughout the body, but more specifically in the interstitial space and in blood at serum concentrations of 40 g/L which is equivalent to 0.7 mM (Yeh et al., Proc. Natl. Acad. Sci. USA, 89:1904-1908 (1992)). HSA is considered to be the most abundant protein of the serum and is responsible for maintaining osmolarity. HSA has favorable pharmacokinetic properties and is cleared very slowly by the liver and kidney displaying in vivo half-lives up to several weeks (Yeh et al., Proc. Natl. Acad. Sci. USA, 89:1904-1908 (1992); Waldmann, T. A., Albumin Structure, Function and Uses, pp. 255-273 (1977); Sarav et al., J Am Soc Nephrol 20:1941-1952(2009)). HSA lacks enzymatic activity and antigenicity thereby eliminating potentially undesirable side effects. HSA acts as a carrier for endogenous as well as exogenous ligands. Combined, these features can be extended, at least partially, onto albumin based fusion protein. The poor pharmacokinetic properties displayed by therapeutic proteins can then be circumvented.

SUMMARY OF THE INVENTION

[0007] Provided herein are multifunctional heteromultimers and methods to design them. In certain embodiments are heteromultimers, each heteromultimer comprising: at least a first monomer unit that comprises at least one cargo molecule, and a first transporter polypeptide; and at least a second monomer unit that comprises at least one cargo molecule and a second transporter polypeptide; wherein at least one transporter polypeptide is derived from a monomeric protein and wherein said transporter polypeptides self-assemble to form a quasi-native structure of said monomeric protein or analog thereof. In certain embodiments, at least one cargo molecule is a drug, or a therapeutic agent. In certain embodiments, at least one cargo molecule is a biomolecule. In an embodiment, the at least one biomolecule is a DNA, RNA, PNA or polypeptide. In an embodiment, at least one cargo molecule is a polypeptide. In certain embodiments, each monomeric transporter polypeptide is unstable and preferentially forms a heteromultimer with at least one other transporter polypeptide. In certain embodiments, each monomeric transporter polypeptide is stable and preferentially forms a heteromultimer with at least one other transporter polypeptide. In certain embodiments, the heteromultimerization interface comprises at least one disulfide bond. In certain embodiments, the heteromultimerization interface does not comprise a disulfide bond.

[0008] In specific embodiments is a heteromultimer that comprises: at least two monomers, wherein each monomer comprises at least one cargo molecule attached to a transporter polypeptide, such that said monomers self-assemble to form the heteromultimer. In certain embodiments is a heteromultimer that comprises: at least two monomeric proteins, wherein each monomeric protein comprises at least one cargo polypeptide, attached to a transporter polypeptide, wherein at least one transporter polypeptide is derived from a monomeric protein and wherein said transporter polypeptides self-assemble to form a quasi-native structure of said monomeric protein or analog thereof. In certain embodiments is a heteromultimer that comprises: at least two monomeric proteins, wherein each monomeric protein comprises at least one cargo polypeptide attached to a transporter polypeptide, such that said monomeric proteins self-assemble via the transporter polypeptide to form the heteromultimer, and wherein at least one transporter polypeptide is derived from a monomeric protein and wherein said transporter polypeptides self-assemble to form a quasi-native structure of said monomeric protein or analog thereof. In certain embodiments, the heteromultimer is a heterodimer. In an

embodiment, the heteromultimer is bispecific. In an embodiment, the heteromultimer is multispecific. In certain embodiments, the heteromltimer is bivalent. In an embodiment the heteromultimer is multivalent. In an embodiment, the heteromultimer is multifunctional. In certain embodiments, at least one transporter polypeptide is not derived from an antibody. In certain embodiments, the transporter polypeptides are not derived from an antibody. In certain embodiments, the transporter polypeptides are derivatives of albumin. In certain embodiments of the hetermultimer described herein, the transporter polypeptides are derived from human serum albumin (HSA or HA) of SEQ ID No. 1. In certain embodiments of the hetermultimer described herein, the transporter polypeptides are derived from alloalbumins (HAA). In certain embodiments of the hetermultimer described herein, the transporter polypeptides are derived from sequence homologous to the human serum albumin (HSA or HA) of SEQ ID No. 1.

[0009] In some embodiments of the heteromultimer described herein, the transporter polypeptides are derivatives of an annexin protein. In an embodiment, the transporter polypeptides are derived from different annexin proteins. In certain embodiments, the transporter polypeptides are derived from the same annexin protein. In an embodiment, at least one transporter polypeptide is derived from Annexin A1 or lipocortin I. In certain embodiments of the heteromultimer, all transporter polypeptides are derived from Annexin A1 of SEQ ID NO: 14. In certain embodiments of the heteromultimer, at least one transporter polypeptides is derived from a sequence homologous to SEQ ID NO: 14. In an embodiment, at least one transporter polypeptide is derived from Annexin A2 or annexin II. In certain embodiments of the heteromultimer, all transporter polypeptides are derived from Annexin A2 or lipocortin II. In an embodiment, at least one transporter polypeptide is derived from Annexin like protein. In certain embodiments of the heteromultimer, all transporter polypeptides are derived from Annexin like protein. In an embodiment, at least one transporter polypeptide is derived from the group comprising Annexin A1-Annexin A7. In an embodiment of the heteromultimer described herein, all transporter polypeptides are derived from the group comprising Annexin A1-Annexin A7. 14. In certain embodiments, the first annexin based transporter polypeptide has a sequence comprising SEQ ID NO:15, and the second annexin based transporter polypeptide has a sequence comprising SEQ ID NO: 16.

[0010] In some embodiments of the heteromultimer described herein, the transporter polypeptides are derivatives of transferrin. In an embodiment, at least one transporter polypeptide is derived from transferrin. In certain embodiments of the heteromultimer, at

least one transporter polypeptides are derived from transferrin of SEQ ID NO: 19 or analog thereof. In certain embodiments of the heteromultimer, at least one transporter polypeptide is derived from a polypeptide seuquence homologous to the transferrin. In certain embodiments of the heteromultimer described herein, at least one transporter polypeptide is derived from apo-transferrin. In certain embodiments, the first transferrin based transporter polypeptide has a sequence comprising SEQ ID NO:15 and the second transferrin based transporter polypeptide has a sequence comprising SEQ ID NO: 16.

[0011] In certain embodiments of the heteromultimer, at least one cargo molecule is a cargo polypeptide. In an embodiment of the heteromultimer described herein, all cargo molecules are cargo polypeptides. In certain embodiments, the cargo polypeptides are therapeutic proteins or fragments or variants thereof. In certain embodiments, the cargo polypeptides are antigens or fragments or variants thereof. In certain embodiments, the cargo polypeptides are antigen receptors or fragments or variants thereof. In some embodiments, the cargo polypeptide is an antibody, an antibody domain, a ligand or a receptor that binds a target polypeptide. In some embodiments, at least one cargo polypeptide is fused to the transporter polypeptide. In certain embodiments, at least one cargo polypeptide is attached to the N-terminus of the transporter polypeptide. In some embodiments, at least one cargo polypeptide is attached to the C-terminus of the transporter polypeptide. In some embodiments, at least one cargo polypeptide is chemically linked to the transporter polypeptide. In some embodiments of the heteromultimers described herein, at least one cargo polypeptide comprises GLP-1 or fragment or variant thereof. In some embodiments, at least one cargo polypeptide comprises glucagon or fragment or variant thereof. In an embodiment, at least one cargo polypeptide comprises an EGF-A like domain.

[0012] Provided herein are heteromultimers, each heteromultimer comprising: at least a first monomeric protein that comprises at least one cargo polypeptide and a first transporter polypeptide; and at least a second monomeric protein that comprises at least one cargo polypeptide and a second transporter polypeptide. In certain embodiments, the heteromultimer is a heterodimer. In an embodiment, the heteromultimer is multispecific. In an embodiment, the heteromultimer is bispecific. In certain embodiments of the heteromultimer, the transporter polypeptides are derivatives of the same protein. In certain embodiments, the transporter polypeptides are derivatives of albumin. In certain embodiments of the hetermultimer described herein, the transporter polypeptides are derived from human serum albumin of SEQ ID No. 1. In certain embodiments, the

transporter polypeptides are derivatives of an annexin. In an embodiment, the transporter polypeptides are derivatives of Annexin A2. In some embodiments, the transporter polypeptides are derivatives of transferrin.

[0013] In certain embodiments, are heteromultimers, each heteromultimer comprising: at least a first monomeric protein that comprises at least one cargo polypeptide and a first transporter polypeptide comprising a first segment of human serum albumin; and at least a second monomeric protein that comprises at least one cargo polypeptide, fragment and a second transporter polypeptide comprising a second segment of human serum albumin; wherein said transporter polypeptides self-assemble to form a quasi-native structure of albumin or analog thereof. In certain embodiments, the first and second segments of human serum albumin are from non-overlapping regions of the protein. In certain embodiments, there is an overlap between the sequences of the first and second segments of human serum albumin. In some embodiments, the overlap is a 5% overlap. In an embodiment, the overlap is a 10% overlap. In certain embodiments, the first segment of human serum albumin comprises a sequence of SEQ ID NO:2, and the second segment of human serum albumin comprises a sequence of SEQ ID NO: 3. In certain embodiments, the first segment of human serum albumin comprises a sequence of SEQ ID NO:8, and the second segment of human serum albumin comprises a sequence of SEQ ID NO: 10.

[0014] In certain embodiments, are heteromultimers, each heteromultimer comprising: at least a first monomeric protein that comprises at least one cargo polypeptide and a first transporter polypeptide comprising a sequence of SEQ ID NO:2; and at least a second monomeric protein that comprises at least one cargo polypeptide, and a second transporter polypeptide comprising a sequence of SEQ ID NO: 3. In certain embodiments, are heteromultimers, each heteromultimer comprising: at least a first monomeric protein that comprises at least one cargo polypeptide and a first transporter polypeptide comprising a sequence of SEQ ID NO:8; and at least a second monomeric protein that comprises at least one cargo polypeptide and a second transporter polypeptide comprising a sequence of SEQ ID NO: 10. In certain embodiments of the hetermultimer described herein, at least one transporter polypeptide is derived from alloalbumins. In certain embodiments, both transporter polypeptides are derived from alloalbumins. In certain embodiments, all transporter polypeptides are derivatives of the same alloalbumin. In some other embodiments, the transporter polypeptides are derivatives of different alloalbumins. In some embodiments, each transporter polypeptide is an alloalbumin derivative based on an alloalbumin selected from Table 2. In certain embodiments, the

first monomeric protein comprises two cargo polypeptides. In some embodiments, the second monomeric protein comprises two cargo polypeptides. In some embodiment, at least one of the monomeric proteins is engineered by introducing mutations. In certain embodiments, the introduced mutations improve the functionality of the monomeric protein as compared to the native, non-mutated form of the monomer. In certain embodiments the introduced mutations improve one or more of the stability, half-life and heteromultimer formation of the transporter polypeptide.

[0015] Provided herein are heteromultimers, each heteromultimer comprising: at least a first monomeric protein that comprises at least one cargo polypeptide and a first transporter polypeptide; and at least a second monomeric protein that comprises at least one cargo polypeptide and a second transporter polypeptide. In certain embodiments, at least one cargo polypeptide is selected from the proteins listed in Table 2 or fragments, variants or derivatives thereof. In certain embodiments, at least one cargo polypeptide is selected from ligand, receptor, or antibody to one or more proteins listed in Table 2, or fragment, variant or derivative of said ligand, receptor or antibody. In certain embodiments, at least one cargo polypeptide targets a cell surface antigen from the group consisting of CD19, CD20, CD22, CD25, CD30, CD33, CD40, CD56, CD64, CD70, CD74, CD79, CD105, Cd138, CD174, CD205, CD227, CD326, CD340, MUC16, GPNMB, PSMA, Cripto, ED-B, TMEFF2, EphB2, EphA2, FAP, integrin, Mesothelin, EGFR, TAG-72, GD2, CAIX, 5T4. In certain embodiments, are heteromultimers, each heteromultimer comprising: at least a first monomeric protein that comprises at least one cargo polypeptide and a first transporter polypeptide; and at least a second monomeric protein that comprises at least one cargo polypeptide and a second transporter polypeptide, wherein at least one at least one cargo polypeptide is an antibody, or fragment or variant thereof. In certain embodiments, all cargo polypeptides are antibodies or fragments or variants thereof. In some embodiments, the cargo polypeptide is an antibody that binds to a protein listed in Table 2. In some embodiments, the antibody fragment comprises antibody Fc or Fab or Fv region. In some embodiment the cargo polypeptide is a non-antibody protein like nanobodies, affibody, maxibody, adnectins, domain antibody, evibody, ankyrin repeat proteins, anticalins, camlids or ligand protein or polypeptide binding to a therapeutically relavant target. In some embodiments, the antibody or its fragment is derived from an immunoglobulin selected from the group consisting of IgG, IgA, IgD, IgE, and IgM. In certain embodiments, the IgG is of subtype selected from IgG1, IgG2a, IgG2b, IgG3 and IgG4. In certain embodiments, the antibody is multispecific.

[0016] Provided herein are heteromultimers, each heteromultimer comprising: at least a first monomeric protein that comprises at least one cargo polypeptide and a first transporter polypeptide; and at least a second monomeric protein that comprises at least one cargo polypeptide and a second transporter polypeptide, wherein at least one cargo polypeptide is a therapeutic antibody. In some embodiments of the heteromultimers described herein, at least one cargo polypeptide is a therapeutic antibody or fragment or variant thereof, wherein the antibody is selected from antibody is selected from abagovomab, adalimumab, alemtuzumab, aurograb, bapineuzumab, basiliximab, belimumab, bevacizumab, briakinumab, canakinumab, catumaxomab, certolizumab pegol, certuximab, daclizumab, denosumab, efalizumab, galiximab, gemtuzumab ozagamicin, golimumab, ibritumomab tiuxetan, infliximab, ipilimumab, lumiliximab, mepolizumab, motavizumab, muromonab, mycograb, natalizumab, nimotuzumab, ocrelizumab, ofatumumab, omalizumab, palivizumab, panitumumab, pertuzumab, ranizumab, reslizumab, rituximab, teplizumab, toclizumab, tositumomab, trastuzumab, Proxinium, Rencarex, ustekinumab, and zalutumumab. In certain embodiments, the therapeutic antibody binds a disease related target antigen such as cancer antigen, inflammatory disease antigen or a metabolic disease antigen. In certain embodiments, the target antigen could be a protein on a cell surface and the cell could belong to the group of B-cell, T-cell, stromal cell, endothelial cell, vascular cell, myeloid cell, hematopoietic cell or carcinoma cell.

[0017] Provided herein are heteromultimers, each heteromultimer comprising: at least a first monomer that comprises at least one cargo molecule, fragment; and at least a second monomer that comprises at least one cargo molecule and a second transporter polypeptide, wherein at least one cargo polypeptide is an enzyme, enzyme inhibitor, hormone, therapeutic polypeptide, antigen, radiotoxin and chemotoxin inclusive of but not restricted to neurotoxins, interferons, cytokine fusion toxins and chemokine fusion toxins, cytokine, antibody fusion protein or variant or fragment thereof. In some embodiments of the heteromultimers described herein, at least one cargo polypeptide comprises GLP-1 or fragment or variant thereof. In some embodiments, at least one cargo polypeptide comprises glucagon or fragment or variant thereof. In an embodiment, at least one cargo polypeptide comprises an EGF-A like domain. In certain embodiments, the toxin is an immunotoxin such as Denileukin diftitox and Anti-CD22 immunotoxin such as CAT-3888 and CAT-8015. In certain embodiments, the toxin is saporin. In some embodiments, the toxin is a mitotoxin. In some embodiments, the toxin is a diphtheria

toxin. In some embodiments, the toxin is botulinux toxin type A. In some embodiments, the toxin is ricin or a fragment there of. In some embodiments, the toxin is a toxin from RTX family of toxins.

[0018] Provided herein are heteromultimers, each heteromultimer comprising: at least a first monomeric protein that comprises at least one cargo polypeptide and a first transporter polypeptide; and at least a second monomeric protein that comprises at least one cargo polypeptide and a second transporter polypeptide, wherein the cargo polypeptide is attached to the transporter polypeptide by chemical conjugation, native ligation, chemical ligation, a disulfide bond or direct fusion or fusion via a linker. In certain embodiments, linkers for attaching cargo molecules such as cargo polypeptides to transporter polypeptides are selected from the linkers described in US5482858, US5258498 and US5856456, US2009060721, US6492123, US4946778, US5869620, US7385032, US5073627, US5108910, US7977457, US5856456, US7138497,US5837846, US5990275, EP1088888 incorporated by reference herein.

[0019] Provided herein are host cells comprising nucleic acid encoding a heteromultimer described herein. In certain embodiments, the nucleic acid encoding the first monomeric protein and the nucleic acid encoding the second monomeric protein are present in a single vector. In certain embodiments, the nucleic acid encoding the first monomeric protein and the nucleic acid encoding the second monomeric protein are present in separate vectors.

[0020] Provided herein is a method of making a heteromultimer, wherein said method comprises: culturing a host cell described herein such that the nucleic acid encoding a heteromultimer described herein is expressed; and recovering the heteromultimer from the cell culture. In some embodiments, the host cell is a prokaryotic cell or a eukaryotic cell. In some embodiments, the host cell is E. coli. In certain embodiments, the host cell is yeast cell. In some embodiments, the yeast is S. cerevisiae. In some embodiments, the yeast is Pichia. In a certain embodiment, the yeast is Pichia pastoris. In some embodiments, the yeast is glycosylation deficient, and/or protease deficient. In some embodiments, the host cell is a bacterial cell. In some embodiments, the host cell expressing a heteromultimer descried herein is a mammalian cell. In certain embodiments, the mammalian cell is a CHO cell, a BHK cell, NSO cell, COS cell or a human cell.

[0021] Provided is a pharmaceutical composition that comprises a heteromultimer described herein and a pharmaceutically acceptable adjuvant. Also provided are methods of treating

an individual suffering from a disease or disorder, said method comprising administering to the individual an effective amount of a formulation or pharmaceutical composition described herein. In certain embodiments is a method of treating cancer in a patient, said method comprising administering to the patient a therapeutically effective amount of a heteromultimer described herein. In some embodiments is a method of treating an immune disorder in a patient, said method comprising administering to the patient a therapeutically effective amount of a heteromultimer described herein. Also provided is a method of treating an infectious disease in a patient, said method comprising administering to the patient a therapeutically effective amount of a heteromultimer described herein. In certain embodiments is a method of treating a cardiovascular disorder in a patient, said method comprising administering to the patient a therapeutically effective amount of a heteromultimer described herein. In certain embodiments is a method of treating a respiratory disorder in a patient, said method comprising administering to the patient a therapeutically effective amount of a heteromultimer described herein. In certain embodiments is a method of treating a metabolic disorder in a patient, said method comprising administering to the patient a therapeutically effective amount of a heteromultimer described herein. In certain embodiments is a method of treating one or more of Congenital adrenal hyperplasia, Gaucher's disease, Hunter syndrome, Krabbe disease, Metachromatic leukodystrophy, Niemann-Pick disease, Phenylketonuria (PKU), Porphyria, Tay-Sachs disease, and Wilson's disease in a patient, said method comprising administering to the patient a therapeutically effective amount of a heteromultimer described herein.

[0022] Provided is a kit for detecting the presence of a biomarker of interest in an individual, said kit comprising (a) an amount of a heteromultimer described herein, wherein said heteromultimer comprises at least one cargo polypeptide such that said cargo polypeptide is capable of binding to the biomarker of interest; and (b) instructions for use.

[0023] Provided herein are heteromultimer proteins that comprise at least two monomeric proteins, wherein each monomeric protein comprises at least one cargo polypeptide, and an albumin based polypeptide, such that said monomeric proteins self-assemble to form the heteromultimer.

[0024] In certain embodiments, the cargo polypeptide is fused to the albumin or alloalbumin based transporter polypeptide. In some embodiments, the cargo polypeptide is fused to the transferrin based transporter polypeptide. In certain embodiments, the cargo polypeptide is fused to the annexin based transporter polypeptide. In some embodiments,

the fusion is at the N terminus of the transporter polypeptide. In certain embodiments, the fusion is at the C terminus of the transporter polypeptide. In some embodiments, the fusion involves a bridging linker or spacer molecule. In some embodiments, the cargo polypeptide is chemically conjugated to the transporter polypeptide. In certain embodiments, the cargo polypeptide is attached to the transporter polypeptide by means of chemical ligation or a disulfide bond.

[0025] Provided herein are heteromultimer proteins that comprise at least two monomeric proteins, wherein each monomeric protein comprises at least one cargo polypeptide, and a transporter polypeptide, such that said transporter polypeptides self-assemble to form the heteromultimer. In some embodiments, each transporter polypeptide is an alloalbumin based polypeptide, such that said alloalbumin based polypeptides self-assemble to form the heteromultimer. In some embodiments, each transporter polypeptide is a transferrin based polypeptide. In some embodiments, each transporter polypeptide is an annexin based polypeptide. In certain embodiments, each monomeric transporter polypeptide is unstable and preferentially forms a heteromultimer with at least one other transporter polypeptide.

[0026] In some embodiments, a heteromultimer described herein is a heterodimer. In some embodiments cargo polypeptide is an antibody, enzyme, hormone, therapeutic polypeptide, antigen, chemotoxin, radiotoxin, cytokine or variant or fragment thereof. In some embodiments, the cargo polypeptide of one monomeric protein functions in synergy with the cargo polypeptide of another monomeric protein.

[0027] Provided herein are heteromultimer proteins that comprise at least two monomeric proteins, wherein each monomeric protein comprises at least one cargo polypeptide, and an annexin based polypeptide, such that said annexin based polypeptides self-assemble to form the heteromultimer with a quasi-native structure of annexin or analog thereof. In some embodiments, the annexin is Annexin A1. In some embodiments, a heteromultimer described herein is a heterodimer. In some embodiments cargo polypeptide is an antibody, enzyme, hormone, therapeutic polypeptide, antigen, chemotoxin, radiotoxin, cytokine, ligand to a receptor, receptor or variant or fragment thereof. In some embodiments, the cargo polypeptide of one monomeric protein functions in synergy with the cargo polypeptide of another monomeric protein. In some embodiments the cargo polypeptide can be an agonist or antagonist to the cargo polypeptide of another monomeric protein.

[0028] Provided herein are heterodimer proteins that comprise at least two monomeric fusion proteins, wherein each monomeric fusion proteins comprises at least one cargo polypeptide fused to an albumin derived polypeptide, such that said albumin derived polypeptides self-assemble to form the multifunctional heterodimer. In certain embodiments are heterodimeric proteins comprising a first monomer which comprises at least one cargo polypeptide fused to an albumin derived polypeptide; and a second monomer that comprises at least one cargo polypeptide fused to an albumin derived polypeptide. In certain embodiments, the at least one cargo polypeptide of the first monomer is different from the at least one cargo polypeptide of the second monomer. In certain embodiments, the at least one cargo polypeptide of the first monomer is the same as the at least one cargo polypeptide of the second monomer.

[0029] In certain embodiments are heteromultimer proteins that comprise at least two monomeric fusion proteins, wherein each monomeric fusion proteins comprises at least one cargo polypeptide fused to an alloalbumin derived polypeptide, such that said alloalbumin derived polypeptides self-assemble to form the multifunctional heteromultimer. In certain embodiments are heteromultimer proteins that comprise at least two monomeric fusion proteins, wherein each monomeric fusion proteins comprises at least one cargo polypeptide fused to a transferrin derived polypeptide, such that said transferrin derived polypeptides self-assemble to form the heteromultimer. In certain embodiments are heteromultimer proteins that comprise at least two monomeric fusion proteins, wherein each monomeric fusion proteins comprises at least one cargo polypeptide fused to an annexin derived polypeptide, such that said annexin derived polypeptides self-assemble to form the heteromultimer. In certain embodiments, the annexin is Annexin A2.

[0030] In certain embodiments are heteromultimer proteins comprising a first monomer which comprises at least one cargo polypeptide fused to an alloalbumin derived polypeptide; and a second monomer that comprises at least one cargo polypeptide fused to an alloalbumin derived polypeptide. In certain embodiments, the at least one cargo polypeptide of the first monomer is different from the at least one cargo polypeptide of the second monomer. In certain embodiments, the at least one cargo polypeptide of the first monomer is the same as the at least one cargo polypeptide of the second monomer.

[0031] Provided herein is a heteromultimer that comprises: at least two monomers, each comprising a transporter polypeptide and optionally at least one cargo molecule attached to said transporter polypeptide, wherein each transporter polypeptide is obtained by segmentation of a whole protein such that said transporter polypeptides self-assemble to

form quasi-native whole protein. In certain embodiments, the heteromultimer is multispecific. In certain embodiments, the transporter polypeptides are not derived from an antibody. In some embodiments, each monomer preferentially forms the heteromultimer as compared to a monomer or a homomultimer. In an embodiment of the heteromultimer, at least one cargo molecule is a theraputic agent, or a biomolecule. In some embodiments, at least one cargo molecule is a biomolecule which is selected from a polypeptide, DNA, PNA, or RNA.In some embodiments, each transporter polypeptide is a derivate of albumin or alloalbumin. In an embodiment, each transporter polypeptide is a derivate of annexin. In certain embodiments, each transporter polypeptide is a derivate of transferrin.

[0032] In certain embodiments are pharmaceutical formulations that comprise an albumin-based and/or alloalbumin-based heteromultimeric protein described herein and a pharmaceutically acceptable diluent or carrier. In certain embodiments are pharmaceutical formulations that comprise a transferrin-based heteromultimeric protein described herein and a pharmaceutically acceptable diluent or carrier. In certain embodiments are pharmaceutical formulations that comprise an annexin-based heteromultimeric protein described herein and a pharmaceutically acceptable diluent or carrier. In certain embodiments are pharmaceutical formulations that comprise an Annexin-A2 based heteromultimeric protein described herein and a pharmaceutically acceptable diluent or carrier. In certain embodiments, a formulation described herein is provided as part of a kit or container. In certain embodiments, the kit or container is packaged with instructions pertaining to extended shelf life of the therapeutic protein. In some embodiments, a heteromultimer described herein is used in a method of treating (e.g., ameliorating) preventing, or diagnosing a disease or disease symptom in an individual, comprising the step of administering said formulation to the individual.

[0033] Provided herein is a method of obtaining fusion protein scaffolds with a known number of conjugation sites based on any transport protein of interest.

[0034] Also provided are transgenic organisms modified to contain nucleic acid molecules described herein to encode and express monomeric fusion proteins described herein.

[0035] Other aspects and features of the present invention will become apparent to those ordinarily skilled in the art upon review of the following description of specific embodiments of the invention in conjunction with the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[0036] In drawings which illustrate embodiments of the invention,

[0037] **Figure 1** depicts the structure of the Human Serum Albumin (HSA) molecule. The alpha helical sections of the secondary structure are shown schematically along with the bonds represented as sticks.

[0038] **Figure 2** is a plot of buried solvent accessible surface area at the interface of two albumin-based polypeptides.

[0039] **Figure 3** depicts two albumin-based polypeptides expressed separately. The two polypeptides are shown in light and dark grey respectively. Each polypeptide comprises two fusion sites for functional cargo proteins and these sites are represented as spheres. The disulphide residues in structure are shown as sticks.

[0040] **Figure 4** is a schematic representation of bispecific and other multifunctional therapeutics based on the multispecific heteromultimer described herein. The albumin-based, or alloalbumin-based polypeptides are denoted A1 and A2. Multifunctional heteromultimers are obtained by conjugating antigen binding motifs, cytokines and other forms of signaling molecules, chemotoxin, radiotoxins or other functionally relevant immunoconjugates to N and/or C terminal sites on A1 and A2 and this is represented by the + symbol.

[0041] **Figure 5** is a schematic of a bispecific antibody derived from a heterodimeric Fc domain. Albumin or alloalbumin based polypeptides are connected to the C-terminal of the Fc to selectively drive the formation of heterodimers.

[0042] **Figures 6A-6C** show native gel electrophoresis profiles of full-length HSA and heterodimer scaffolds Albumin-based heteromultimer -1 (ABH1) and Albumin-based heteromultimer-2 (ABH2) formed by coexpression of HSA based transporter polypeptides.

[0043] **Figure 7** shows stability of wild type HSA and heterodimer scaffolds ABH1 and ABH2 stuided using Differential Scanning Calorimetry

[0044] **Figures 8A-8B** show equilibrium binding isotherms 3000 nM FcRN 3x dilution series over 3000 RUs. Figure 8A shows Albumin and Figure 8B shows heteromultimer scaffold ABH1

[0045] **Figure 9** shows scheme for multivalent Albumin based heteromultimers comprising anti-Her2/neu and anti-CD16 scFv bioactive fusions

[0046] **Figures 10A-10B** contain a non-reducing SDS PAGE analysis of the heteromultimer ABH2 fusions described in table 8. The gel indicates all constructs form the correct complex with expected MW.

[0047] **Figure 11** shows structure of Annexin molecule based on the PDB structure 1MCX. The two monomers that will be derived by splitting the Annexin molecule are color coded as light and dark grey units. The sites of fusion for the cargo protein are represented as spheres.

[0048] **Figure 12** shows a plot of the buried solvent accessible surface area at the interface of Annexin based tranporter polypeptide-1, and Annexin based tranporter polypeptide-2.

[0049] **Figure 13** shows structure of transferrin molecule based on the PDB structure 1H76. The two monomers derived by splitting the transferrin molecule are color coded as light and dark grey units. The sites of fusion for the cargo protein are represented as spheres.

[0050] **Figure 14** shows a plot of the buried solvent accessible surface area at the interface of two transferrin based transporter polypeptides described herein. A split transferrin near residue position 330 as designed herein, forms a heterodimer with about 1800 $Å^2$ of buried surface area.

[0051] Figure 15 shows sequences of multimers comprising transporter polypeptides based on human serum albumin.

DETAILED DESCRIPTION

[0052] In the realm of therapeutic proteins, bispecific molecules exhibit dual target specificities or are able to simultaneously perform multiple functional roles by providing the necessary spatiotemporal organization necessary for drug action. In one aspect, bispecific molecules are particularly interesting when the mode of therapeutic action involves retargeting of effector cells or molecules to a target such as a tumor cell [Muller D. and Kontermann R.E. (2010) *Biodrugs* **24**, 89-98]. The development of bispecific therapeutic proteins with favorable pharmacokinetics and functional activity in stable and homogeneous condition has been a challenge. Attempts have been made to assemble bispecific units from multiple antigen binding domains using a number of approaches. These techniques have involved using heterodimeric antibody IgG molecule, using leucine zipper proteins such as the Fos/Jun pair or other scaffolds assembled from the alternate organizations of the light and heavy chains of the variable domains in an antibody. Kipriyanov and Le Gall

have reviewed the design of a variety of bispecific constructs [Kipriyanov S.M. & Le Gall F. (2004) *Curr Opin Drug Discov Dev* **7**, 233-242]. The use of a heterodimeric antibody IgG molecule wherein mutations are introduced in the CH3 domain of the antibody to achieve the heterodimer and hence introduce the two unique antigen binding sites into one molecule is very attractive because of the natural immunoglobulin like structure of this construct. Further, the Fc portion of the antibody is involved in interactions with the neonatal Fc receptor (FcRn) which mediates an endocytic salvage pathway and this is attributed to improved serum half-life of the antibody molecule [Roopenian D. & Akilesh S. (2007) *Nature Rev Immunol* **7**, 715-725]. On the other hand, antibody based bispecific molecules have been problematic in clinical trials because of the strong cytokine responses as a result of the concurrent effector activity induced via the Fc portion of the bispecific antibody [Weiner L.M.; Alpaugh R.K. et al. (1996) *Cancer Immunol Immunother* **42**, 141-150]. This highlights the needs for novel scaffolds that can aid in the design of bispecific and immunoconjugate molecules.

[0053] The human serum album (HSA) protein is the most abundant component of blood, accounting for close to 60% of the total protein in blood serum at a concentration of about 40 mg/ml. Albumin is also one of the longest-lived proteins in the circulatory system with a half-life of about 19 days. Interestingly, the same endocytic salvage pathway dependent on FcRn molecules that prevents antibody degradation is known to interact with the HSA molecule as well [Chaudhary C.; Mehnaz S. et al. (2003) *J Exp Med* **197**, 315-322].

[0054] HSA (shown in Figure 1) is a non-glycosylated 585-residue single polypeptide protein and the 3-dimensional structure of the protein was first observed using X-ray crystallography by Carter and coworkers [reviewed in Carter, D.C. & Ho, J.X. (1994) *Adv Prot Chem* **45**, 153-203]. The HSA protein consists of three homologous domains: DI, DII, DIII, attributed to gene duplication, a feature common to the serum albumin in other species as well [Gray J.E. & Doolittle R.F. (1992) Protein Sci 1, 289-302]. Each of the three domains have been expressed and characterized separately and shown to be independently stable [Dockal M., Carter D.C. & Ruker F. (1999) *J Biol Chem* **274**, 29303-29310]. Each domain is made up of 10 helical segments and based on the inter-helical organization each domain can be further classified into 2 sub-domains comprised of helix 1-6 and 7-10 respectively. HSA has 17 disulphide bonds in total and all these cysteine pairs forming the linkages are within the individual domains. In general, HSA is a very stable due to the large number of disulphide bonds as well as the predominantly helical fold. The sequence identities of albumin molecules across a number of species is

quite large, greater than 70% among albumin cDNA derived from humans, horse, bovine, rat, etc. [Carter, D.C. & Ho, J.X. (1994) *Adv Prot Chem* **45**, 153-203].

[0055] Split protein pairs have been used as sensors to understand protein-protein interactions in the area of functional proteomics. The approach involves identifying suitable segments from a protein that can reconstitute to form an active native-like protein. Generating new split proteins is technically demanding. For a protein to be split in a functionally useful manner, the segmentation site has to yield two segments that efficiently reconstitute into the quasi-native protein when associated to each other. Further, the component protein segments should be soluble enough to stay in solution and selectively associate with the partner segments such that manufacture yields and purification will be economical. Deriving split protein segments that would recombine to form the quasi-native structure is quite challenging [Tafelmeyer P., Johnsson N. & Johnsson K. *Chem & Biol* **11**, 681-689]. Such split proteins have not been used in the design of protein therapeutics, or as cargo delivery vehicles in the past.

[0056] Definitions

[0057] It is to be understood that this invention is not limited to the particular protocols; cell lines, constructs, and reagents described herein and as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which will be limited only by the appended claims.

[0058] As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly indicates otherwise. Thus, for example, reference to a "HSA", "HA", "albumin", "human serum albumin" and various capitalized, hyphenated and unhyphenated forms is a reference to one or more such proteins and includes variants, derivatives, fragments, equivalents thereof known to those of ordinary skill in the art, and so forth.

[0059] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs. Although any methods, devices, and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods, devices and materials are now described.

[0060] All publications and patents mentioned herein are incorporated herein by reference for the purpose of describing and disclosing, for example, the constructs and methodologies that are described in the publications, which might be used in connection with the presently described invention. The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason.

[0061] A "heteromultimer" or "heteromultimeric polypeptide" is a molecule comprising at least a first monomer comprising a first transporter polypeptide and a second monomer comprising a second transporter polypeptide, wherein the second polypeptide differs in amino acid sequence from the first polypeptide by at least one amino acid residue. The heteromultimer can comprise a "heterodimer" formed by the first and second transporter polypeptides. In certain embodiments, the heteromultimer can form higher order tertiary structures such as, but not restricted to trimers and tetramers. In some embodiments, transporter polypeptides in addition to the first and second transporter polypeptides are present. In certain embodiments, the assembly of transporter polypeptides to form the heteromultimer is driven by surface area burial. In some embodiments, the transporter polypeptides interact with each other by means of electrostatic interactions and/or salt-bridge interactions that drive heteromultimer formation by favoring heteromultimer formation and/or disfavoring homomultimer formation. In some embodiments, the transporter polypeptides inteact with each other by means of hydrophobic interactions that drive heteromultimer formation by favoring heteromultimer formation and/or disfavoring homomultimer formation. In certain embodiments, the transporter polypeptides inteact with each other by means of covalent bond formation. In certain embodiments, the covalent bonds are formed between naturally present or introduced cysteines that drive heteromultimer formation. In certain embodiments of the heteromultimers described herein, no covalent bonds are formed between the monomers. In some embodiments, the transporter polypeptides inteact with each other by means of packing/size-complementarity/knobs-into-holes/protruberance-cavity type interactions that drive heteromultimer formation by favoring heteromultimer formation and/or disfavoring homomultimer formation. In some embodiments, the transporter polypeptides inteact with each other by means of cation-pi interactions that drive heteromultimer formation by favoring heteromultimer formation and/or disfavoring homomultimer formation. In certain embodiments the individual transporter polypeptides cannot exist as

isolated monomers in solution. In certain embodiments, the heteromultimer is the preferred state of the individual transporter polypeptides as compared to the monomer.

[0062] The term "bispecific" is intended to include any agent, e.g., heteromultimer, monomer, protein, peptide, or protein or peptide complex, which has two different binding specificities. For example, in some embodiments, the molecule may bind to, or interact with, (a) a cell surface target molecule and (b) an Fc receptor on the surface of an effector cell. In certain embodiments of a heteromultimer described herein, at least one monomer is bispecific formed by attaching to the same transporter polypeptide, two cargo molecules with different binding specificities. In certain embodiments of a heteromultimer described herein, the heteromultimer is itself bispecific formed by attaching to the transporter polypeptides, at least two cargo molecules with different specificities. The term "multispecific molecule" or "heterospecific molecule" is intended to include any agent, e.g., a protein, peptide, or protein or peptide complex, which has more than two different binding specificities. For example, the molecule may bind to, or interact with, (a) a cell surface target molecule such as but not limited to cell surface antigens, (b) an Fc receptor on the surface of an effector cell, and (c) at least one other component. Accordingly, embodiments of the heteromultimers described herein, are inclusive of, but not limited to, bispecific, trispecific, tetraspecific, and other multispecific molecules. In certain embodiments, these molecules are directed to cell surface antigens, such as CD30, and to other targets, such as Fc receptors on effector cells.

[0063] Unless indicated otherwise, the expression "multivalent" is used throughout this specification to denote a heteromultimer comprising at least two sites of attachment for target molecules. The multivalent heteromultimer is designed to have multiple binding sites for desired targets. In certain embodiments, the binding sites are on at least one cargo molecules attached to a transporter polypeptide. In certain embodiments, at least one binding site is on a transporter polypeptide. The expression “bivalent” is used throughout this specification to denote a heteromultimer comprising two target binding sites. In certain embodiments of a bivalent heteromultimer, both binding sites are on the same monomer. The expression “trivalent” is used throughout this specification to denote a heteromultimer comprising three target binding sites. The expression “tetravalent” is used throughout this specification to denote a heteromultimer comprising four target binding sites.

[0064] “Fusion proteins” and polypeptides are created by joining two or more genes that originally code for separate polypeptides. Translation of this fusion gene results in a

single polypeptide with functional properties derived from each of the original polypeptides. In embodiments of the heteromultimers described herein, at least one monomer may comprise a fusion protein formed by the fusion of at least one cargo polypeptide to the N- or C-terminus of a transporter polypeptide.

[0065] The term "substantially purified" refers to a heteromultimer described herein, or variant thereof that may be substantially or essentially free of components that normally accompany or interact with the protein as found in its naturally occurring environment, i.e. a native cell, or host cell in the case of recombinantly produced heteromultimer that in certain embodiments, is substantially free of cellular material includes preparations of protein having less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1% (by dry weight) of contaminating protein. When the heteromultimer or variant thereof is recombinantly produced by the host cells, the protein in certain embodiments is present at about 30%, about 25%, about 20%, about 15%, about 10%, about 5%, about 4%, about 3%, about 2%, or about 1% or less of the dry weight of the cells. When the heteromultimer or variant thereof is recombinantly produced by the host cells, the protein, in certain embodiments, is present in the culture medium at about 5 g/L, about 4 g/L, about 3 g/L, about 2 g/L, about 1 g/L, about 750 mg/L, about 500 mg/L, about 250 mg/L, about 100 mg/L, about 50 mg/L, about 10 mg/L, or about 1 mg/L or less of the dry weight of the cells. In certain embodiments, "substantially purified" heteromultimer produced by the methods described herein, has a purity level of at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, specifically, a purity level of at least about 75%, 80%, 85%, and more specifically, a purity level of at least about 90%, a purity level of at least about 95%, a purity level of at least about 99% or greater as determined by appropriate methods such as SDS/PAGE analysis, RP-HPLC, SEC, and capillary electrophoresis.

[0066] A "recombinant host cell" or "host cell" refers to a cell that includes an exogenous polynucleotide, regardless of the method used for insertion, for example, direct uptake, transduction, f-mating, or other methods known in the art to create recombinant host cells. The exogenous polynucleotide may be maintained as a nonintegrated vector, for example, a plasmid, or alternatively, may be integrated into the host genome.

[0067] As used herein, the term "medium" or "media" includes any culture medium, solution, solid, semi-solid, or rigid support that may support or contain any host cell, including

bacterial host cells, yeast host cells, insect host cells, plant host cells, eukaryotic host cells, mammalian host cells, CHO cells, prokaryotic host cells, E. coli, or Pseudomonas host cells, and cell contents. Thus, the term may encompass medium in which the host cell has been grown, e.g., medium into which the protein has been secreted, including medium either before or after a proliferation step. The term also may encompass buffers or reagents that contain host cell lysates, such as in the case where a heteromultimer described herein is produced intracellularly and the host cells are lysed or disrupted to release the heteromultimer.

[0068] "Refolding," as used herein describes any process, reaction or method which transforms disulfide bond containing polypeptides from an improperly folded or unfolded state to a native or properly folded conformation with respect to disulfide bonds.

[0069] "Cofolding," as used herein, refers specifically to refolding processes, reactions, or methods which employ at least two monomeric polypeptides which interact with each other and result in the transformation of unfolded or improperly folded polypeptides to native, properly folded polypeptides.

[0070] As used herein, the term "modulated serum half-life" means the positive or negative change in circulating half-life of a cargo polypeptide that is comprised by a heteromultimer described herein relative to its native form. Serum half-life is measured by taking blood samples at various time points after administration of heteromultimer, and determining the concentration of that molecule in each sample. Correlation of the serum concentration with time allows calculation of the serum half-life. Increased serum half-life desirably has at least about two-fold, but a smaller increase may be useful, for example where it enables a satisfactory dosing regimen or avoids a toxic effect. In some embodiments, the increase is at least about three-fold, at least about five-fold, or at least about ten-fold.

[0071] The term "modulated therapeutic half-life" as used herein means the positive or negative change in the half-life of the therapeutically effective amount of a cargo polypeptide comprised by a heteromultimer described herein, relative to its non-modified form. Therapeutic half-life is measured by measuring pharmacokinetic and/or pharmacodynamic properties of the molecule at various time points after administration. Increased therapeutic half-life desirably enables a particular beneficial dosing regimen, a particular beneficial total dose, or avoids an undesired effect. In some embodiments, the increased therapeutic half-life results from increased potency, increased or decreased binding of the modified molecule to its target, increased or decreased breakdown of the

molecule by enzymes such as proteases, or an increase or decrease in another parameter or mechanism of action of the non-modified molecule or an increase or decrease in receptor-mediated clearance of the molecule.

[0072] The term "isolated," when applied to a nucleic acid or protein, denotes that the nucleic acid or protein is free of at least some of the cellular components with which it is associated in the natural state, or that the nucleic acid or protein has been concentrated to a level greater than the concentration of its in vivo or in vitro production. It can be in a homogeneous state. Isolated substances can be in either a dry or semi-dry state, or in solution, including but not limited to, an aqueous solution. It can be a component of a pharmaceutical composition that comprises additional pharmaceutically acceptable carriers and/or excipients. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein which is the predominant species present in a preparation is substantially purified. In particular, an isolated gene is separated from open reading frames which flank the gene and encode a protein other than the gene of interest. The term "purified" denotes that a nucleic acid or protein gives rise to substantially one band in an electrophoretic gel. Particularly, it may mean that the nucleic acid or protein is at least 85% pure, at least 90% pure, at least 95% pure, at least 99% or greater pure.

[0073] The term "nucleic acid" refers to deoxyribonucleotides, deoxyribonucleosides, ribonucleosides, or ribonucleotides and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides which have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless specifically limited otherwise, the term also refers to oligonucleotide analogs including PNA (peptidonucleic acid), analogs of DNA used in antisense technology (phosphorothioates, phosphoroamidates, and the like). Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (including but not limited to, degenerate codon substitutions) and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka

et al., J. Biol. Chem. 260:2605-2608 (1985); Rossolini et al., Mol. Cell. Probes 8:91-98 (1994)).

[0074] The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. That is, a description directed to a polypeptide applies equally to a description of a peptide and a description of a protein, and vice versa. The terms apply to naturally occurring amino acid polymers as well as amino acid polymers in which one or more amino acid residues is a non-naturally encoded amino acid. As used herein, the terms encompass amino acid chains of any length, including full length proteins, wherein the amino acid residues are linked by covalent peptide bonds.

[0075] The term "amino acid" refers to naturally occurring and non-naturally occurring amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally encoded amino acids are the 20 common amino acids (alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, praline, serine, threonine, tryptophan, tyrosine, and valine) and pyrrolysine and selenocysteine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an a carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, such as, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (such as, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Reference to an amino acid includes, for example, naturally occurring proteogenic L-amino acids; D-amino acids, chemically modified amino acids such as amino acid variants and derivatives; naturally occurring non-proteogenic amino acids such as β-alanine, ornithine, etc.; and chemically synthesized compounds having properties known in the art to be characteristic of amino acids. Examples of non-naturally occurring amino acids include, but are not limited to, α-methyl amino acids (e.g. α-methyl alanine), D-amino acids, histidine-like amino acids (e.g., 2-amino-histidine, β-hydroxy-histidine, homohistidine), amino acids having an extra methylene in the side chain ("homo" amino acids), and amino acids in which a carboxylic acid functional group in the side chain is replaced with a sulfonic acid group (e.g., cysteic acid). The incorporation of non-natural amino acids, including synthetic non-native amino acids, substituted amino acids, or one or more D-amino acids into the proteins of the present invention may be advantageous in a number of different ways. D-amino acid-containing peptides, etc., exhibit increased stability in vitro or in

vivo compared to L-amino acid-containing counterparts. Thus, the construction of peptides, etc., incorporating D-amino acids can be particularly useful when greater intracellular stability is desired or required. More specifically, D-peptides, etc., are resistant to endogenous peptidases and proteases, thereby providing improved bioavailability of the molecule, and prolonged lifetimes in vivo when such properties are desirable. Additionally, D-peptides, etc., cannot be processed efficiently for major histocompatibility complex class II-restricted presentation to T helper cells, and are therefore, less likely to induce humoral immune responses in the whole organism.

[0076] Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0077] "Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, "conservatively modified variants" refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of ordinary skill in the art will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence.

[0078] As to amino acid sequences, one of ordinary skill in the art will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the deletion of an amino acid, addition of an amino acid, or substitution of an

amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are known to those of ordinary skill in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

[0079] Conservative substitution tables providing functionally similar amino acids are known to those of ordinary skill in the art. The following eight groups each contain amino acids that are conservative substitutions for one another:

[0080] 1) Alanine (A), Glycine (G);

[0081] 2) Aspartic acid (D), Glutamic acid (E);

[0082] 3) Asparagine (N), Glutamine (Q);

[0083] 4) Arginine (R), Lysine (K);

[0084] 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);

[0085] 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W);

[0086] 7) Serine (S), Threonine (T); and [0139] 8) Cysteine (C), Methionine (M) (see, e.g., Creighton, Proteins: Structures and Molecular Properties (W H Freeman & Co.; 2nd edition (December 1993)

[0087] The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same. Sequences are "substantially identical" if they have a percentage of amino acid residues or nucleotides that are the same (i.e., about 60% identity, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95% identity over a specified region), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms (or other algorithms available to persons of ordinary skill in the art) or by manual alignment and visual inspection. This definition also refers to the complement of a test sequence. The identity can exist over a region that is at least about 50 amino acids or nucleotides in length, or over a region that is 75-100 amino acids or nucleotides in length, or, where not specified, across the entire sequence of a polynucleotide or polypeptide. A polynucleotide encoding a polypeptide of the present invention, including homologs from species other than human, may be obtained by a process comprising the steps of screening a library under stringent hybridization conditions with a labeled probe having a polynucleotide sequence of the invention or a fragment thereof, and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Such hybridization techniques are well known to the skilled artisan.

[0088] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

[0089] A "comparison window", as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are known to those of ordinary skill in the art. Optimal alignment of sequences for comparison can be conducted, including but not limited to, by the local homology algorithm of Smith and Waterman (1970) Adv. Appl. Math. 2:482c, by the homology alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443, by the search for similarity method of Pearson and Lipman (1988) Proc. Nat'l. Acad. Sci. USA 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by manual alignment and visual inspection (see, e.g., Ausubel et al., Current Protocols in Molecular Biology (1995 supplement)).

[0090] One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al. (1997) Nuc. Acids Res. 25:3389-3402, and Altschul et al. (1990) J. Mol. Biol. 215:403-410, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information available at the World Wide Web at ncbi.nlm.nih.gov. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) or 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1992) Proc. Natl. Acad. Sci. USA 89:10915) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a

comparison of both strands. The BLAST algorithm is typically performed with the "low complexity" filter turned off.

[0091] The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-5787). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, or less than about 0.01, or less than about 0.001.

[0092] The phrase "selectively (or specifically) hybridizes to" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent hybridization conditions when that sequence is present in a complex mixture (including but not limited to, total cellular or library DNA or RNA).

[0093] The phrase "stringent hybridization conditions" refers to hybridization of sequences of DNA, RNA, or other nucleic acids, or combinations thereof under conditions of low ionic strength and high temperature as is known in the art. Typically, under stringent conditions a probe will hybridize to its target subsequence in a complex mixture of nucleic acid (including but not limited to, total cellular or library DNA or RNA) but does not hybridize to other sequences in the complex mixture. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Probes, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993).

[0094] As used herein, the term "eukaryote" refers to organisms belonging to the phylogenetic domain Eucarya such as animals (including but not limited to, mammals, insects, reptiles, birds, etc.), ciliates, plants (including but not limited to, monocots, dicots, algae, etc.), fungi, yeasts, flagellates, microsporidia, protists, etc.

[0095] As used herein, the term "prokaryote" refers to prokaryotic organisms. For example, a non-eukaryotic organism can belong to the Eubacteria (including but not limited to, Escherichia coli, Thermus thermophilus, Bacillus stearothermophilus, Pseudomonas fluorescens, Pseudomonas aeruginosa, Pseudomonas putida, etc.) phylogenetic domain, or the Archaea (including but not limited to, Methanococcus jannaschii,

Methanobacterium thermoautotrophicum, Halobacterium such as Haloferax volcanii and Halobacterium species NRC-1, Archaeoglobus fulgidus, Pyrococcus furiosus, Pyrococcus horikoshii, Aeuropyrum pernix, etc.) phylogenetic domain.

[0096] The term "subject" as used herein, refers to an animal, in some embodiments a mammal, and in other embodiments a human, who is the object of treatment, observation or experiment. An animal may be a companion animal (e.g., dogs, cats, and the like), farm animal (e.g., cows, sheep, pigs, horses, and the like) or a laboratory animal (e.g., rats, mice, guinea pigs, and the like).

[0097] The term "effective amount" as used herein refers to that amount of heteromultimer being administered, which will relieve to some extent one or more of the symptoms of the disease, condition or disorder being treated. Compositions containing the heteromultimer described herein can be administered for prophylactic, enhancing, and/or therapeutic treatments.

[0098] The terms "enhance" or "enhancing" means to increase or prolong either in potency or duration a desired effect. Thus, in regard to enhancing the effect of therapeutic agents, the term "enhancing" refers to the ability to increase or prolong, either in potency or duration, the effect of other therapeutic agents on a system. An "enhancing-effective amount," as used herein, refers to an amount adequate to enhance the effect of another therapeutic agent in a desired system. When used in a patient, amounts effective for this use will depend on the severity and course of the disease, disorder or condition, previous therapy, the patient's health status and response to the drugs, and the judgment of the treating physician.

[0099] The term "modified," as used herein refers to any changes made to a given polypeptide, such as changes to the length of the polypeptide, the amino acid sequence, chemical structure, co-translational modification, or post-translational modification of a polypeptide. The form "(modified)" term means that the polypeptides being discussed are optionally modified, that is, the polypeptides under discussion can be modified or unmodified.

[00100] The term "post-translationally modified" refers to any modification of a natural or non-natural amino acid that occurs to such an amino acid after it has been incorporated into a polypeptide chain. The term encompasses, by way of example only, co-translational in vivo modifications, co-translational in vitro modifications (such as in a cell-free translation system), post-translational in vivo modifications, and post-translational in vitro modifications.

[00101] The term "segmentation" refers to a precise internal splice of the original protein sequence which results in "segments" of the protein sequence that preferentially associate as heteromultimers to form a quasi-protein.

[00102] Quasi-native Structure:

[00103] With reference to a native protein or its structure, quasi-native proteins and/or 'quasi-native structures' present the native protein like functional and structural characteristics. Proteins are naturally dynamics molecules and display an ensemble of structural configurations although we ascribe a native structure to it, such as the one obtained by X-ray crystallography. The alternate structural configurations observed in the ensemble of geometries of that protein can be deemed to be quasi-native structures relative to each other or relative to the structure observed in the crystal. On a different front, homologous proteins sequences or proteins belonging to common structural families tend to fold into similar structural geometries. The member proteins belonging to this family can be deemed to achieve a quasi-native structure relative to each other. Some of the unique sequences in the protein family could also exhibit similar functional attributes and hence can be referred to as quasi-native proteins relative to each other. In the case of heteromultimers described here comprising of two or more monomeric proteins each of which have a transporter polypeptide component, the transporter polypeptides assemble to form a quasi-native structure. The reference native protein in this case is the protein from which the transporter polypeptide is derived and the reference native structure is the structure of the protein from which the transporter polypeptide is derived. We describe a case where two or more different polypeptides self-assemble to form a heteromultimeric structural and exhibit functional characteristics like a native protein which itself is a monomeric entity. In certain embodiments, we present polypeptide segments derived from albumin that self-assemble to form a heteromultimer that exhibits native albumin like functional characteristics such as FcRn binding and structural characteristics. In certain embodiments, we present polypeptide segments derived from transferrin that self-assemble to form a heteromultimer that exhibits native transferrin like structural and functional characteristics. In certain embodiments, we present polypeptide segments derived from annexin that self-assemble to form a heteromultimer that exhibits native annexin like structural and functional characteristics. These heteromultimers are referred to as being quasi-native.

[00104] Transporter polypeptide

[00105] As used herein, the term “transporter polypeptide” or “transporter polypeptide” or “transporter peptide” or “transporter” refers to a polypeptide, such that said transporter polypeptide is capable of forming heteromultimeric proteins with other such transporter polypeptides in solution, and wherein said heteromultimeric proteins have a quasi-native structure of a monomeric protein from which at least one transporter polypeptide is derived. In certain embodiments of the heteromultimers described herein, all transporter polypeptides are derived from the same albumin or alloalbumin protein. In certain other embodiments, the heteromultimers are formed by transporter polypeptides derived from various albumin and alloalbumin proteins. In certain embodiments of the heteromultimers described herein, the transporter polypeptides are derived from transferrin. In certain embodiments of the heteromultimers described herein, all transporter polypeptides are derived from annexin proteins. In certain embodiments, the heteromultimers are formed by transporter polypeptides derived from the same annexin protein. In some embodiments, the heteromultimers are formed by transporter polypeptides derived from different annexin proteins. In an embodiment, the heteromultimers are formed by transporter polypeptides derived from annexin A2.

[00106] In certain embodiments, transporter polypeptides are segments of a whole protein, wherein said segments are capable of assembling to form a heteromultimer. In certain embodiments, the transporter polypeptides are segments derived from a coiled coil protein. In certain embodiments, the transporter polypeptides are segments derived from a leucine-zipper protein. In an embodiment, the transporter polypeptides are segments from a beta-barrel protein. In an embodiment, transporter polypeptides are segments obtained from a beta-propeller protein. In some embodiments, the transporter polypeptides are segments obtained from a helical bundle protein. In embodiments, the trasporter polypeptides are generated from for instance, but not restricted to proteins comprising a zinc finger motif, a helix-turn-helix motif or a beta-hairpin motif. In some embodiments, the transporter polypeptides are segments obtained from non-immunogenic proteins that are structurally stable, and have favorable biological properties.

[00107] Albumin

[00108] As used herein, "albumin" refers collectively to albumin protein or amino acid sequence, or an albumin segment or variant, having one or more functional activities (e.g., biological activities) of albumin. In particular "albumin" refers to human albumin or segments thereof (see for example, EP 201 239, EP 322 094 WO 97/24445, WO95/23857) especially the mature form of human albumin as shown in FIG. 1, or

albumin from other vertebrates, or segments thereof, or analogs or variants of these molecules or fragments thereof. Inc ertain embodiments, albumin refers to a truncated version of albumin.

[00109] The term "quasi-albumin" refers to a heteromultimer molecule that has structure and/or function similar to the whole albumin, and wherein said heteromultimer molecule is formed by the assembly of two or more monomeric polypeptides designed based on the sequence of the whole albumin. In certain embodiments, the monomeric polypeptides are "segments" that preferentially associate as heteromultimeric pairs to form a quasi-protein. In some embodiments, the quasi-albumin has 90% of the activity of the whole albumin. In some embodiments, the quasi-albumin has 75% of the activity of whole-albumin. In an embodiment, the quasi-albumin has 50% of the activity of whole albumin. In some embodiments, the quasi-albumin has 50-75% of the activity of whole albumin. In an embodiment, quasi-albumin has 80% of the activity of whole albumin. In some embodiments, the quasi-albumin has 90% of the structure of whole albumin as determined by molecular modeling. In some embodiments, the quasi-albumin has 80% of the structure of whole albumin as determined by molecular modeling. In some embodiments, the quasi-albumin has 70% of the structure of whole albumin as determined by molecular modeling. In some embodiments, the quasi-albumin has 50% of the structure of whole albumin as determined by molecular modeling. In some embodiments, the quasi-albumin has 50%-75% of the structure of whole albumin as determined by molecular modeling.

[00110] The terms, human serum albumin (HSA) and human albumin (HA) are used interchangeably herein. The terms, "albumin and serum albumin" are broader, and encompass human serum albumin (and fragments and variants thereof) as well as albumin from other species (and fragments and variants thereof).

[00111] In certain embodiments, each albumin-based monomer of the heteromultimeric proteins described herein is based on a variant of normal HA. Each cargo polypeptide portion of the heteromultimeric proteins of the invention may also be variants of the Therapeutic proteins as described herein. The term "variants" includes insertions, deletions and substitutions, either conservative or non conservative, where such changes do not substantially alter one or more of the oncotic, useful ligand-binding and non-immunogenic properties of albumin, or the active site, or active domain which confers the therapeutic activities of the Therapeutic proteins.

[00112] In certain embodiments, the heteromultimeric proteins described herein include naturally occurring polymorphic variants of human albumin and fragments of human albumin, for example those fragments disclosed in EP 322 094 (namely HA (Pn), where n is 369 to 419).

[00113] In certain embodiments, the albumin is derived from any vertebrate, especially any mammal that includes but is not limited to human, cow, sheep, rat, mouse, rabbit, horse, dog or pig. In certain embodiments, the albumin is derived from non-mammalian albumins including, but are not limited to hen and salmon.

[00114] The sequence of human albumin is as shown:

[00115] SEQ ID NO: 1

[00116] MKWVTFISLLFLFSSAYSRGVFRRDAHKSEVAHRFKDLGEENFKALVLIAF
AQYLQQCPFEDHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLR
ETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFL
KKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEG
KASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHT
ECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEM
PADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKT
YETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNAL
LVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLC
VLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLS
EKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAE
EGKKLVAASQAALGL

[00117] Alloalbumin

[00118] An alloalbumin is a genetic variant of albumin. In certain embodiments the alloalbumin is human alloalbumin (HAA). Alloalbumins that differ in electrophoretic mobility from albumin have been identified through population genetics surveys in the course of clinical electrophoresis, or in blood donor surveys. As markers of mutation and migration, alloalbumins are of interest to geneticists, biochemists, and anthropologists, but most of these alloalbumin are not associated with disease (Minchioti et al. Human Mutations 29(8), 1007-1016(2008)).

[00119] **Table 1:** List of substitutions comprised by various alloalbumins as compared to HA of SEQ ID NO: 1. Thermostability, half-life information and other HAAs are provided in Krogh-hansen et al. Biochim Biophys Acta 1747, 81-88(2005); and WO2011051489 incorporated by reference herein.

Mutation	Thermostability (C) (positive=stabilizing, negative=destabilizing)	Effect on half-life (% change)
H3Y	N/A	N/A
H3Q	N/A	N/A
Q32Stop	N/A	N/A
E60K	N/A	N/A
D63N	6.07	N/A
L66P	N/A	N/A
E82K	2.03	N/A
R114G	N/A	N/A
R114Stop	N/A	N/A
E119K	N/A	N/A
V122E	0.57	N/A
H128R	N/A	N/A
Y140C	N/A	N/A
A175Stop	N/A	N/A
C177F	-1.59	N/A
R218H	N/A	N/A
R218P	N/A	N/A
K225Q*	-4.86	N/A
K240E	N/A	N/A
E244Stop	N/A	N/A
Q268R	N/A	N/A
D269G	3.67	N/A
K276N	4.87	N/A
K313N	-7.16	N/A

D314G	-0.38	N/A
D314V	N/A	N/A
N318K	N/A	N/A
A320T, & -1R	N/A	6.16
E321K	1.42	N/A
E333K	-2.56	N/A
E354K	N/A	N/A
E358K	N/A	N/A
K359K	-6.56	N/A
D365H	0.89	N/A
D365V	N/A	N/A
E368G	N/A	N/A
K372E	N/A	N/A
D375N	N/A	N/A
D375H	-0.09	N/A
E376K	N/A	N/A
E376Q	N/A	N/A
E382K	N/A	N/A
Q385Stop	N/A	N/A
Y401Stop	N/A	N/A
R410C	N/A	N/A
E479K	N/A	N/A
D494N	N/A	0.84
E501K	0.13	N/A
E505K	1.87	N/A
I513N	N/A	N/A
V533M	N/A	N/A

K536E	N/A	N/A
K541E	6.12	N/A
D550G	N/A	N/A
D550A	N/A	N/A
K560E	0.70	N/A
D563N	4.17	N/A
E565K	N/A	N/A
E570K	-6.53	N/A
K573E	2.08	2.7
K574N	N/A	N/A
L575insertion(TCCCKSSCLR LITSHLKASQPTMRIRERK)	-5.30	N/A
Frameshift after 567; Stop at 582	N/A	-5.7 %
Frameshift after 572; Stop at 578	N/A	-8.9 %

[00120] Annexin:

[00121] As used herein, "annexin" refers to a group of cellular proteins found in eukaryotic organisms. Annexin is also known as lipocortin. As used herein “annexin” may refer to any annexin protein, or to specific annexin proteins such as “annexin A1,” “annexin A2,” and “annexin A5.” Annexins are characterized by their calcium dependent ability to bind negatively charged phospholipids (i.e. membrane walls). Annexins are characterized by a repeat protein scaffold limited to 30-50 kDa in size with fairly ubiquitous tissue distribution. The basic structure of an annexin is composed of two domains: a structurally conserved C terminal “core” region and a divergent N terminal domain. The core region binds the phospholipid cellular membrane in a Ca^{2+} dependent manner. The N terminal region binds cytoplasmic proteins. Annexins are important in various cellular and physiological processes and provide a membrane scaffold. The C terminal core is composed of four annexin repeats. Annexin is

characterized by its flexible repeat-like nature that influences its intrinsic membrane-sensing abilities. For instance, the affinity towards specific biomembranes can be controlled by the number of repeats. With the characteristic phospholipid sensing, annexin can be useful to sense/target intestinal junctions for drug delivery. Another potential application for an annexin is targeting intestinal tight junctions and the Zonula Occludens region (ZO-1), which is known to be particularly difficult to traverse for larger protein therapeutics, significantly impairing drug absorption.

[00122] The term "quasi-annexin" refers to a heteromultimer molecule that has structure and/or function similar to the whole annexin, and wherein said heteromultimer molecule is formed by the assembly of two or more monomeric polypeptides designed based on the sequence of the whole annexin. In certain embodiments, the monomeric polypeptides are "segments" that preferentially associate as heteromultimeric pairs to form a quasi-protein. In some embodiments, the quasi- annexin has 90% of the activity of the whole annexin. In some embodiments, the quasi- annexin has 75% of the activity of whole- annexin. In an embodiment, the quasi- annexin has 50% of the activity of whole annexin. In some embodiments, the quasi- annexin has 50-75% of the activity of whole annexin. In an embodiment, quasi- annexin has 80% of the activity of whole annexin. In some embodiments, the quasi- annexin has 90% of the structure of whole annexin as determined by molecular modeling. In some embodiments, the quasi- annexin has 80% of the structure of whole annexin as determined by molecular modeling. In some embodiments, the quasi- annexin has 70% of the structure of whole annexin as determined by molecular modeling. In some embodiments, the quasi- annexin has 50% of the structure of whole annexin as determined by molecular modeling. In some embodiments, the quasi- annexin has 50%-75% of the structure of whole annexin as determined by molecular modeling.

[00123] The sequence of Human wild-type Annexin A2 is as shown:

[00124] SEQ ID NO: 14

[00125] GSAVSPYPTFNPSSDVAALHKAIMVKGVDEATIIDILTKRNNAQRQQIKAAYLQE TGKPLDETLKKALTGHLEEVVLALLKTPAQFDADELRAAMKGLGTDEDTLIEILA SRTNKEIRDINRVYREELKRDLAKDITSDTSGDFRNALLSLAKGDRSEDFGVNED LADSDARALYEAGERRKGTDVNVFNTILTTRSYPQLRRVFQKYTKYSKHDMNK VLDLELKGDIEKCLTAIVKCATSKPAFFAEKLHQAMKGVGTRHKALIRIMVSRSEI DMNDIKAFYQKMYGISLCQAILDETKGDYEKILVALCGGN

[00126] Transferrin:

[00127] Transferrins are monomeric proteins of about 76 kDa molecular weight present in all vertebrates and function as a iron-binding and transporting protein. Recombinant human transferrin and its fusions is being considered for the management of various diseases including thalassemia, atransferrinemia, age related macular degeneration, type 2 diabetes, during stem cell transplantation and in the treatment of acute infectious disease caused by the anthrax bacteria. Transferrin is stable in the gastrointestinal environment and a number of studies have shown that intact protein-transferrin conjugates can be orally delivered and remain bioactive.

[00128] The term "quasi-transferrin" refers to a heteromultimer molecule that has structure and/or function similar to the whole transferrin, and wherein said heteromultimer molecule is formed by the assembly of two or more monomeric polypeptides designed based on the sequence of the whole transferrin. In certain embodiments, the monomeric polypeptides are "segments" that preferentially associate as heteromultimeric pairs to form a quasi-protein. In some embodiments, the quasi- transferrin has 90% of the activity of the whole transferrin. In some embodiments, the quasi- transferrin has 75% of the activity of whole- transferrin. In an embodiment, the quasi- transferrin has 50% of the activity of whole transferrin. In some embodiments, the quasi- transferrin has 50-75% of the activity of whole transferrin. In an embodiment, quasi- transferrin has 80% of the activity of whole transferrin. In some embodiments, the quasi- transferrin has 90% of the structure of whole transferrin as determined by molecular modeling. In some embodiments, the quasi- transferrin has 80% of the structure of whole transferrin as determined by molecular modeling. In some embodiments, the quasi- transferrin has 70% of the structure of whole transferrin as determined by molecular modeling. In some embodiments, the quasi- transferrin has 50% of the structure of whole transferrin as determined by molecular modeling. In some embodiments, the quasi- transferrin has 50%-75% of the structure of whole transferrin as determined by molecular modeling.

[00129] The sequence of wildtype Human Transferrin is as shown:

[00130] SEQ ID NO: 19

[00131] MRLAVGALLV CAVLGLCLAV PDKTVRWCAV SEHEATKCQS FRDHMKSVIP SDGPSVACVK KASYLDCIRA IAANEADAVT LDAGLVYDAY LAPNNLKPVV AEFYGSKEDP QTFYYAVAVV KKDSGFQMNQ LRGKKSCHTG LGRSAGWNIP IGLLYCDLPE PRKPLEKAVA NFFSGSCAPC ADGTDFPQLC QLCPGCGCST LNQYFGYSGA FKCLKDGAGD VAFVKHSTIF ENLANKADRD QYELLCLDNT RKPVDEYKDC HLAQVPSHTV VARSMGGKED LIWELLNQAQ EHFGKDKSKE

FQLFSSPHGK DLLFKDSAHG FLKVPPRMDA KMYLGYEYVT AIRNLREGTC PEAPTDECKP VKWCALSHHE RLKCDEWSVN SVGKIECVSA ETTEDCIAKI MNGEADAMSL DGGFVYIAGK CGLVPVLAEN YNKSDNCEDT PEAGYFAVAV VKKSASDLTW DNLKGKKSCH TAVGRTAGWN IPMGLLYNKI NHCRFDEFFS EGCAPGSKKD SSLCKLCMGS GLNLCEPNNK EGYYGYTGAF RCLVEKGDVA FVKHQTVPQN TGGKNPDPWA KNLNEKDYEL LCLDGTRKPV EEYANCHLAR APNHAVVTRK DKEACVHKIL RQQQHLFGSN VTDCSGNFCL FRSETKDLLF RDDTVCLAKL HDRNTYEKYL GEEYVKAVGN LRKCSTSSLL EACTFRRP

[00132] Cargo molecule:

[00133] A heteromultimer described herein comprises monomers that comprise at least one cargo molecule, and at least one transporter polypeptide, said cargo molecule and transporter polypeptide associated with one another, by means inclusive of, but not restricted to genetic fusion or chemical conjugation. In certain embodiments, at least one cargo molecule is a therapeutic agent. In certain agents, the cargo molecule is a toxin. In certain embodiments, the cargo molecule is an antigen, or analogs thereof. In an embodiment, the cargo molecule is a natural product, analog, or prodrug thereof. In certain embodiments, the cargo molecule is a therapeutic agent such as a cytotoxin, e.g., a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, 213Bi. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6mercaptopurine, 6thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

[00134] In certain embodiment, the cargo molecule is a biomolecule. In an embodiment, the cargo molecule is a natural or synthetic nucleic acid. In some embodiments, at least

one cargo molecule is one or more of a DNA, PNA, and/or RNA oligomer. In certain embodiments, a heteromultimer described herein comprises monomeric proteins that comprise at least one cargo polypeptide, or fragments or variants thereof, and at least one transporter polypeptide, said cargo polypeptide and transporter polypeptide associated with one another, by means inclusive of, but not restricted to genetic fusion or chemical conjugation

[00135] As used herein, "Cargo polypeptide" refers to proteins, polypeptides, antibodies, peptides or fragments or variants thereof, having one or more therapeutic and/or biological activities. Cargo polypeptides encompassed by the invention include but are not limited to, proteins, polypeptides, peptides, antibodies, substrates or ligands to therapeutically relevant target proteins and biologics. (The terms peptides, proteins, and polypeptides are used interchangeably herein.) Specifically the term "Cargo polypeptide" encompasses antibodies and fragments and variants thereof. Thus a heteromultimer described herein may contain at least a fragment or variant of a cargo polypeptide, and/or at least a fragment or variant of an antibody. Additionally, in certain embodiments, the term "Cargo polypeptide" refers to the endogenous or naturally occurring correlate of a cargo polypeptide.

[00136] As a non-limiting example, a “Cargo biomolecule” is a biomolecule such as but not restricted to a protein, DNA, or RNA that is useful to treat, prevent or ameliorate a disease, condition or disorder. As a non-limiting example, a "Cargo polypeptide" may be one that binds specifically to a particular cell type (normal (e.g., lymphocytes) or abnormal e.g., (cancer cells)) and therefore may be used to target a compound (drug, or cytotoxic agent) to that cell type specifically.

[00137] In another non-limiting example, a "Cargo molecule" is a molecule that has a biological, activity, and in particular, a biological activity that is useful for treating preventing or ameliorating a disease. A non-inclusive list of biological activities that may be possessed by a Cargo molecule, for instance a Cargo polypeptide includes, enhancing the immune response, promoting angiogenesis, inhibiting angiogenesis, regulating hematopoietic functions, stimulating nerve growth, enhancing an immune response, inhibiting an immune response, or any one or more of the biological activities described herein.

[00138] Cargo polypeptides corresponding to a cargo polypeptide portion of a heteromultimer protein described herein, such as cell surface and secretory proteins, are often modified, by the attachment of one or more oligosaccharide groups. The modification, referred to as glycosylation, can dramatically affect the physical properties of proteins and can be

important in protein stability, secretion, and localization. Glycosylation occurs at specific locations along the polypeptide backbone. There are usually two major types of glycosylation: glycosylation characterized by O-linked oligosaccharides, which are attached to serine or threonine residues; and glycosylation characterized by N-linked oligosaccharides, which are attached to asparagine residues in an Asn-X-Ser/Thr sequence, where X can be any amino acid except proline. N-acetylneuramic acid (also known as sialic acid) is usually the terminal residue of both N-linked and Blinked oligosaccharides. Variables such as protein structure and cell type influence the number and nature of the carbohydrate units within the chains at different glycosylation sites. Glycosylation isomers are also common at the same site within a given cell type.

[00139] Table 2 provides a non-exhaustive list of Cargo polypeptides that correspond to a Cargo polypeptide portion of a heteromultimer described herein. The "Cargo Polypeptide" column discloses Cargo polypeptide molecules followed by parentheses containing scientific and brand names that comprise, or alternatively consist of, that Cargo polypeptide molecule or a fragment or variant thereof. In an embodiment the cargo molecule is a molecule that binds to a protein disclosed in the “Cargo polypeptide” column, or in Zhu et al. (Nucleic Acids Res. 38(1), D787-D791 (2009)); Wishart et al. (Nucleic Acids Res 36, D901-D906 (2008)); Ahmed et al. (Nucleic Acids Res 39, D960-D967 (2011)) incorporated by reference herein, or a protein that belongs in the class of therapeutic target molecules.

[00140] "Cargo polypeptide" as used herein may refer either to an individual Cargo polypeptide molecule (as defined by the amino acid sequence obtainable from the CAS and Genbank accession numbers), or to the entire group of Cargo polypeptide associated with a given Cargo polypeptide molecule disclosed in this column, or a Cargo polypeptide that binds to a polypeptide molecule disclosed in this column.

Table 2: Non-exhaustive list of Cargo polypeptides that correspond to a Cargo polypeptide portion of a heteromultimer

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
EPO (Erythropoietin; Epoetin alfa; Epoetin beta; Gene- activated erythropoietin; Darbepoetin- alpha; NESP; Epogen; Procrit;	Stimulates cellular differentiation of bone-marrow stem cells at an early stage of erythropoiesis; accelerates the	Cell proliferation assay using a erythroleukemic cell line TF-1. (Kitamura et al. 1989 J.Cell. Physiol. 140: 323)	Anemia; Anemia in Renal Disease; Anemia in Oncology Patients; Bleeding Disorders; Chronic Renal Failure; Chronic Renal Failure in Pre-Dialysis Patients; Renal Disease; End-

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
Eprex; Erypo; Espo; Epoimmun; EPOGIN; NEORECORMON; HEMOLINK; Dynepo; ARANESP)	proliferation and maturation of terminally differentiating cells into erythrocytes; and modulates the level of circulating erythrocytes.		Stage Renal Disease; End-Stage Renal Disease in Dialysis Patients; Chemotherapy; Chemotherapy in Cancer Patients; Anemia in zidovudine-treated HIV patients; Anemia in zidovudine-treated patients; Anemia in HIV patients; Anemia in premature infants; Surgical patients (pre and/or post surgery); Surgical patients (pre and/or post surgery) who are anemic; Surgical patients (pre and/or post surgery) who are undergoing elective surgery; Surgical patients (pre and/or post surgery) who are undergoing elective, non-cardiac surgery; Surgical patients (pre and/or post surgery) who are undergoing elective, non-cardiac, non-vascular surgery; Surgical patients (pre and/or post surgery) who are undergoing elective, non-vascular surgery; Surgical patients (pre and/or post surgery) who are undergoing cardiac and/or vascular surgery; Aplastic anemia; Refractory anemia; Anemia in Inflammatory Bowel Disease; Refractory anemia in Inflammatory Bowel Disease; Transfusion avoidance; Transfusion avoidance for surgical patients; Transfusion avoidance for elective surgical patients; Transfusion avoidance for elective orthopedic surgical patients; Patients who want to Increase Red Blood Cells.

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
G-CSF (Granulocyte colony- stimulating factor; Granulokine; KRN 8601; Filgrastim; Lenograstim; Meograstim; Nartograstim; Neupogen; NOPIA; Gran; GRANOCYTE; Granulokine; Neutrogin; Neu-up; Neutromax)	Stimulates the proliferation and differentiation of the progenitor cells for granulocytes and monocytes-macrophages.	Proliferation of murine NFS-60 cells (Weinstein et al, Proc Natl Acad Sci USA 1986; 83, pp5010-4)	Chemoprotection; Adjunct to Chemotherapy; Inflammatory disorders; Cancer; Leukemia; Myelocytic leukemia; Neutropenia, Primary neutropenias (e.g.; Kostmann syndrome); Secondary neutropenia; Prevention of neutropenia; Prevention and treatment of neutropenia in HIV-infected patients; Prevention and treatment of neutropenia associated with chemotherapy; Infections associated with neutropenias; Myelopysplasia; Autoimmune disorders; Psoriasis; Mobilization of hematopoietic progenitor cells; Wound Healing; Autoimmune Disease; Transplants; Bone marrow transplants; Acute myelogeneous leukemia; Lymphoma, Non-Hodgkin's lymphoma; Acute lymphoblastic leukemia; Hodgkin's disease; Accelerated myeloid recovery; Glycogen storage disease.
GM-CSF (Granulocyte-macrophage colony-stimulating factor; rhuGM- CSF; BI 61012; Prokine; Molgramostim; Sargramostim; GM-CSF/IL 3 fusion; Milodistim; Leucotropin; PROKINE; LEUKOMAX;	Regulates hematopoietic cell differentiation, gene expression, growth, and function.	Colony Stimulating Assay: Testa, N. G., et al., “Assays for hematopoietic growth factors.” Balkwill FR (edt) Cytokines, A practical Approach, pp 229-44; IRL Press Oxford 1991.	Bone Marrow Disorders; Bone marrow transplant; Chemoprotection; Hepatitis C; HIV Infections; Cancer; Lung Cancer; Melanoma; Malignant melanoma; Mycobacterium avium complex; Mycoses; Leukemia; Myeloid Leukemia; Infections; Neonatal infections; Neutropenia; Mucositis; Oral Mucositis; Prostate Cancer; Stem Cell Mobilization; Vaccine Adjuvant;

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
Interberin; Leukine; Leukine Liquid; Pixykine)			Ulcers (such as Diabetic, Venous Stasis, or Pressure Ulcers); Prevention of neutropenia; Acute myelogenous leukemia; Hematopoietic progenitor cell mobilization; Lymphoma; Non-Hodgkin's lymphoma; Acute Lymphoblastic Leukemia; Hodgkin's disease; Accelerated myeloid recovery; Transplant Rejection; Xenotransplant Rejection.
Human growth hormone (Pegvisamont; Somatrem; Somatropin; TROVERT; PROTROPIN; BIO-TROPIN; HUMATROPE; NUTROPIN; NUTROPIN AQ; NUTROPHIN; NORDITROPIN; GENOTROPIN; SAIZEN; SEROSTIM)	Binds to two GHR molecules and Induces signal transduction through receptor dimerization	Ba/F3-hGHR proliferation assay, a novel specific bioassay for serum human growth hormone. J Clin Endocrinol Metab 2000 Nov; 85(11): 4274-9 Plasma growth hormone (GH) immunoassay and tibial bioassay, Appl Physiol 2000 Dec; 89(6): 2174-8 Growth hormone (hGH) receptor mediated cell mediated proliferation, Growth Horm IGF Res 2000 Oct; 10(5): 248-55 International standard for growth hormone, Horm Res 1999; 51 Suppl 1: 7-12	Acromegaly; Growth failure; Growth hormone replacement; Growth hormone deficiency; Pediatric Growth Hormone Deficiency; Adult Growth Hormone Deficiency; Idiopathic Growth Hormone Deficiency; Growth retardation; Prader- Willi Syndrome; Prader-Willi Syndrome in children 2 years or older; Growth deficiencies; Growth failure associated with chronic renal insufficiency; Osteoporosis; Postmenopausal osteoporosis; Osteopenia, Osteoclastogenesis; burns; Cachexia; Cancer Cachexia; Dwarfism; Metabolic Disorders; Obesity; Renal failure; Turner's Syndrome; Fibromyalgia; Fracture treatment; Frailty, AIDS wasting; Muscle Wasting; Short Stature; Diagnostic Agents; Female Infertility; lipodystrophy.
Insulin (Human insulin; Insulin aspart; Insulin Glargine; Insulin lispro;	Stimulates glucose uptake and promotes glycogenesis and	Insulin activity may be assayed in vitro using a [3-H]-glucose uptake	Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
Lys-B28 Pro- B29; lyspro; LY 275585; diarginylinsulin; Des-B26- B30-insulin- B25-amide; Insulin detemir; LABI; NOVOLIN; NOVORAPID; HUMULIN; NOVOMIX 30; VELOSULIN; NOVOLOG; LANTUS; ILETIN; HUMALOG; MACRULIN; EXUBRA; INSUMAN; ORALIN; ORALGEN; HUMAHALE; HUMAHALIN)	lipogenesis.	assay. (J Biol Chem 1999 Oct 22; 274(43): 30864-30873).	diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non- insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X.
Interferon alfa (Interferon alfa-2b; recombinant; Interferon alfa- n1; Interferon alfa-n3; Peginterferon alpha-2b; Ribavirin and interferon alfa- 2b; Interferon alfacon-1; interferon consensus; YM 643; CIFN; interferon- alpha consensus; recombinant methionyl consensus interferon; recombinant consensus interferon; CGP 35269; RO 253036; RO 258310; INTRON A; PEG-INTRON; OIF; OMNIFERON; PEG-OMNIFERON; VELDONA; PEG-	Confers a range of cellular responses including antiviral, antiproliferative, antitumor and immunomodulatory activities; stimulate production of two enzymes: a protein kinase and an oligoadenylate synthetase.	Anti-viral assay: Rubinstein S, Familletti PC, Pestka S. (1981) Convenient assay for interferons. J. Virol. 37(2): 755-8; Anti-proliferation assay: Gao Y, et al (1999) Sensitivity of an epstein-barr virus-positive tumor line, Daudi, to alpha interferon correlates with expression of a GC-rich viral transcript. Mol Cell Biol. 19(11): 7305-13.	Viral infections; HIV Infections; Hepatitis; Chronic Hepatitis; Hepatitis B; Chronic Hepatitis B; Hepatitis C; Chronic Hepatitis C; Hepatitis D; Chronic Hepatitis D; Human Papillomavirus; Herpes Simplex Virus Infection; External Condylomata Acuminata; HIV; HIV Infection; Oncology; Cancer; Solid Tumors; Melanoma; Malignant Melanoma; Renal Cancer (e.g., Renal Cell Carcinoma); Lung Cancer (e.g,. Non-Small Cell Lung Cancer or Small Cell Lung Cancer) Colon Cancer; Breast Cancer; Liver Cancer; Prostate Cancer; Bladder Cancer; Gastric Cancer; Sarcoma; AIDS- Related Kaposi's Sarcoma; Lymphoma; T Cell Lymphoma; Cutaneous T-Cell Lymphoma; Non-Hodgkin's Lymphoma; Brain

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
REBETRON; ROFERON A; WELLFERON; ALFERON N/LDO; REBETRON; ALTEMOL; VIRAFERON PEG; PEGASYS; VIRAFERON; VIRAFON; AMPLIGEN; INFERGEN; INFAREX; ORAGEN)			Cancer; Glioma; Glioblastoma Multiforme; Cervical Dysplasia; Leukemia; Preleukemia; Bone Marrow Disorders; Bone Disorders; Hairy Cell Leukemia; Chronic Myelogeonus Leukemia; Hematological Malignancies; Hematological Disorders; Multiple Myeloma; Bacterial Infections; Chemoprotection; Thrombocytopenia; Multiple Sclerosis; Pulmonary Fibrosis; Age-Related Macular Degeneration; Macular Degeneration; Crohn's Disease; Neurological Disorders; Arthritis; Rheumatoid Arthritis; Ulcerative Colitis; Osteoporosis, Osteopenia, Osteoclastogenesis; Fibromyalgia; Sjogren's Syndrome; Chronic Fatigue Syndrome; Fever; Hemmorhagic Fever; Viral Hemmorhagic Fevers; Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non- insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity;

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X.
Calcitonin (Salmon Calcitonin (Salcatonin); Calcitonin human-salmon hybrid; Forcaltonin; Fortical; Calcitonin; Calcitonina Almirall; Calcitonina Hubber; Calcimar; Calsynar; Calogen; Miacalcic; Miacalcin; SB205614; Macritonin; Cibacalcin; Cibacalcina; Cibacalcine; Salmocalcin; PowderJect Calcitonin) (CAS-21215-62-3)	Regulates levels of calcium and phosphate in serum; causes a reduction in serum calcium--an effect opposite to that of human parathyroid hormone.	Hypocalcemic Rat Bioassay, bone resorbing assay and the pit assay, CT receptor binding assay, CAMP stimulation assay: J Bone Miner Res 1999 Aug; 14(8): 1425-31	Bone Disorders; Fracture prevention; Hypercalcemia; Malignant hypercalcemia; Osteoporosis; Paget's disease; Osteopenia, Osteoclastogenesis; osteolysis; osteomyelitis; osteonecrosis; periodontal bone loss; osteoarthritis; rheumatoid arthritis; osteopetrosis; periodontal, lytic, or metastatic bone disease; osteoclast differentiation inhibition; bone disorders; bone healing and regeneration.
Interferon beta (Interferon beta-1a; Interferon beta 1b; Interferon- beta-serine; SH 579; ZK 157046; BCDF; beta-2 IF; Interferon- beta-2; rhIL-6; SJ0031; DL 8234; FERON; IFNbeta; BETASERON; AVONEX; REBIF; BETAFERON; SIGOSIX)	Modulates MHC antigen expression, NK cell activity and IFNg production and IL12 production in monocytes.	Anti-viral assay: Rubinstein S, Familletti PC, Pestka S. (1981) Convenient assay for interferons. J. Virol. 37(2): 755-8; Anti-proliferation assay: Gao Y, et al (1999) Sensitivity of an epstein-barr virus-positive tumor line, Daudi, to alpha interferon correlates with expression of a GC-rich viral transcript. Mol Cell Biol. 19(11): 7305-13.	Multiple Sclerosis; Oncology; Cancer; Solid Tumors; Melanoma; Malignant Melanoma; Renal Cancer (e.g., Renal Cell Carcinoma); Lung Cancer (e.g,. Non-Small Cell Lung Cancer or Small Cell Lung Cancer) Colon Cancer; Breast Cancer; Liver Cancer; Prostate Cancer; Bladder Cancer; Gastric Cancer; Sarcoma; AIDS-Related Kaposi's Sarcoma; Lymphoma; T Cell Lymphoma; Cutaneous T-Cell Lymphoma; Non-Hodgkin's Lymphoma; Brain Cancer; Glioma; Glioblastoma Multiforme; Cervical Dysplasia; Leukemia; Preleukemia; Bone Marrow Disorders; Bone Disorders; Hairy Cell Leukemia; Chronic Myelogeonus Leukemia;

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			Hematological Malignancies; Hematological Disorders; Multiple Myeloma; Bacterial Infections; Chemoprotection; Thrombocytopenia; Viral infections; HIV Infections; Hepatitis; Chronic Hepatitis; Hepatitis B; Chronic Hepatitis B; Hepatitis C; Chronic Hepatitis C; Hepatitis D; Chronic Hepatitis D; Human Papillomavirus; Herpes Simplex Virus Infection; External Condylomata Acuminata; HIV; HIV Infection; Pulmonary Fibrosis; Age-Related Macular Degeneration; Macular Degeneration; Crohn's Disease; Neurological Disorders; Arthritis; Rheumatoid Arthritis; Ulcerative Colitis; Osteoporosis, Osteopenia, Osteoclastogenesis; Fibromyalgia; Sjogren's Syndrome; Chronic Fatigue Syndrome; Fever; Hemmorhagic Fever; Viral Hemmorhagic Fevers; Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non- insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X.
Growth hormone releasing factor; Growth hormone releasing hormone (Sermorelin acetate; Pralmorelin; Somatorelin; Somatoliberin; Geref; Gerel; Groliberin)	Acts on the anterior pituitary to stimulate the production and secretion of growth hormone and exert a trophic effect on the gland.	Growth hormone-releasing peptides (GHRPs) are known to release growth hormone (GH) in vivo and in vitro by a direct action on receptors in anterior pituitary cells. Biological activity can be measured in cell lines expressing growth hormone releasing factor receptor (Mol Endocrinol 1992 Oct; 6(10): 1734-44, Molecular Endocrinology, Vol 7, 77-84).	Acromegaly; Growth failure; Growth hormone replacement; Growth hormone deficiency; Pediatric Growth Hormone Deficiency; Adult Growth Hormone Deficiency; Idiopathic Growth Hormone Deficiency; Growth retardation; Prader- Willi Syndrome; Prader-Willi Syndrome in children 2 years or older; Growth deficiencies; Growth failure associated with chronic renal insufficiency; Osteoporosis; Osteopenia, Osteoclastogenesis; Postmenopausal osteoporosis; burns; Cachexia; Cancer Cachexia; Dwarfism; Metabolic Disorders; Obesity; Renal failure; Turner's Syndrome; Fibromyalgia; Fracture treatment; Frailty, AIDS wasting; Muscle Wasting; Short Stature; Diagnostic Agents; Female Infertility; lipodystrophy.
IL-2 (Aldesleukin; interleukin-2 fusion toxin; T cell growth factor; PROLEUKIN; IMMUNACE; CELEUK; ONCOLIPIN 2; MACROLIN)	Promotes the growth of B and T cells and augments NK cell and CTL cell killing activity.	T cell proliferation assay "Biological activity of recombinant human interleukin-2 produced in Escherichia coli." Science 223: 1412-1415, 1984. natural killer (NK) cell and CTL cytotoxicity assay "Control of homeostasis of CD8+	Cancer; Solid Tumors; Metastatic Renal Cell Carcinoma; Metastatic Melanoma; Malignant Melanoma; Melanoma; Renal Cell Carcinoma; Renal Cancer; Lung Cancer (e.g,. Non-Small Cell Lung Cancer or Small Cell Lung Cancer); Colon Cancer; Breast Cancer; Liver Cancer; Leukemia; Preleukemia; Hematological

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
		memory T cells by opposing cytokines. Science 288: 675-678, 2000; CTLL-2 Proliferation: Gillis et al (1978) J. Immunol. 120, 2027	Malignancies; Hematological Disorders; Acute Myeloid Leukemia; Melanoma; Malignant Melanoma; Non-Hodgkin's Lymphoma; Ovarian Cancer; Prostate Cancer; Brain Cancer; Glioma; Glioblastoma Multiforme; Hepatitis; Hepatitis C; Lymphoma; HIV Infection (AIDS); Inflammatory Bowel Disorders; Kaposi's Sarcoma; Multiple Sclerosis; Arthritis; Rheumatoid Arthritis; Transplant Rejection; Diabetes; Type 1 Diabetes Mellitus; Type 2 Diabetes.
Parathyroid hormone; parathyrin (PTH; Ostabolin; ALX1-11; hPTH 1-34; LY 333334; MN 10T; parathyroid hormone (1-31); FORTEO; PARATHAR)	Acts in conjuction with calcitonin to control calcium and phosphate metabolism; elevates blood calcium level; stimulates the activity of osteocytes; enhances absorption of Ca+/Pi from small intestine into blood; promotes reabsorption of Ca+ and inhibits Pi by kidney tubules.	Adenylyl cyclase stimulation in rat osteosarcoma cells, ovariectomized rat model of osteoporosis: IUBMB Life 2000 Feb; 49(2): 131-5	Bone Disorders; Fracture prevention; Hypercalcemia; Malignant hypercalcemia; Osteoporosis; Paget's disease; Osteopenia, Osteoclastogenesis; osteolysis; osteomyelitis; osteonecrosis; periodontal bone loss; osteoarthritis; rheumatoid arthritis; osteopetrosis; periodontal, lytic, or metastatic bone disease; osteoclast differentiation inhibition; bone disorders; bone healing and regeneration.
Resistin	Mediates insulin resistance in Type II diabetes; inhibits insulin-stimulated glucose uptake	Ability of resistin to influence type II diabetes can be determined using assays known in the art: Pontoglio et al., J Clin Invest 1998 May 15; 101(10): 2215-22.	Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non- insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			(IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X.
TR6 (DcR3; Decoy Receptor 3; FASTR)	Inhibits Fas Ligand and AIM-2 (TL5, LIGHT) mediated apoptosis.	Cellular apoptosis can be measured by annexin staining, TUNEL staining, measurement of caspase levels. Inhibition of cell growth can also be directly measured, for example by ALOMAR Blue staining. Assay refs: cytotoxicity assay on human fibrosarcoma (Epsevik and Nissen-Meyer, 1986, J. Immunol. methods).	Fas Ligand or LIGHT induced apoptotic disorders: hepatitis; liver failure (including fulminant liver failure); graft versus host disease; graft rejection; myelodysplastic syndrome; renal failure; insulin dependent diabetes mellitus; rheumatoid arthritis; inflammatory bowel disease; autoimmune disease; toxic epidermal necrolysis; multiple sclerosis.
DeCAF (D- SLAM; BCM-like membrane protein; BLAME (B lymphocyte activator macrophage expressed))x	Inhibits proliferation and differentiation of B cells; Antagonize BLyS activity	DeCAF activity can be determined using assays known in the art, such as for example, those described in Examples 32-33 of International Publication No. WO0111046.	B cell and/or T cell mediated immune disorders; Immunodeficiency (e.g., Common Variable Immunodeficiency, Selective IgA Deficiency)
BLyS (B Lymphocyte Stimulator; Neutrokine alpha; TL7; BAFF; TALL-1; THANK; radiolabeled BLyS)	Promotes proliferation, differentiation and survival of B cells; Promotes immunoglobulin production by B cells.	BLyS activity can be determined using assays known in the art, such as, for example, the costimulatory proliferation assay and other assays disclosed by	B cell and/or T cell mediated immune disorders, particularly immune system disorders associated with low B cell numbers or low serum immunoglobulin; Immunodeficiency (e.g., Common

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
		Moore et al., 1999, Science, 285(5425): 260-3.	Variable Immunodeficiency, Selective IgA Deficiency). Radiolabeled forms: lymphoma, non-Hodgkins lymphoma, chronic lymphocytic leukemia, multiple myeloma.
Anti-BLyS single chain antibody (scFvI116A01, scFvI050B11, scFvI006D08) and others.	Agonize or antagonize BlyS activity.	BLyS agonist or antagonist activity can be determined using assays known in the art, such as, for example, a modified version the costimulatory proliferation assay disclosed by Moore et al., 1999, Science, 285(5425): 260-3, in which BlyS is mixed or preincubated with the anti-BlyS antibody prior to being applied to the responder B lymphocytes.	B cell and/or T cell mediated immune disorders; Autoimmune disorders, particularly autoimmune diseases associated with the production of autoantibodies; Rheumatoid Arthritis, Systemic Lupus Erythmatosus; Sjogren's Syndrome, cancers expressing Blys as an autocrine growth factor, e.g. certain chronic lymphocytic leukemias.
MPIF-1 (Myeloid Progenitor Inhibitory Factor; CK beta-8; Mirostipen)	Inhibits myeloid progenitor cells; and activates monocytes	MPIF-1 activity can be measured using the myeloprotection assay and chemotaxis assay described in U.S. Pat. No. 6,001,606.	Chemoprotection; Adjunct to Chemotherapy; Inflammatory disorders; Cancer; Leukemia; Myelocytic leukemia; Neutropenia, Primary neutropenias (e.g.; Kostmann syndrome); Secondary neutropenia; Prevention of neutropenia; Prevention and treatment of neutropenia in HIV-infected patients; Prevention and treatment of neutropenia associated with chemotherapy; Infections associated with neutropenias; Myelopysplasia; Autoimmune disorders; Psoriasis; Mobilization of hematopoietic

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			progenitor cells; Wound Healing; Autoimmune Disease; Transplants; Bone marrow transplants; Acute myelogeneous leukemia; Lymphoma, Non-Hodgkin's lymphoma; Acute lymphoblastic leukemia; Hodgkin's disease; Accelerated myeloid recovery; Glycogen storage disease.
KDI (Keratinocyte Derived Interferon; Interferon Kappa Precursor)	Inhibits bone marrow proliferation; and shows antiviral activity.	KDI activity can be measured using the antiviral and cell proliferation assays described in Examples 57-63 of International Publication No. WO0107608.	Multiple sclerosis; Hepatitis; Cancer; Viral infections, HIV infections, Leukemia.
TNFR2 (p75) (ENBREL)	Binds both TNFα and TNFβ; mediates T-cell proliferation by TNF; reduces signs and structural damage in patients with moderately to severely active rheumatoid arthritis (RA).	T-cell proliferation can be measured using assays known in the art. For example, "Lymphocytes: a practical approach" edited by: SL Rowland, AJ McMichael - chapter 6, pages 138-160 Oxford University Press (2000); and "Current Protocols on CD-ROM" section 3.12 Proliferation Assays for T-cell Function John Wiley & Soncs, Inc. (1999).	Autoimmune disease; Rheumatoid Arthritis; Psoriatic arthritis; Still's Disease; Ankylosing Spondylitis; Cardiovascular Diseases; Vasulitis; Wegener's granulomatosis; Amyloidosis; Systemic Lupus Erythematosus, Insulin-Dependent Diabetes Mellitus; Immunodeficiency Disorders; Infection; Inflammation; Inflammatory Bowel Disease; Chrohn's Disease; Psoriasis; AIDS; Graft Rejection; Graft Versus Host Disease.
Keratinocyte growth factor 2 (Repifermin; KGF-2; Fibroblast Growth Factor-10; FGF-10)	Stimulates epithelial cell growth.	KGF-2 activity can be measured using the wound healing assays and epithelial cell proliferation assays described in U.S. Pat. No.	Stimulate Epithelial Cell Proliferation; Stimulate Basal Keratinocytes; Wound Healing; Stimulate Hair Follicle Production; Healing Of Dermal Wounds. Wound Healing; Eye

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
		6,077,692.	Tissue Wounds, Dental Tissue Wounds, Oral Cavity Wounds, Diabetic Ulcers, Dermal Ulcers, Cubitus Ulcers, Arterial Ulcers, Venous Stasis Ulcers, Burns Resulting From Heat Exposure Or Chemicals, or Other Abnormal Wound Healing Conditions such as Uremia, Malnutrition, Vitamin Deficiencies or Complications Associated With Systemic Treatment With Steroids, Radiation Therapy or Antineoplastic Drugs or Antimetabolites; Promote Dermal Reestablishment Subsequent To Dermal Loss; Increase the Adherence Of Skin Grafts To A Wound Bed; Stimulate Re-Epithelialization from The Wound Bed; To Promote Skin Strength; Improve The Appearance Of Aged Skin; Proliferate Hepatocytes, Lung, Breast, Pancreas, Stomach, Bladder, Small Intestine, Large Intestine; Sebocytes, Hair Follicles, Type II Pneumocytes, Mucin- Producing Goblet Cells, or Other Epithelial Cells, Endothelial Cells, Keratinocytes, or Basal Keratinocytes (and Their Progenitors) Contained Within The Skin, Lung, Liver, Bladder, Eye, Salivary Glands, or Gastrointestinal Tract; Reduce The Side Effects Of Gut Toxicity That Result From Radiation, Chemotherapy Treatments Or

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			Viral Infections; Cytoprotector, especially of the Small Intestine Mucosa or Bladder; Mucositis (Mouth Ulcers); Regeneration Of Skin; Full and/or Partial Thickness Skin Defects, including Burns, (e.g., Repopulation Of Hair Follicles, Sweat Glands, And Sebaceous Glands); Psoriasis; Epidermolysis Bullosa; Blisters; Gastric and/or Doudenal Ulcers; Reduce Scarring; Inflamamatory Bowel Diseases; Crohn's Disease; Ulcerative Colitis; Gut Toxicity; Lung Damage; Repair Of Alveoli And/or Brochiolar Epithelium; Acute Or Chronic Lung Damage; Emphysema, ARDS; Inhalation Injuries; Hyaline Membrane Diseases; Infant Respiratory Distress Syndrome; Bronchopulmonary Displasia In Premature Infants; Fulminant Liver Failure; Cirrhosis, Liver Damage caused by Viral Hepatitis and/or Toxic Substances; Diabetes Mellitus; Inflammation.
TR2 (and TR2sv1, TR2SV2; TNFRSF14; HVEM; Herpes Virus Entry Mediator; ATAR)	Inhibits B cell proliferation, and mediates and inhibits Herpes Simplex Virus (HSV) infection.	Co-stimulation B-cell proliferation assay and Ig production assay (Moore et al., 1999, Science, 285(5425): 260-3.). HSV-1 and HSV-2 Infectivity Assay: International Publication No. WO 97/	Herpes; immune disorders; autoimmune disease; graft versus host disease; graft rejection; variable immunodeficiency; immunodeficiency syndromes; cancer.
Macrophage derived chemokine, MDC (Ckbeta-13)	Chemotactic for monocyte-derived dendritic cells and IL-	04658 Chemokine activities can be determined using assays	Inflammatory diseases; wound healing; angiogenesis; AIDS infection.

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
	2-activated natural killer cells.	known in the art: Methods in Molecular Biology, 2000, vol. 138: Chemokine Protocols. Edited by: A. E. I. Proudfoot, T. N. C. Wells, and C. A. Power. © Humana Press Inc., Totowa, NJ	
HAGDG59 (Retinal short-chain dehydrogenase)	Activates MIP1a release in Dendritic Cells.	Dendritic cell assays are well known in the art. For example, J. Immunol. 158: 2919-2925 (1997); J. Leukoc. Biol. 65: 822-828 (1999).	Immune disorders; cancer; viral infection; inflammation; sepsis; arthritis; asthma.
GnRH (Gonadotropin Releasing Hormone)	Promotes release of follicle-stimulating hormone and luteinizing hormone from anterior pituitary.	GnRH is known to cause the release of follicle stimulating hormone (FSH) and/or luteinizing hormone (LH) in vivo by a direct action on receptors in anterior pituitary gonadotropes. GnRH activity can be determined by measuring FSH levels in the medium of cultured gonadotropes before and after GnRH supplementation. For example, Baker et al. Biol Reprod 2000 Sep; 63(3): 865-71.	Infertility; Kallmann's syndrome or other forms of hypergonadotropic hypergonadism (failure to go through puberty naturally).
Teprotide	Inhibits angiotensin converting enzyme (ACE).	Inhibition of ACE can be determined using assays known in the art. For example, Anzenbacherova et al.,	Hypertension; congestive heart failure.

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
		J.Pharma Biomed Anal 2001 Mar; 24(5-6): 1151-6.	
Human chemokine HCC-1 (ckBeta-1; HWFBD)	Involved in inflammation, allergy, tissue rejection, viral infection, and tumor biology; enhances proliferation of CD34+ myeloid progenitor cells.	Chemokine activities can be determined using assays known in the art: Methods in Molecular Biology, 2000, vol. 138: Chemokine Protocols. Edited by: A. E. I. Proudfoot, T. N. C. Wells, and C. A. Power. © Humana Press Inc., Totowa, NJ	Autoimmune disorders; Immunity; Vascular and Inflammatory disorders; HIV; AIDS; infectious diseases.
ACE2 inhibitor (DX512)	Inhibits production of angiotensin II which induces aldosterone production, arteriolar smooth muscle vasoconstriction, and proliferation of cardiac fibroblasts, Induces angiogenesis; an enzyme that converts angiotensin I to angiotensin1-9; also cleaves des-Arg, bradykinin and neurotensin.	Inhibition of angiotensin can be determined using assays known in the art. For example, in vitro using a proliferation assay with rat cardiac fibroblasts as described in Naunyn Schmiedebergs Arch Pharmacol 1999 May; 359(5): 394-9.	Treatment for elevated angiotensin II and/or aldosterone levels, which can lead to vasoconstriction, impaired cardiac output and/or hypertension; Cardiovascular Disease; Cardiac Failure; Diabetes; Type II Diabetes; Proteinuria; Renal disorders, congestive heart failure.
TR1 (OCIF; Osteoclastogenesis inhibitory factor; osteoprotegerin, OPG; tumor necrosis factor receptor superfamily member 11B precursor;)	Inhibits osteoclastogenesis and bone resorption, and induces fibroblast proliferation.	Coculture Assay for Osteoclastogenesis, Bone resorption assay using fetal long-bone organ culture system, dentine resorption assay, and fibroblast proliferation assays are each described in Kwon et al., FASEB J. 12: 845-854 (1998).	Osteoporosis; Paget's disease; osteopenia; osteolysis; osteomyelitis; osteonecrosis; periodontal bone loss; osteoarthritis; rheumatoid arthritis; osteopetrosis; periodontal, lytic, or metastatic bone disease; osteoclast differentiation inhibition; bone disorders; bone healing and

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			regeneration; organ calcification; vascular calcification.
Human chemokine Ckbeta-7	Chemotactic for both activated (CD3+) T cells and nonactivated (CD14−) lymphocytes and (CD4+) and (CD8+) T lymphocytes and (CD45RA+) T cells	Chemokine activities can be determined using assays known in the art: Methods in Molecular Biology, 2000, vol. 138: Chemokine Protocols. Edited by: A. E. I. Proudfoot, T. N. C. Wells, and C. A. Power. © Humana Press Inc., Totowa, NJ	Cancer; Wound healing; Inflammatory disorders; Immmunoregulatory disorders; Atherosclerosis; Parasitic Infection; Rheumatoid Arthritis; Asthma; Autoimmune disorders.
CKbeta4 (HGBAN46; HE9DR66)	Attracts and activates microbicidal leukocytes; Attracts CCR6-expressing immature dendritic cells and memory/effector T cells; B-cell chemotaxis; inhibits proliferation of myeloid progenitors; chemotaxis of PBMC's.	Chemokine activities can be determined using assays known in the art: Methods in Molecular Biology, 2000, vol. 138: Chemokine Protocols. Edited by: A. E. I. Proudfoot, T. N. C. Wells, and C. A. Power. © Humana Press Inc., Totowa, NJ	Cancer; Solid Tumors; Chronic Infection; Autoimmune Disorders; Psoriasis; Asthma; Allergy; Hematopoiesis; Wound Healing; Bone Marrow Failure; Silicosis; Sarcoidosis; Hyper-Eosinophilic Syndrome; Lung Inflammation; Fibrotic Disorders; Atherosclerosis; Periodontal diseases; Viral diseases; Hepatitis.
Leptin	Controls obesity through regulation of appetite, reduction of body weight, and lowering of insulin and glucose level.	in vivo modulation of food intake, reduction in body weight, and lowering of insulin and glucose levels in ob/ob mice, radioimmunoassay (RIA) and activation of the leptin receptor in a cell-based assay. Protein Expr Purif 1998 Dec; 14(3): 335-42	Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); a Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X; Immunological Disorders; Immunosuppression.
IL-1 receptor antagonist (Anakinra; soluble interleukin-1 receptor; IRAP; KINERET; ANTRIL)	Binds IL1 receptor without activating the target cells; inhibits the binding of IL1-alpha and IL1-beta; and neutralizes the biologic activity of IL1-alpha and IL1- beta.	1) Competition for IL-1 binding to IL-1 receptors in YT-NCI or C3H/HeJ cells (Carter et al., Nature 344: 633-638, 1990); 2) Inhibition of IL-1-induced endothelial cell-leukocyte adhesion (Carter et al., Nature 344: 633-638, 1990); 3) Proliferation assays on A375-C6 cells, a human melanoma cell line highly susceptible to the antiproliferative action of IL-1 (Murai T et al., J. Biol. Chem. 276: 6797-6806, 2001).	Autoimmune Disease; Arthritis; Rheumatoid Arthritis; Asthma; Diabetes; Diabetes Mellitus; GVHD; Inflammatory Bowel Disorders; Chron's Disease; Ocular Inflammation; Psoriasis; Septic Shock; Transplant Rejection; Inflammatory Disorders; Rheumatic Disorders; Osteoporosis; Postmenopausal Osteoporosis; Stroke.
TREM-1 (Triggering Receptor Expressed on Monocytes 1)	Mediates activation of neutrophil and monocytes; Stimulates neutrophil and monocyte-mediated inflammatory response; Promotes secretion of TNF, IL-8, and MCP-1; Induces neutrophil degranulation, Ca2+ mobilization and tyrosine phosphorylation of extracellular signal-	Secretion of cytokines, chemokines, degranulation, and cell surface activation markers can be determined using assays described in Bouchon et al, J Immunol 2000 May 15; 164(10): 4991-5.	Inflammation; Sepsis; bacterial infection; autoimmune diseases; GVHD.

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
	related kinase 1 (ERK1), ERK2 and phospholipase C-gamma.		
HCNCA73	Induces T-cell activation- expression of CD152 marker; Stimulates release of TNF-a and MIP- 1a from immature, monocyte-derived dendritic cells; Promotes maturation of dendritic cells.	FMAT can be used to measure T-cell surface markers (CD69, CD152, CD71, HLA- DR) and T-cell cytokine production (e.g., IFNg production). J. of Biomol. Screen. 4: 193-204 (1999). Other T-cell proliferation assays: "Lymphocytes: a practical approach" edited by: SL Rowland, AJ McMichael - Chapter 6, pages 138-160 Oxford University Press (2000); WO 01/21658 Examples 11-14, 16-17 and 33.	Autoimmune disorders; Inflammation of the gastrointestinal tract; Cancer; Colon Cancer; Allergy; Crohn's disease.
VEGF-2 (Vascular Endothelial Growth Factor-2; VEGF-C)	Promotes endothelial cell proliferation.	VEGF activity can be determined using assays known in the art, such as those disclosed in International Publication No. WO0045835, for example.	Coronary artery disease; Critical limb ischemia; Vascular disease; proliferation of endothelial cells, both vascular and lymphatic. Antagonists may be useful as anti-angiogenic agents; Cancer.
HCHNF25 (jumping translocation breakpoint)	Activates MIP1a Release in Dendritic Cells.	Dendritic cell assays are well known in the art. For example, J. Immunol. 158: 2919-2925 (1997); J. Leukoc. Biol. 65: 822-828 (1999).	Immune disorders; cancer.
HLDOU18 (Bone Morphogenic Protein 9 (BMP9); Growth differentiation factor-2 precursor (GDF-2	Activates L6/GSK3 kinase assay.	Assays for activation of GSK3 kinase activity are well known in the art. For example, Biol. Chem. 379(8-9): (1998) 1101-	Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency;

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
precursor))		1110.; Biochem J. 1993 Nov 15; 296 (Pt 1): 15-9.	Hyperlipidemia; Hyperketonemia; Non- insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X.
Glucagon- Like-Peptide 1 (GLP1; Insulinotropin)	Stimulates the synthesis and release of insulin; enhances the sensitivity of adipose, muscle, and liver tissues towards insulin; stimulates glucose uptake; slows the digestive process; suppresses appetite; blocks the secretion of glucagon.	GLP1 activity may be assayed in vitro using a [3-H]-glucose uptake assay. (J Biol Chem 1999 Oct 22; 274(43): 30864-30873).	Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non- insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X.
Exendin-4 (AC-2993)	Stimulates the synthesis and release of insulin; enhances the sensitivity of adipose, muscle, and liver tissues towards insulin;	Exendin-4 activity may be assayed in vitro using a [3-H]-glucose uptake assay. (J Biol Chem 1999 Oct 22; 274(43): 30864-30873).	Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia;

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
	stimulates glucose uptake; slows the digestive process; suppresses appetite; blocks the secretion of glucagon.		Non- insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X.
T20 (T20 HIV inhibitory peptide, DP178; DP178 HIV inhibitory peptide)	a peptide from residues 643-678 of the HIV gp41 transmembrane protein ectodomain which binds to gp41 in its resting state and prevents transformation to the fusogenic state	Virus inhibition assays as described in Zhang et al., Sep. 26 2002, Sciencexpress (www.sciencexpress.org).	HIV; AIDS; SIV (simian immunodeficiency virus) infection.
T1249 (T1249 HIV inhibitory peptide; T1249 anti-HIV peptide)	a second generation HIV fusion inbitor	Virus inhibition assays as described in Zhang et al., Sep. 26 2002, Sciencexpress (www.sciencexpress.org).	HIV; AIDS; SIV (simian immunodeficiency virus) infection
Interferon Hybrids, specifically preferred: IFNalpha A/D hybrid (BgIII version) IFNalpha A/D hybrid (PvuII version) IFNalpha A/F hybrid IFNalpha A/B hybrid IFNbeta 1/alpha D hybrid (IFNbeta-1/alpha-1 hybrid) IFNalpha/beta hybrid	Confers a range of cellular responses including antiviral, antiproliferative, antitumor and immunomodulatory activities; stimulate production of two enzymes: a protein kinase and an oligoadenylate synthetase. Also, modulates MHC	Anti-viral assay: Rubinstein S, Familletti PC, Pestka S. (1981) Convenient assay for interferons. J. Virol. 37(2): 755-8; Anti-proliferation assay: Gao Y, et al (1999) Sensitivity of an epstein-barr virus-positive tumor line, Daudi, to alpha interferon correlates with expression of a GC-rich	Viral infections; HIV Infections; Hepatitis; Chronic Hepatitis; Hepatitis B; Chronic Hepatitis B; Hepatitis C; Chronic Hepatitis C; Hepatitis D; Chronic Hepatitis D; Human Papillomavirus; Herpes Simplex Virus Infection; External Condylomata Acuminata; HIV; HIV Infection; Oncology; Cancer; Solid Tumors; Melanoma; Malignant Melanoma; Renal Cancer (e.g., Renal Cell Carcinoma); Lung Cancer (e.g.,

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
	antigen expression, NK cell activity and IFNg production and IL12 production in monocytes.	viral transcript. Mol Cell Biol. 19(11): 7305-13.	Non-Small Cell Lung Cancer or Small Cell Lung Cancer) Colon Cancer; Breast Cancer; Liver Cancer; Prostate Cancer; Bladder Cancer; Gastric Cancer; Sarcoma; AIDS- Related Kaposi's Sarcoma; Lymphoma; T Cell Lymphoma; Cutaneous T-Cell Lymphoma; Non-Hodgkin's Lymphoma; Brain Cancer; Glioma; Glioblastoma Multiforme; Cervical Dysplasia; Leukemia; Preleukemia; Bone Marrow Disorders; Bone Disorders; Hairy Cell Leukemia; Chronic Myelogeonus Leukemia; Hematological Malignancies; Hematological Disorders; Multiple Myeloma; Bacterial Infections; Chemoprotection; Thrombocytopenia; Multiple Sclerosis; Pulmonary Fibrosis; Age-Related Macular Degeneration; Macular Degeneration; Crohn's Disease; Neurological Disorders; Arthritis; Rheumatoid Arthritis; Ulcerative Colitis; Osteoporosis, Osteopenia, Osteoclastogenesis; Fibromyalgia; Sjogren's Syndrome; Chronic Fatigue Syndrome; Fever; Hemmorhagic Fever; Viral Hemmorhagic Fevers; Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non- insulin dependent Diabetes

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X.
B-type natriuretic peptide (BNP, brain natriuretic peptide)	stimulates smooth muscle relaxation and vasodilation, natriuresis, and suppression of renin-angiotensin and endothelin.	Inhibition of angiotensin can be determined using assays known in the art, for example using an in vitro proliferation assay with rat cardiac fibroblasts as described in Naunyn Schmiedebergs Arch Pharmacol 1999 May; 359(5): 394-9. Vasodilation can be measured in animals by measuring the myogenic responses of small renal arteries in an isobaric arteriograph system (see Am J Physiol Regul Integr Comp Physiol 2002 Aug; 283(2): R349-R355). Natriuesis is determined by measuring the amount of sodium in the urine.	Congestive heart failure; cardiac volume overload; cardiac decompensation; Cardiac Failure; Left Ventricular Dysfunction; Dyspnea
α-defensin, including alpha 1 defensin, alpha 2 defensin, alpha 3 defensin (myeloid-	Suppression of HIV replication; active against bacteria, fungi, and enveloped viruses.	Virus inhibition assays as described in Zhang et al., Sep. 26 2002, Sciencexpress	HIV, AIDS; ARC.

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
related defensin; DEFA1; neutrophil-specific defensin; CAF)		(www.sciencexpress.org).	
Phosphatonin (matrix extracellular phosphoglycoprotein; MEPE)	Regulation of phosphate metabolism.	Blood phosphate levels can be measured using methods known in the art such as the Hypophosphatemic Rat Bioassay. Zoolog Sci 1995 Oct; 12(5): 607-10.	Hyperphosphatemia; Hyperphosphatemia in chronic renal failure; hypophosphatemia; Osteomalacia; Rickets; X-linked dominant hypophosphatemic rickets/osteomalacia (XLH); autosomal dominant hypophosphatemic rickets/osteomalacia (ADHR); tumor-induced rickets/osteomalacia (TIO).
P1pal-12 (pepducin, PAR1-based pepducin)	Regulation of protease-activated receptor (PAR) signal transduction and thrombin-mediated aggregation of human platelets.	Platelet aggregation can be measured using methods known in the art such as described in Nature Medicine 2002 Oct; 8(10): 1161-1165.	Protection against systemic platelet activation, thrombus, heart attack, stroke, and/or coagulation disorders.
P4pal-10 (pepducin, PAR4-based pepducin)	Regulation of protease-activated receptor (PAR) signal transduction and thrombin-mediated aggregation of human platelets.	Platelet aggregation can be measured using methods known in the art such as described in Nature Medicine 2002 Oct; 8(10): 1161-1165.	Protection against systemic platelet activation, thrombus, heart attack, stroke, and/or coagulation disorders.
HRDFD27	Involved in the proliferation of T cells; Production of TNFgamma.	T-cell proliferation can be measured using assays known in the art. For example, “Lymphocytes: a practical approach” edited by: SL Rowland, AJ McMichael - chapter 6, pages 138-160 Oxford University Press (2000); and “Current Protocols on CD-ROM” section	Chemoprotection; Adjunct to Chemotherapy; Inflammatory disorders; Cancer; Leukemia; Myelocytic leukemia; Neutropenia, Primary neutropenias (e.g.; Kostmann syndrome); Secondary neutropenia; Prevention of neutropenia; Prevention and treatment of neutropenia in HIV-infected patients; Prevention and

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
		3.12 Proliferation Assays for T-cell Function John Wiley & Soncs, Inc. (1999).	treatment of neutropenia associated with chemotherapy; Infections associated with neutropenias; Myelopysplasia; Autoimmune disorders; Psoriasis; Mobilization of hematopoietic progenitor cells; Wound Healing; Autoimmune Disease; Transplants; Bone marrow transplants; Acute myelogeneous leukemia; Lymphoma, Non-Hodgkin's lymphoma; Acute lymphoblastic leukemia; Hodgkin's disease; Accelerated myeloid recovery; Glycogen storage disease
HWHGZ51 (CD59; Metastasis- associated GPI-adhered protein homolog)	Stimulates an immune response and induces inflammation by inducing mononuclear cell, eosinophil and PMN infiltration; Inhibits growth of breast cancer, ovarian cancer, leukemia, and melanoma; Overexpressed in colon, lung, breast and rectal tumors; Regulates glucose and/or FFA update by adipocytes and skeletal muscle; Induces redifferentiation of chondrocytes	The ability to affect chondrocyte differentiation can be measured using methods known in the art, such as described in Bone (1995) Sep; 17(3): 279-86.	Skeletal diseases and disorders; Musculoskeletal diseases and disorders; Bone fractures and/or breaks; Osteoporosis (postmenopausal, senile, or idiopathic juvenile); Gout and/or pseudogout; Paget's disease; Osteoarthritis; Tumors and/or cancers of the bone (osteochondromas, benign chondromas, chondroblastomas, chondromyxoid fibromas, osteoid osteomas, giant cell tumors, multiple myelomas, osteosarcomas, fibrosarcomas, malignant fibrous histiocytomas, chondrosarcomas, Ewing's tumors, and/or malignant lymphomas); Bone and joint infections (osteomyelitits and/or infectious arthritis); Charcot's joints; Heel spurs; Sever's disease; Sport's injuries; Cancer; Solid

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			Tumors; Melanoma; Malignant Melanoma; Renal Cancer (e.g., Renal Cell Carcinoma); Lung Cancer (e.g,. Non-Small Cell Lung Cancer or Small Cell Lung Cancer) Colon Cancer; Breast Cancer; Liver Cancer; Prostate Cancer; Bladder Cancer; Gastric Cancer; Sarcoma; AIDS-Related Kaposi's Sarcoma; Lymphoma; T Cell Lymphoma; Cutaneous T-Cell Lymphoma; Non-Hodgkin's Lymphoma; Brain Cancer; Glioma; Glioblastoma Multiforme; Cervical Dysplasia; Leukemia; Preleukemia; Bone Marrow Disorders; Bone Disorders; Hairy Cell Leukemia; Chronic Myelogeonus Leukemia; Hematological Malignancies; Hematological Disorders; Multiple Myeloma; Kidney diseases and disorders; Shonlein-Henoch purpura, Berger disease, celiac disease, dermatitis herpetiformis, Chron disease; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non- insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X; Kidney disorders; Hyperinsulinemia; Hypoinsulinemia; Immunological disorders (e.g. arthritis, asthma, immunodeficiency diseases, AIDS, rheumatoid arthritis, granulomatous disease, inflammatory bowl disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, T-cell mediated cytotoxicity, host-versus-graft disease, autoimmunity disorders, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjorgren's disease, scleroderma)
C17 (cytokine- like protein C17)	Inhibits glucose and/or FFA uptake by adipocytes; Induces proliferation of kidney mesangial cells; Regulation of cytokine production and antigen presentation	Proliferation of kidney mesangial cells can be assayed using techniques described in J. Investig. Med. (1998) Aug; 46(6): 297-302.	Kidney diseases and disorders; Shonlein- Henoch purpura, Berger disease, celiac disease, dermatitis herpetiformis, Chron disease; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X; Kidney disorders; Hyperinsulinemia; Hypoinsulinemia; Hematopoietic disorders; Immunological diseases and disorders; Developmental diseases and disorders; Hepatic diseases and disorders; Cancer (particularly leukemia); Immunological disorders (e.g. arthritis, asthma, immunodeficiency diseases, AIDS, rheumatoid arthritis, granulomatous disease, inflammatory bowl disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, T-cell mediated cytotoxicity, host- versus-graft disease, autoimmunity disorders, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjorgren's disease, scleroderma)
HDPBQ71	Regulates production and secretion of IFNgamma; Activation of myeloid cells and/or hematopoietic cells	Such assays that may be used or routinely modified to test immunomodulatory activity of polypeptides of the invention (including antibodies and agonists or antagonists of the invention) include the assays disclosed in Miraglia et al., J	Blood disorders and infection (e.g., viral infections, tuberculosis, infections associated with chronic granulomatosus disease and malignant osteoporosis); Autoimmune disease (e.g., rheumatoid arthritis, systemic lupus erythematosis, multiple sclerosis); Immunodeficiency, boosting a T cell-mediated immune response,

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
		Biomolecular Screening 4: 193-204 (1999); Rowland et al., ""Lymphocytes: a practical approach"" Chapter 6: 138-160 (2000); Gonzalez et al., J Clin Lab Anal 8(5): 225-233 (1995); Billiau et al., Ann NY Acad Sci 856: 22-32 (1998); Boehm et al., Annu Rev Immunol 15: 749-795 (1997), and Rheumatology (Oxford) 38(3): 214-20 (1999)	and suppressing a T cell-mediated immune response; Inflammation and inflammatory disorders; Idiopathic pulmonary fibrosis; Neoplastic diseases (e.g., leukemia, lymphoma, melanoma); Neoplasms and cancers, such as, for example, leukemia, lymphoma, melanoma, and prostate, breast, lung, colon, pancreatic, esophageal, stomach, brain, liver and urinary cancer;. Benign dysproliferative disorders and pre-neoplastic conditions, such as, for example, hyperplasia, metaplasia, and/or dysplasia; Anemia; Pancytopenia; Leukopenia; Thrombocytopenia; Hodgkin's disease; Acute lymphocytic anemia (ALL); Plasmacytomas; Multiple myeloma; Burkitt's lymphoma; Arthritis; AIDS; Granulomatous disease; Inflammatory bowel disease; Sepsis; Neutropenia; Neutrophilia; Psoriasis; Suppression of immune reactions to transplanted organs and tissues; Hemophilia; Hypercoagulation; Diabetes mellitus; Endocarditis; Meningitis; Lyme Disease; Asthma; Allergy
Oscar (osteoclast-associated receptor isoform-3)	Regulator of osteoclast differentiation; regulator of innate and adaptive immune responses	Assay to detect osteoclast differentiation is described in J. Exp. Med. (2002) Jan 21; 195(2): 201-9.	Skeletal diseases and disorders; Musculoskeletal diseases and disorders; Bone fractures and/or breaks; Osteoporosis (postmenopausal, senile, or idiopathic juvenile); Gout and/or pseudogout; Paget's disease;

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			Osteoarthritis; Tumors and/or cancers of the bone (osteochondromas, benign chondromas, chondroblastomas, chondromyxoid fibromas, osteoid osteomas, giant cell tumors, multiple myelomas, osteosarcomas, fibrosarcomas, malignant fibrous histiocytomas, chondrosarcomas, Ewing's tumors, and/or malignant lymphomas); Bone and joint infections (osteomyelitits and/or infectious arthritis); Charcot's joints; Heel spurs; Sever's disease; Sport's injuries
Tumstatin (T5, T7 or T8 peptide; α3(IV)NC1)	Inhibits angiogenesis; Inhibits tumor growth; Inhibits protein synthesis	A tumor cell proliferation assay is described in J. Biol. Chem. (1997) 272: 20395-20401. Protein synthesis can be measured as described in Science (2002) Jan 4; 295(5552): 140-3.	Cancer; Solid Tumors; Melanoma; Malignant Melanoma; Renal Cancer (e.g., Renal Cell Carcinoma); Lung Cancer (e.g,. Non-Small Cell Lung Cancer or Small Cell Lung Cancer) Colon Cancer; Breast Cancer; Liver Cancer; Prostate Cancer; Bladder Cancer; Gastric Cancer; Sarcoma; AIDS- Related Kaposi's Sarcoma; Lymphoma; T Cell Lymphoma; Cutaneous T-Cell Lymphoma; Non-Hodgkin's Lymphoma; Brain Cancer; Glioma; Glioblastoma Multiforme; Cervical Dysplasia; Leukemia; Preleukemia; Bone Marrow Disorders; Bone Disorders; Hairy Cell Leukemia; Chronic Myelogeonus Leukemia; Hematological Malignancies; Hematological Disorders; Multiple Myeloma; Angiogenesis
CNTF (Ciliary	Enhances myelin	Regulation of myelin	Neurological and neural diseases

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
neurotrophic factor)	formation; Reduces photoreceptor degradation; Regulates calcium currents	formation can be assayed as described in J. Neurosci. (2002) Nov. 1; 22(21): 9221-7.	and disorders, particularly diseases and disorders associated with myelin and demyelination, such as, for example, ALS, multiple sclerosis, Huntington's disease; Neuronal and spinal cord injuries; Disorders of the eye, such as, for example, retinitis pigmentosa, blindness, color-blindness, macular degeneration.
Somatostatin (Octreotide; octreotide acetate; Sandostating LAR®)	Inhibits growth hormone, glucagons and insulin; Suppresses LF response to GnRH; Decreases splanchnic blood flow; Inhibits release of serotonin, gastrin, vasoactive intestinal peptide, secretin, motilin, and pancreatic polypeptide.	Inhibition of growth hormone release in humans by somatostatin can be measured as described in J. Clin. Endocrinol. Metab. (1973) Oct; 37(4): 632-4. Inhibition of insulin secretion by somatostatin can be measured as described in the Lancet (1973) Dec. 8; 2(7841): 1299-1301.	Cancer; Metastatic carcinoid tumors; Vasoactive Intestinal Peptide secreting adenomas; Diarrhea and Flushing; Prostatic disorders and cancers; Breast cancer; Gastrointestinal disorders and cancers; Cancers of the endocrine system; Head and neck paragangliomas; Liver disorders and cancers; Nasopharyngeal cancers; Thyroid disorders and cancers; Acromegaly; Carcinoid Syndrome; Gallbladder disorders, such as gallbladder contractility diseases and abnormal bile secretion; Psoriasis; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non- insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X; Kidney disorders; Neurological disorders and diseases, including Alzheimers Disease, Parkinson's disease and dementia; Neuropsychotic disorders, including Bipolar affective disorder; Rheumatoid arthritis; Hypertension; Intracranial hypertension; Esophageal varices; Graves' disease; Seizures; Epilepsy; Gastritis; Angiogenesis;
IL-22 (IL22, interleukin-22; IL17D, IL27)	Stimulates glucose uptake in skeletal muscle cells; increases skeletal muscle insulin sensitivity.	IL-22 activity may be assayed in vitro using a [3-H]-glucose uptake assay. (J Biol Chem 1999 Oct 22; 274(43): 30864-30873).	Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non- insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X.
HCE1P80	Stimulates glucose uptake in; increases insulin sensitivity,	HCE1P80 activity may be assayed in vitro using a [3-H]-glucose uptake assay. (J Biol Chem 1999	Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance;

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
		Oct 22; 274(43): 30864-30873).	Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non- insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X.
HDRMI82	Stimulates glucose uptake; increases insulin sensitivity.	HDRMI82 activity may be assayed in vitro using a [3-H]-glucose uptake assay. (J Biol Chem 1999 Oct 22; 274(43): 30864-30873).	Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non- insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X.
HDALV07 (adiponectin; gelatin-binding 28k protein precursor; adipose most abundant gene	Modulates insulin action	Insulin activity may be assayed in vitro using a [3-H]-glucose uptake assay. (J Biol Chem 1999 Oct 22; 274(43): 30864-	Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia;

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
transcript; APM-1; GBP28; ACRP30; ADIPOQ)		30873).	Non-insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X; Hyperglycemia; Familial combined hyperlipidemia; Metabolic syndrome; Inflammatory disorders; Atherogenic disorders
C Peptide	An insulin precursor involved in insulin regulation	C-peptide concentrations can be measured using assays well known in the art, such as the one described in PNAS (1970) Sep; 67(1): 148-55	Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X; Hyperglycemia; Familial combined hyperlipidemia; Metabolic syndrome

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
HCBOG68 (enteric adipokine; Fat SID; proline rich acidic protein)	Controls proliferation/ differentiation or metabolism/ physiology/pathology/ of adipocytes and adipose tissue in response to dietary conditions.	Activation of cAMP-mediated transcription in adipocytes can be assayed using methods known in the art (Berger et al., Gene 66: 1-10 (1998); Cullen and Malm, Methods in Enzymol 216: 362-368 (1992); Henthorn et al., Proc Natl Acad Sci USA 85: 6342-6346 (1988); Reusch et al., Mol Cell Biol 20(3): 1008-1020 (2000); and Klemm et al., J Biol Chem 273: 917-923 (1998)).	Treatment of Obesity; treatment of Diabetes; suppression of body weight gain; suppression of appetite. Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non- insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X. Other indications for antibodies and/or antagonists, include treatment of weight loss; treatment of AIDS wasting; appetite stimulant; treatment of cachexia.
PYY (Peptide YY), including PYY_{3-36} (amino acid residues 31-64 of full length PYY, amino acid residues 3-36 of mature PYY)	Decreases appetite; increases satiety; decreases food intake.	Appetite and food intake can be can be measured by methods known in the art (Batterham et al. Nature 2002; 418: 650654)	Most preferred: Treatment of Obesity; treatment of Diabetes; suppression of body weight gain; suppression of appetite. Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non- insulin dependent Diabetes Mellitus (NIDDM); Insulin-

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X. Other indications for antibodies, antagonists: treatment of weight loss; treatment of AIDS wasting; appetite stimulant; treatment of cachexia.
WNT10b	Inhibits adipogenesis.	WNT10b activity can be measured using adipogenesis inhibition assays (Ross et al., Science 2000; 289(5481): 950-953	Most preferred: Treatment of Obesity; suppression of body weight gain; suppression of appetite. Other indications: Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM).
WNT11	Promotes cardiogenesis.	WNT11 activity can be measured using assays known in the art, including cardiogenesis assays (Eisenberg et al., Dev Dyn 1999 Sep; 216(1): 45-58).	Treatment of Cardiovascular disorders; Congestive Heart Failure; Myocardial Infarction.
Herstatin	Inhibits cancer proliferation.	Herstatin activity can be measured using cell	Oncology; Cancer; Solid Tumors; Melanoma; Malignant Melanoma;

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
		proliferation assays known in the art (Doherty et al., PNAS 1999; 96(19): 10869-10874.	Renal Cancer (e.g., Renal Cell Carcinoma); Lung Cancer (e.g,. Non-Small Cell Lung Cancer or Small Cell Lung Cancer); Colon Cancer; Breast Cancer; Liver Cancer; Prostate Cancer; Bladder Cancer; Gastric Cancer; Sarcoma; AIDS- Related Kaposi's Sarcoma; Lymphoma; T Cell Lymphoma; Cutaneous T-Cell Lymphoma; Non-Hodgkin's Lymphoma; Brain Cancer; Glioma; Glioblastoma Multiforme; Cervical Dysplasia; Leukemia; Preleukemia; Hairy Cell Leukemia; Chronic Myelogeonus Leukemia; Hematological Malignancies; Hematological Disorders; Multiple Myeloma.
Adrenomedullin	stimulates vasodilation; promotes bone growth.	Vasodilation can be measured using assays known in the art (Ashton et al. Pharmacology 2000; 61(2): 101-105. The promotion of bone growth can be measured using assays known in the art, such as the osteoblast proliferation assay (Cornish et al. Am J Physiol 1997 Dec; 273(6 Pt 1): E1113- 20).	Treatment of Congestive Heart Failure; Hypertension; Myocardial Infarction; Septic Shock; Osteoporosis; Postmenopausal osteoporosis; Osteopenia.
Nogo Receptor	Receptor for the axon growth inhibitor, Nogo.	The promotion of axon regeneration and growth can be measured using assays known in the art (Fournier et al. Nature 2001; 409(6818): 341-346).	Treatment of Central Nervous System Damage; Spinal Cord Injury; Peripheral Nerve Damage; Neurodegenerative Diseases; Parkinson's Disease; Alzheimer's Disease; Huntington's Disease; Amyotrophic Lateral Sclerosis;

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			Progressive Supranuclear Palsy; Creutzfeld-Jacob Disease; Motor Neuron Disease.
CART (Cocaine- and Amphetamine-Regulated Transcript)	Inhibits food intact and fat storage; promotes lipid oxidation.	Appetite and food intake can be can be measured by methods known in the art (Batterham et al. Nature 2002; 418: 650654)	Most preferred: Treatment of Obesity; suppression of body weight gain; suppression of appetite. Other indications: Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non-insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM).
RegIV (Colon Specific Gene; Colon Specific Protein)	Stimulates glucose uptake; increases insulin sensitivity.	RegIV activity may be assayed in vitro using a [3-H]-glucose uptake assay. (J Biol Chem 1999 Oct 22; 274(43): 30864-30873).	Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non- insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X.
Cosyntropin (Cortrosyn) (CAS-16960- 16-0)	Synthetic corticotropin; stimulates the release of cortisol.	The activity of cosyntropin can be assessed in vivo by	Endocrine; Addison's disease; Cushing's syndrome; pituitary dysfunction; acute adrenal crisis

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
		measuring serum cortisol levels. (Frank et al. J. Am. Vet. Med. Assoc. 1998 212(10): 1569-71).	
Pexiganan Acetate (CAS-172820- 23-4)	Disrupts bacterial membranes.	Pexiganan acetate activity can be assessed using in vitro antibacterial assays known in the art. (Zasloff et al., Antimicrobial Agents and Chemotherapy 1999, 43: 782-788).	Treatment of Infectious Diseases; Treatment of Bacterial Infections.
Pramlintide (Amylin) (CAS-151126- 32-8)	Slows gastric emptying; decreases food intake.	Appetite and food intake can be can be measured by methods known in the art (Batterham et al. Nature 2002; 418: 650654)	Treatment of Obesity; treatment of Diabetes; suppression of body weight gain; suppression of appetite; treatment of endocrine disorders; Hyperglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non- insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X. Other indications for antibodies, antagonists: treatment of weight loss; treatment of AIDS wasting;

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			appetite stimulant; treatment of cachexia.
Teriparatide (CAS-52232- 67-4)	Acts in conjuction with calcitonin to control calcium and phosphate metabolism; elevates blood calcium level; stimulates the activity of osteocytes; enhances absorption of Ca+/Pi from small intestine into blood; promotes reabsorption of Ca+ and inhibits Pi by kidney tubules.	Adenylyl cyclase stimulation in rat osteosarcoma cells, ovariectomized rat model of osteoporosis: IUBMB Life 2000 Feb; 49(2): 131-5	Bone Disorders; Fracture prevention; Hypercalcemia; Malignant hypercalcemia; Osteoporosis; Paget's disease; Osteopenia, Osteoclastogenesis; osteolysis; osteomyelitis; osteonecrosis; periodontal bone loss; osteoarthritis; rheumatoid arthritis; osteopetrosis; periodontal, lytic, or metastatic bone disease; osteoclast differentiation inhibition; bone disorders; bone healing and regeneration.
Terlipressin (triglycyl lycine vasopressin) (CAS-14636- 12-5)	Analog of vasopressin; induces vasoconstriction.	Terlipressin activity can be measured using assays of vasoconstriction, such as the isolated arterial ring preparation. (Landstrom et al., Hum Reprod 1999 Jan; 14(1): 151-5).	Variceal hemorrhage; cirrhosis; portal hypertension; hepatorenal syndrome; Blood-related disorders
Ularitide (CAS-118812-69-4)	Stimulates natriuresis, diuresis, and vasodilation.	Ularitide activity can be assessed by measuring cGMP accumulation in rat renal cells. (Valentin et al., Hypertension 1993 Apr; 21(4): 432-8).	Excretory disorders; Acute renal failure; asthma; congestive heart failure; hypertension; pulmonary hypertension; cardiovascular disorders
Aprotinin (Trasylol) (CAS-9087- 70-1; CAS-11061-94-2; CAS-12407- 79-3)	Serine protease inhibitor; attenuates Systemic Inflammatory Response, fibrinolysis and thrombin-induced platelet aggregation.	Inhibition of thrombin-induced platelet aggregation can be measured using methods known in the art. (Poullis et al., J Thorac Cardiovasc Surg 2000 Aug; 120(2): 370-8).	Inhibition of fibrinolysis; reduction of blood loss during surgery; Treatment of Inflammation and Immune Disorders.
Aspartocin (CAS-4117-	Antibacteria	Aspartocin activity can	Treatment of Infectious Diseases;

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
65-1; CAS- 1402-89-7)		be assessed using in vitro antibacterial assays known in the art. (Zasloff et al., Antimicrobial Agents and Chemotherapy 1999, 43: 782-788).	treatment of bacterial infections.
Calcitonin (Calcimar) (CAS-21215- 62-3)	Regulates levels of calcium and phosphate in serum; causes a reduction in serum calcium--an effect opposite to that of human parathyroid hormone.	Hypocalcemic Rat Bioassay, bone resorbing assay and the pit assay, CT receptor binding assay, CAMP stimulation assay: J Bone Miner Res 1999 Aug; 14(8): 1425-31	Musculoskeletal; Osteroporosis; Paget's disease; hypercalcemia; Bone Disorders; Fracture prevention; Malignant hypercalcemia; Osteopenia, Osteoclastogenesis; osteolysis; osteomyelitis; osteonecrosis; periodontal bone loss; osteoarthritis; rheumatoid arthritis; osteopetrosis; periodontal, lytic, or metastatic bone disease; osteoclast differentiation inhibition; bone disorders; bone healing and regeneration.
Carperitide (HANP; recombinant human atrial natriuretic peptide) (CAS-89213-87-6)	Stimulates natriuresis, diuresis, and vasodilation.	Carperitide activity can be assessed in vitro by measuring cGMP accumulation in a number of cell lines, including PC12 cells and cultured human glomerular cells. (Medvede et al., Life Sci 2001 Aug 31; 69(15): 1783-90; Green et al., J Am Soc Nephrol 1994 Oct; 5(4): 1091-8).	Treatment of Heart Failure; Cardiovascular disorders; Respiratory disorders; Acute respiratory distress syndrome.
Desirudin (recombinant hirudin; Revasc) (CAS-120993- 53-5)	Inhibits thrombin; inhibits blood clotting.	Desirudin activity can be assessed using blood clotting assays known in the art, such as in vitro platelet aggragation	Blood-related disorder; Thrombosis; thrombocytopenia; hemorrhages.

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
		assays. (Glusa, Haemostasis 1991; 21 Suppl 1: 116-20).	
Emoctakin (interleukin 8) (CAS-142298- 00-8)	proinflammatory cytokine		Treatment of Inflammation, Immune disorders, RSV infection.
Felypressin (CAS-56-59-7)	Derivative of Vasopressin; Stimulates vasoconstriction; Induces local anesthesia.	Felypressin vasoconstriction activity can be measured using assays of vasoconstriction, such as the isolated arterial ring preparation. (Landstrom et al., Hum Reprod 1999 Jan; 14(1): 151-5).	Treatment of pain; to induce local anesthesia.
Glucagon (CAS-16941-32-5)	Induces hyperglycemia.	Glucagon activity may be assayed in vitro using a [3-H]-glucose uptake assay. (J Biol Chem 1999 Oct 22; 274(43): 30864-30873).	Hypoglycemia; Diabetes; Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non- insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X; Endocrine disorders.
Nagrestipen (CAS-166089- 33-4)			Inflammation; Immune
Pentigetide (Pentyde) (CAS-62087- 72-3)			Respiratory; Allergy; Immune
Proinsulin (CAS-67422-	Stimulates glucose	Insulin activity may be	Hyperglycemia; Diabetes;

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
14-4)	uptake and promotes glycogenesis and lipogenesis.	assayed in vitro using a [3-H]-glucose uptake assay. (J Biol Chem 1999 Oct 22; 274(43): 30864-30873).	Diabetes Insipidus; Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency; Hyperlipidemia; Hyperketonemia; Non- insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity, Heart Disease, Hyperglycemia, Infections, Retinopathy, And/Or Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X.
Becaplermin (Regranex; recombinant PDGF-BB) (CAS-165101- 51-9)	Promotes wound healing.	Becaplermin activity can be assessed using animal wound healing models known in the art. (Saba et al., Ann Plast Surg 2002 Jul; 49(1): 62-6).	Stimulate Epithelial Cell Proliferation; Stimulate Basal Keratinocytes; Promote Wound Healing; Stimulate Hair Follicle Production; Healing Of Dermal Wounds. Wound Healing; Eye Tissue Wounds, Dental Tissue Wounds, Oral Cavity Wounds, Diabetic Ulcers, Dermal Ulcers, Cubitus Ulcers, Arterial Ulcers, Venous Stasis Ulcers, Burns Resulting From Heat Exposure Or Chemicals, or Other Abnormal Wound Healing Conditions such as Uremia, Malnutrition, Vitamin Deficiencies or Complications Associated With Systemic Treatment With Steroids, Radiation Therapy or Antineoplastic Drugs or Antimetabolites; Promote Dermal

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			Reestablishment Subsequent To Dermal Loss; Increase the Adherence Of Skin Grafts To A Wound Bed; Stimulate Re-Epithelialization from The Wound Bed; To Promote Skin Strength; Improve The Appearance Of Aged Skin; Proliferate Hepatocytes, Lung, Breast, Pancreas, Stomach, Bladder, Small Intestine, Large Intestine; Sebocytes, Hair Follicles, Type II Pneumocytes, Mucin-Producing Goblet Cells, or Other Epithelial Cells, Endothelial Cells, Keratinocytes, or Basal Keratinocytes (and Their Progenitors) Contained Within The Skin, Lung, Liver, Bladder, Eye, Salivary Glands, or Gastrointestinal Tract; Reduce The Side Effects Of Gut Toxicity That Result From Radiation, Chemotherapy Treatments Or Viral Infections; Cytoprotector, especially of the Small Intestine Mucosa or Bladder; Mucositis (Mouth Ulcers); Regeneration Of Skin; Full and/or Partial Thickness Skin Defects, including Burns, (e.g., Repopulation Of Hair Follicles, Sweat Glands, And Sebaceous Glands); Psoriasis; Epidermolysis Bullosa; Blisters; Gastric and/or Doudenal Ulcers; Reduce Scarring; Inflamamatory Bowel Diseases; Crohn's Disease; Ulcerative Colitis; Gut Toxicity; Lung Damage; Repair Of Alveoli

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			And/or Brochiolar Epithelium; Acute Or Chronic Lung Damage; Emphysema, ARDS; Inhalation Injuries; Hyaline Membrane Diseases; Infant Respiratory Distress Syndrome; Bronchopulmonary Displasia In Premature Infants; Fulminant Liver Failure; Cirrhosis, Liver Damage caused by Viral Hepatitis and/or Toxic Substances; Diabetes Mellitus; Inflammation; Cancer; Digestive disorders.
Ghrelin (Genbank Accession No. AB029434)	Stimulates release of growth hormone from anterior pituitary. Stimulates appetite and reduces fat burning.	Appetite and food intake can be can be measured by methods known in the art (Batterham et al. Nature 2002; 418: 650654)	Endocrine; loss of body weight; loss of body weight associated with cancer or anorexia nervosa; loss of appetite; excessive appetite; body weight gain; Obesity; Diabetes; Acromegaly; Growth failure; Growth hormone deficiency; Growth failure and growth retardation Prader-Willi syndrome in children 2 years or older; Growth deficiencies; Growth failure associated with chronic renal insufficiency; Postmenopausal osteoporosis; burns; cachexia; cancer cachexia; dwarfism; metabolic disorders; obesity; renal failure; Turner's

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			Syndrome, pediatric and adult; fibromyalgia; fracture treatment; frailty, AIDS wasting
Ghrelin - binding antibody including antibody fragment, or dominant- negative form of Ghrelin	Inhibits growth hormone release in response to Ghrelin; inhibits increase in appetite.	Appetite and food intake can be can be measured by methods known in the art (Batterham et al. Nature 2002; 418: 650654)	Endocrine; Obesity; Diabetes; body weight gain; excessive appetite; loss of appetite; loss of body weight.
receptor NOGO-66 peptide fragment (Genbank Accession No. NP_008939 (amino acids 62-101))			Neurodegenerative disorders; spinal cord injury; neuronal injury; brain trauma; stroke; multiple sclerosis; demyelinating disorders; neural activity and neurological diseases; neural cell (e.g., neuron, glial cell, and schwann cell) regeneration and/or growth
Gastric inhibitory polypeptide (GIP), including GIP fragments (Genbank Accession No. NM_004123)	Increases nutrient uptake and tryglyceride accumulation in adipocytes, which leads to obesity and insulin resistance.	Nutrient uptake and tryglyceride accumulation can be measured by methods described in Miyawaki et al., Nat. Medicine, 2002, Vol 8(7): 738-742.	Most preferred: loss of body weight, AIDS wasting, cachexia, and loss of appetite. Other: Obesity; Diabetes; insulin resistance; body weight gain; excessive appetite.
Gastric inhibitory polypeptide antibody, or antibody fragments	Increased use of fat as predominant energy source; decreased accumulation of fat in adipocytes.	Fat utilization as an energy source can be measured as described in Miyawaki et al., Nat. Medicine, 2002, Vol 8(7): 738-742.	Obesity; Diabetes; Insulin resistance; body weight gain.
Gastric inhibitory peptide receptor or receptor fragments or variants including soluble fragments or variants (Genbank Accession Number	Increased use of fat as predominant energy source; decreased accumulation of fat in adipocytes.	Fat utilization as an energy source can be measured as described in Miyawaki et al., Nat. Medicine, 2002, Vol 8(7): 738-742.	Most preferred: Obesity; Diabetes; body weight gain; excessive appetite; insulin resistance. Other: loss of body weight, AIDS wasting, loss of appetite.

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
NM_000164)			
POMC (proopiomelanocortin), including fragments or variants (such as, for example, alpha-melanocyte stimulating hormone, αMSH, gamma melanocyte stimulating hormone, γMSH, beta-melanocyte stimulating hormone, βMSH, adrenocorticotropin, ACTH, beta- endorphin, met- enkephalin) (Genbank Accession No. NM_000930)	Activity of POMC-derived fragments are diverse, and well-known in the art. See, for example, Hadley et al., Ann N Y Acad Sci 1999 Oct 20; 885: 1-21; Dores, Prog Clin Biol Res 1990; 342: 22-7; Blalock, Ann N Y Acad Sci. 1999 Oct 20; 885: 161-72).		Preferred: resistance to stress; anti- inflammatory activity; analgesic activity; increased skin pigmentation; increased protein catabolism; increased gluconeogenesis; obesity; diabetes. Other: decreased protein catabolism, decreased skin pigmentation, Addison's disease, Cushing's syndrome
HP 467, HP228 (U.S. Pat. No. 6,350,430)	See U.S. Pat. No. 6,350,430	See U.S. Pat. No. 6,350,430	Resistance to stress; anti-inflammatory activity; analgesic activity; increased skin pigmentation; increased protein catabolism; increased gluconeogenesis.
NDP (U.S. Pat. No. 6,350,430)	See U.S. Pat. No. 6,350,430	See U.S. Pat. No. 6,350,430	Resistance to stress; anti-inflammatory activity; analgesic activity; increased skin pigmentation; increased protein catabolism; increased gluconeogenesis.
Interleukin-21 (IL-21)	Immunomodulator; inhibits interferon gamma production by Th1 cells,	IL-21 activity can be assessed by measuring interferon gamma production in Th1 cells. (Wurster et al.,: J Exp Med 2002 Oct 7; 196(7): 969-77)	Autoimmune disorders; Inflammatory disorders; Treatment of Psoriasis; Rheumatoid Arthritis; Inflammatory bowel disease.
Interleukin-4 (IL-4)	Immunomodulator; promotes the	IL-4 activity can be assessed by measuring	Treatment of Psoriasis; Autoimmune disorders;

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
	differentiation of T cells into Th2 phenotype.	Th1/Th2 cytokine responses of isolated spleen cells in vitro. (Waltz et al., Horm Metab Res 2002 Oct; 34(10): 561-9).	Rheumatoid Arthritis; Inflammatory bowel disease; Inflammatory disorders.
Osteoclast Inhibitory Lectin (OCIL)	Inhibits osteoclast formation.	Osteoclast Inhibitory Lectin activity can be assessed using osteoclast formation assays known in the art. (Zhou et al., J Biol Chem 2002 Dec 13; 277(50): 48808-15)	Treatment of Bone Disorders; Osteoporosis; Fracture prevention; Hypercalcemia; Malignant hypercalcemia; Paget's disease; Osteopenia, Osteoclastogenesis; osteolysis; osteomyelitis; osteonecrosis; periodontal bone loss; osteoarthritis; rheumatoid arthritis; osteopetrosis; periodontal, lytic, or metastatic bone disease; osteoclast differentiation inhibition; bone healing and regeneration.
PCSK9 Inhibitor	Inhibits the interaction of PCSK9 with LDL Receptor.	Further LDL lowering through targeting PCSK9 for coronary artery disease. (Cao et al. Endocrine, Metabolic & Immune Disorders-Drug Targets 2008, 8, 238-243)	Treatment of coronary heart disease.

[00141]

[00142] Functional Activity :

[00143] "A polypeptide having functional activity" refers to a polypeptide capable of displaying one or more known functional activities associated with the full-length, pro-protein, and/or mature form of a cargo polypeptide. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a polypeptide for binding) to an anti-polypeptide antibody], immunogenicity (ability to generate antibody which binds to a specific polypeptide described herein), ability to form multimers with polypeptides described herein, and ability to bind to a receptor or ligand

for a polypeptide. In certain embodiments, the functional activity includes the ability to improve the expression and stability of a partner protein.

[00144] "A polypeptide having biological activity" refers to a polypeptide exhibiting activity similar to, but not necessarily identical to, an activity of a therapeutic protein described herein, including mature forms, as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the polypeptide described herein (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less, or not more than about tenfold less activity, or not more than about three-fold less activity relative to a polypeptide described herein, or presented in Table 2).

[00145] In certain embodiments, a heteromultimer described herein has at least one biological and/or therapeutic activity associated with the cargo molecule when said cargo molecule is not linked to the transporter polypeptide. In certain embodiments, a heteromultimer described herein has at least one biological and/or therapeutic activity associated with the cargo polypeptide when said cargo polypeptide is not linked to the transporter polypeptide. In certain embodiments, a heteromultimeric protein described herein has at least one biological and/or therapeutic activity associated with the cargo polypeptide portion (or fragment or variant thereof) when said cargo polypeptide is not linked to the albumin or alloalbumin based polypeptide.

[00146] The heteromultimeric proteins described herein can be assayed for functional activity (e.g., biological activity) using or routinely modifying assays known in the art, as well as assays described herein. Additionally, one of skill in the art may routinely assay fragments of a protein corresponding to a cargo protein portion of an albumin or alloalbumin based monomeric polypeptide, for activity using assays referenced in its corresponding row of Table 2 (e.g., in column 3 of Table 2). In certain embodiments, are assay of fragments of an albumin protein corresponding to an albumin protein portion of a heteromultimer, for activity using assays known in the art and/or as described in the Examples section below.

[00147] For example, in one embodiment where one is assaying for the ability of a heteromultimeric protein described herein to bind or compete with a Cargo polypeptide for binding to an anti-Cargo polypeptide antibody and/or anti-albumin antibody, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA

(enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

[00148] In certain embodiments, where a binding partner (e.g., a receptor or a ligand) is identified for a cargo molecule comprised by a heteromultimer described herein, binding to that binding partner by a heteromultimer described herein is assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky et al., Microbiol. Rev. 59:94-123 (1995). In another embodiment, the ability of physiological correlates of a heteromultimeric protein to bind to a substrate(s) of polypeptides corresponding to the cargo protein portion of the heteromultimer can be routinely assayed using techniques known in the art.

[00149] Biological Activities

[00150] In certain embodiments, heteromultimers described herein, are used in assays to test for one or more biological activities. If a heteromultimer exhibits an activity in a particular assay, it is likely that at least one cargo protein comprised by one or more monomers of the heteromultimer is implicated in the diseases associated with the biological activity. Thus, the heteromultimer is of use in a treatment of the associated disease.

[00151] In certain embodiments, provided is a method of treating a disease or disorder comprising administering to a patient in which such treatment, prevention or amelioration is desired, a heteromultimer described herein, in an amount effective to treat, prevent or ameliorate the disease or disorder.

[00152] Provided herein are monomeric albumin or alloalbumin based fusion proteins produced by a cell, wherein said proteins are encoded by polynucleotides, wherein said monomeric proteins comprise at least one cargo protein, and an albumin or alloalbumin derived polypeptide, such that said monomers form heteromultimers in solution. In certain

embodiments, when the polynucleotides are used to express the encoded protein from a cell, the cell's natural secretion and processing steps produces a protein that lacks at least one signal sequence. The specific amino acid sequence of the signal sequence is well known in the art.

[00153] In certain embodiments, heteromultimers described herein are used in the diagnosis, prognosis, prevention and/or treatment of diseases and/or disorders of the endocrine system. In some embodiments, heteromultimers described herein are used in the diagnosis, prognosis, prevention and/or treatment of diseases and/or disorders of the nervous system.

[00154] In certain embodiments, heteromultimers described herein are used in the diagnosis, prognosis, prevention and/or treatment of diseases and/or disorders of the immune system. In certain embodiments, heteromultimers described herein are used in the diagnosis, prognosis, prevention and/or treatment of diseases and/or disorders of the respiratory system.

[00155] In certain embodiments, heteromultimers described herein are used in the diagnosis, prognosis, prevention and/or treatment of diseases and/or disorders of the cardiovascular system. In some embodiments, heteromultimers described herein are used in the diagnosis, prognosis, prevention and/or treatment of diseases and/or disorders of the reproductive system.

[00156] In certain embodiments, heteromultimers described herein are used in the diagnosis, prognosis, prevention and/or treatment of diseases and/or disorders of the digestive system. In certain embodiments, heteromultimer proteins described herein are used in the diagnosis, prognosis, prevention and/or treatment of diseases or disorders relating to the blood.

[00157] In certain embodiments, heteromultimers described herein are used in the diagnosis and/or prognosis of diseases and/or disorders associated with at least one tissue(s) in which at least one gene of interest is expressed, wherein a heteromultimer described herein comprises a cargo molecule that binds said at least one gene of interest.

[00158] In some embodiments, heteromultimers described herein and/or polynucleotides encoding the albumin/alloalbumin based monomers that associate to form heteromultimers described herein, are used in the diagnosis, detection and/or treatment of diseases and/or disorders associated with activities that include, but are not limited to, prohormone activation, neurotransmitter activity, cellular signaling, cellular proliferation, cellular differentiation, and cell migration.

[00159] Therapeutic Uses:

[00160] In an aspect, heteromultimers described herein are directed to antibody-based therapies which involve administering heteromultimers described comprising cargo polypeptide(s) which is an antibody, a fragment or variant of an antibody, to a patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds described herein include, but are not limited to, heteromultimers described herein, nucleic acids encoding heteromultimers described herein.

[00161] In a specific embodiment, are antibody-based therapies which involve administering heteromultimers described herein comprising at least a fragment or variant of an antibody to a patient for treating one or more diseases, disorders, or conditions, including but not limited to: neural disorders, immune system disorders, muscular disorders, reproductive disorders, gastrointestinal disorders, pulmonary disorders, cardiovascular disorders, renal disorders, proliferative disorders, and/or cancerous diseases and conditions, and/or as described elsewhere herein.

[00162] A summary of the ways in which the heteromultimer proteins of the invention comprising at least a fragment or variant of an antibody are used therapeutically includes binding locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the heteromultimers described herein for diagnostic, monitoring or therapeutic purposes without undue experimentation.

[00163] The heteromultimers described herein, comprising at least a fragment or variant of an antibody may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in an embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

[00164] Gene Therapy:

[00165] In a specific embodiment, nucleic acids comprising sequences encoding heteromultimer proteins described herein are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of a protein, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a

subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect. Any of the methods for gene therapy available in the art can be used.

[00166] Demonstration of Therapeutic or Prophylactic Activity:

[00167] The heteromultimers or pharmaceutical compositions described herein are tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a heteromultimer, and the effect of such heteromultimer upon the tissue sample is observed.

[00168] Therapeutic/Prophylactic Administration and Composition

[00169] Provided are methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a heteromultimer or pharmaceutical composition described herein. In an embodiment, the heteromultimer is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). In certain embodiments, the subject is an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and in certain embodiments, a mammal, and most preferably human.

[00170] Various delivery systems are known and can be used to administer a heteromultimer formulation described herein, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other

biologically active agents. Administration can be systemic or local. In addition, in certain embodiments, it is desirable to introduce the heteromultimer compositions described herein into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[00171] In a specific embodiment, it is desirable to administer the heteromultimers, or compositions described herein locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

[00172] In another embodiment, the heteromultimers or composition can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, ibid., pp. 317-327; see generally ibid.)

[00173] In yet another embodiment, the heteromultimers or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); see also Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J. Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, e.g., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)).

[00174] Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

[00175] In a specific embodiment comprising a nucleic acid encoding a heteromultimer decribed herein, the nucleic acid can be administered in vivo to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Pat. No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see e.g., Joliot et al., Proc. Natl. Acad. Sci. USA 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

[00176] Also provided herein are pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium

saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

[00177] In certain embodiments, the composition comprising the heteromultimer is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[00178] In certain embodiments, the compositions described herein are formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxide isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[00179] The amount of the composition described herein which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a Therapeutic protein can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses are extrapolated from dose-response curves derived from in vitro or animal model test systems.

[00180] Methods of Recombinant and Synthetic Production of Heteromultimer Proteins:

[00181] In certain embodiments are heteromultimers produced as recombinant molecules by secretion from yeast, a microorganism such as a bacterium, or a human or animal cell line. In embodiments, the polypeptides are secreted from the host cells.

[00182] Embodiments include a cell, such as a yeast cell transformed to express a heteromultimer protein described herein. In addition to the transformed host cells themselves, are provided culture of those cells, preferably a monoclonal (clonally homogeneous) culture, or a culture derived from a monoclonal culture, in a nutrient medium. If the polypeptide is secreted, the medium will contain the polypeptide, with the cells, or without the cells if they have been filtered or centrifuged away. Many expression systems are known and may be used, including bacteria (for example E. coli and Bacillus subtilis), yeasts (for example Saccharomyces cerevisiae, Kluyveromyces lactis and Pichia pastoris, filamentous fungi (for example Aspergillus), plant cells, animal cells and insect cells.

[00183] A heteromultimer described herein is produced in conventional ways, for example from a coding sequence inserted in the host chromosome or on a free plasmid. The yeasts are transformed with a coding sequence for the desired protein in any of the usual ways, for example electroporation. Methods for transformation of yeast by electroporation are disclosed in Becker & Guarente (1990) Methods Enzymol. 194, 182.

[00184] Successfully transformed cells, i.e., cells that contain a DNA construct of the present invention, can be identified by well known techniques. For example, cells resulting from the introduction of an expression construct can be grown to produce the desired polypeptide. Cells can be harvested and lysed and their DNA content examined for the presence of the DNA using a method such as that described by Southern (1975) J. Mol. Biol. 98, 503 or Berent et al. (1985) Biotech. 3, 208. Alternatively, the presence of the protein in the supernatant can be detected using antibodies.

[00185] Useful yeast plasmid vectors include pRS403-406 and pRS413-416 and are generally available from Stratagene Cloning Systems, La Jolla, Calif. 92037, USA. Plasmids pRS403, pRS404, pRS405 and pRS406 are Yeast Integrating plasmids (YIps) and incorporate the yeast selectable markers HIS3, 7RP1, LEU2 and URA3. Plasmids pRS413-416 are Yeast Centromere plasmids (Ycps).

[00186] A variety of methods have been developed to operably link DNA to vectors via complementary cohesive termini. For instance, complementary homopolymer tracts can be added to the DNA segment to be inserted to the vector DNA. The vector and DNA

segment are then joined by hydrogen bonding between the complementary honmopolymeric tails to form recombinant DNA molecules.

[00187] Synthetic linkers containing one or more restriction sites provide an alternative method of joining the DNA segment to vectors. The DNA segment, generated by endonuclease restriction digestion, is treated with bacteriophage T4 DNA polymerase or E. coli DNA polymerase 1, enzymes that remove protruding, _-single-stranded termini with their 3' 5'-exonucleolytic activities, and fill in recessed 3'-ends with their polymerizing activities.

[00188] The combination of these activities therefore generates blunt-ended DNA segments. The blunt-ended segments are then incubated with a large molar excess of linker molecules in the presence of an enzyme that is able to catalyze the ligation of blunt-ended DNA molecules, such as bacteriophage T4 DNA ligase. Thus, the products of the reaction are DNA segments carrying polymeric linker sequences at their ends. These DNA segments are then cleaved with the appropriate restriction enzyme and ligated to an expression vector that has been cleaved with an enzyme that produces termini compatible with those of the DNA segment.

[00189] Synthetic linkers containing a variety of restriction endonuclease sites are commercially available from a number of sources including International Biotechnologies Inc, New Haven, Conn., USA.

[00190] Exemplary genera of yeast contemplated to be useful in the practice of the present invention as hosts for expressing the albumin, fusion proteins are Pichua (formerly classified as Hansenula), Saccharomyces, Kluyveromyces, Aspergillus, Candida, Torulopsis, Torulaspora, Schizosaccharomyces, Citeromyces, Pachysolen, Zygosaccharomyces, Debaromyces, Trichoderma, Cephalosporium, Humicola, Mucor, Neurospora, Yarrowia, Metschunikowia, Rhodosporidium, Leucosporidium, Botryoascus, Sporidiobolus, Endomycopsis, and the like. Preferred genera are those selected from the group consisting of Saccharomyces, Schizosaccharomyces, Kluyveromyces, Pichia and Torulaspora. Examples of Saccharomyces spp. are S. cerevisiae, S. italicus and S. rouxii.

[00191] Examples of Kluyveromyces spp. are K. fragilis, K. lactis and K. marxianus. A suitable Torulaspora species is T. delbrueckii. Examples of Pichia (Hansenula) spp. are P. angusta (formerly H. polymorpha), P. anomala (formerly H. anomala) and P. pastoris. Methods for the transformation of S. cerevisiae are taught generally in EP 251 744, EP 258 067 and WO 90/01063, all of which are incorporated herein by reference.

[00192] Preferred exemplary species of Saccharomyces include S. cerevisiae, S. italicus, S. diastaticus, and Zygosaccharomyces rouxii. Preferred exemplary species of Kluyveromyces include K. fragilis and K. lactis. Preferred exemplary species of Hansenula include H. polymorpha (now Pichia angusta), H. anomala (now Pichia anomala), and Pichia capsulata. Additional preferred exemplary species of Pichia include P. pastoris. Preferred exemplary species of Aspergillusinclude A. niger and A. nidulans. Preferred exemplary species of Yarrowia include Y. lipolytica. Many preferred yeast species are available from the ATCC. For example, the following preferred yeast species are available from the ATCC and are useful in the expression of albumin fusion proteins: Saccharomyces cerevisiae, Hansen, teleomorph strain BY4743 yap3 mutant (ATCC Accession No. 4022731); Saccharomyces cerevisiae Hansen, teleomorph strain BY4743 hsp150 mutant (ATCC Accession No. 4021266); Saccharomyces cerevisiae Hansen, teleomorph strain BY4743 pmt1 mutant (ATCC Accession No. 4023792); Saccharomyces cerevisiae Hansen, teleomorph (ATCC Accession Nos. 20626; 44773; 44774; and 62995); Saccharomyces diastaticus Andrews et Gilliland ex van der Walt, teleomorph (ATCC Accession No. 62987); Kluyveromyces lactis (Dombrowski) van der Walt, teleomorph (ATCC Accession No. 76492); Pichia angusta (Teunisson et al.) Kurtzman, teleomorph deposited as Hansenula polymorpha de Morais et Maia, teleomorph (ATCC Accession No. 26012); Aspergillus niger van Tieghem, anamorph (ATCC Accession No. 9029); Aspergillus niger van Tieghem, anamorph (ATCC Accession No. 16404); Aspergillus nidulans (Eidam) Winter, anamorph (ATCC Accession No. 48756); and Yarrowia lipolytica (Wickerham et al.) van der Walt et von Arx, teleomorph (ATCC Accession No. 201847).

[00193] Suitable promoters for S. cerevisiae include those associated with the PGKI gene, GAL1 or GAL10 genes, CYCI, PH05, TRP1, ADH1, ADH2, the genes for glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, triose phosphate isomerase, phosphoglucose isomerase, glucokinase, alpha-mating factor pheromone, [a mating factor pheromone], the PRBI promoter, the GUT2 promoter, the GPDI promoter, and hybrid promoters involving hybrids of parts of 5' regulatory regions with parts of 5' regulatory regions of other promoters or with upstream activation sites (e.g. the promoter of EP-A-258 067).

[00194] Convenient regulatable promoters for use in Schizosaccharomyces pombe are the thiamine-repressible promoter from the nmt gene as described by Maundrell (1990) J.

Biol. Chem. 265, 10857-10864 and the glucose repressible jbpl gene promoter as described by Hoffman & Winston (1990) Genetics 124, 807-816.

[00195] Methods of transforming Pichia for expression of foreign genes are taught in, for example, Cregg et al. (1993), and various Phillips patents (e.g. U.S. Pat. No. 4,857,467, incorporated herein by reference), and Pichia expression kits are commercially available from Invitrogen BV, Leek, Netherlands, and Invitrogen Corp., San Diego, Calif. Suitable promoters include AOX1 and AOX2. Gleeson et al. (1986) J. Gen. Microbiol. 132, 3459-3465 include information on Hansenula vectors and transformation, suitable promoters being MOX1 and FMD1; whilst EP 361 991, Fleer et al. (1991) and other publications from Rhone-Poulenc Rorer teach how to express foreign proteins in Kluyveromyces spp., a suitable promoter being PGKI.

[00196] The transcription termination signal is preferably the 3' flanking sequence of a eukaryotic gene which contains proper signals for transcription termination and polyadenylation. Suitable 3' flanking sequences may, for example, be those of the gene naturally linked to the expression control sequence used, i.e. may correspond to the promoter. Alternatively, they may be different in which case the termination signal of the S. cerevisiae ADHI gene is preferred.

[00197] In certain embodiments, the desired heteromultimer protein is initially expressed with a secretion leader sequence, which may be any leader effective in the yeast chosen. Leaders useful in S. cerevisiae include that from the mating factor alpha polypeptide (MFα-1) and the hybrid leaders of EP-A-387 319. Such leaders (or signals) are cleaved by the yeast before the mature albumin is released into the surrounding medium. Further such leaders include those of S. cerevisiae invertase (SUC2) disclosed in JP 62-096086 (granted as 911036516), acid phosphatase (PH05), the pre-sequence of MFα-1, 0 glucanase (BGL2) and killer toxin; S. diastaticus glucoarnylase Il; S. carlsbergensis α-galactosidase (MEL1); K. lactis killer toxin; and Candida glucoarnylase.

[00198] Provided are vectors containing a polynucleotide encoding a heteromultimer protein described herein, host cells, and the production of the heteromultimer proteins by synthetic and recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

[00199] In certain embodiments, the polynucleotides encoding heteromultimer proteins described herein are joined to a vector containing a selectable marker for propagation in a

host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

[00200] In certain embodiments, the polynucleotide insert is operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the E. coli lac, trp, phoA and rac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

[00201] As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418, glutamine synthase, or neomycin resistance for eukaryotic cell culture, and tetracycline, kanamycin or ampicillin resistance genes for culturing in E. coli and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as E. coli, Streptomyces and Salmonella typhimurium cells; fungal cells, such as yeast cells (e.g., Saccharomyces cerevisiae or Pichia pastoris (ATCC Accession No. 201178)); insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, NSO, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

[00202] Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A; pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalph, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.

[00203] In one embodiment, polynucleotides encoding a heteromultimer protein described herein are fused to signal sequences that will direct the localization of a protein of the invention to particular compartments of a prokaryotic or eukaryotic cell and/or direct the secretion of a protein of the invention from a prokaryotic or eukaryotic cell. For example, in E. coli, one may wish to direct the expression of the protein to the periplasmic space. Examples of signal sequences or proteins (or fragments thereof) to which the heteromultimeric proteins are fused in order to direct the expression of the polypeptide to the periplasmic space of bacteria include, but are not limited to, the pelB signal sequence, the maltose binding protein (MBP) signal sequence, MBP, the ompA signal sequence, the signal sequence of the periplasmic E. coli heat-labile enterotoxin B-subunit, and the signal sequence of alkaline phosphatase. Several vectors are commercially available for the construction of fusion proteins which will direct the localization of a protein, such as the pMAL series of vectors (particularly the pMAL-.rho. series) available from New England Biolabs. In a specific embodiment, polynucleotides albumin fusion proteins of the invention may be fused to the pelB pectate lyase signal sequence to increase the efficiency of expression and purification of such polypeptides in Gram-negative bacteria. See, U.S. Pat. Nos. 5,576,195 and 5,846,818, the contents of which are herein incorporated by reference in their entireties.

[00204] Examples of signal peptides that are fused to a heteromultimeric protein in order to direct its secretion in mammalian cells include, but are not limited to, the MPIF-1 signal sequence (e.g., amino acids 1-21 of GenBank Accession number AAB51134), the stanniocalcin signal sequence (MLQNSAVLLLLVISASA), and a consensus signal sequence (MPTWAWWLFLVLLLALWAPARG). A suitable signal sequence that may be used in conjunction with baculoviral expression systems is the gp67 signal sequence (e.g., amino acids 1-19 of GenBank Accession Number AAA72759).

[00205] Vectors which use glutamine synthase (GS) or DHFR as the selectable markers can be amplified in the presence of the drugs methionine sulphoximine or methotrexate, respectively. An advantage of glutamine synthase based vectors are the availabilty of cell lines (e.g., the murine myeloma cell line, NSO) which are glutamine synthase negative. Glutamine synthase expression systems can also function in glutamine synthase expressing cells (e.g., Chinese Hamster Ovary (CHO) cells) by providing additional inhibitor to prevent the functioning of the endogenous gene. A glutamine synthase expression system and components thereof are detailed in PCT publications: WO87/04462; WO86/05807; WO89/10036; WO89/10404; and WO91/06657, which are

hereby incorporated in their entireties by reference herein. Additionally, glutamine synthase expression vectors can be obtained from Lonza Biologics, Inc. (Portsmouth, N.H.). Expression and production of monoclonal antibodies using a GS expression system in murine myeloma cells is described in Bebbington et al., Bio/technology 10:169(1992) and in Biblia and Robinson Biotechnol. Prog. 11:1(1995) which are herein incorporated by reference.

[00206] Also provided are host cells containing vector constructs described herein, and additionally host cells containing nucleotide sequences that are operably associated with one or more heterologous control regions (e.g., promoter and/or enhancer) using techniques known of in the art. The host cell can be a higher eukaryotic cell, such as a mammalian cell (e.g., a human derived cell), or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. A host strain may be chosen which modulates the expression of the inserted gene sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus expression of the genetically engineered polypeptide may be controlled. Furthermore, different host cells have characteristics and specific mechanisms for the translational and post-translational processing and modification (e.g., phosphorylation, cleavage) of proteins. Appropriate cell lines can be chosen to ensure the desired modifications and processing of the foreign protein expressed.

[00207] Introduction of the nucleic acids and nucleic acid constructs of the invention into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (1986). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

[00208] In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., the coding sequence corresponding to a Cargo polypeptide is replaced with a heteromultimer protein corresponding to the Cargo polypeptide), and/or to include genetic material. The genetic material operably associated

with the endogenous polynucleotide may activate, alter, and/or amplify endogenous polynucleotides.

[00209] In addition, techniques known in the art may be used to operably associate heterologous polynucleotides (e.g., polynucleotides encoding an albumin protein, or a fragment or variant thereof) and/or heterologous control regions (e.g., promoter and/or enhancer) with endogenous polynucleotide sequences encoding a Therapeutic protein via homologous recombination (see, e.g., U.S. Pat. No. 5,641,670, issued Jun. 24, 1997; International Publication Number WO 96/29411; International Publication Number WO 94/12650; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

[00210] Heteromultimer proteins described herein can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography, hydrophobic charge interaction chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

[00211] In certain embodiments the heteromultimer proteins of the invention are purified using Anion Exchange Chromatography including, but not limited to, chromatography on Q-sepharose, DEAE sepharose, poros HQ, poros DEAF, Toyopearl Q, Toyopearl QAE, Toyopearl DEAE, Resource/Source Q and DEAE, Fractogel Q and DEAE columns.

[00212] In specific embodiments the proteins described herein are purified using Cation Exchange Chromatography including, but not limited to, SP-sepharose, CM sepharose, poros HS, poros CM, Toyopearl SP, Toyopearl CM, Resource/Source S and CM, Fractogel S and CM columns and their equivalents and comparables.

[00213] In addition, heteromultimer proteins described herein can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, Proteins: Structures and Molecular Principles, W. H. Freeman & Co., N.Y and Hunkapiller et al., Nature, 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of a polypeptide can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4diaminobutyric

acid, alpha-amino isobutyric acid, 4aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, fluoro-amino acids, designer amino acids such as β-methyl amino acids, Cα-methyl amino acids, Nα-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

[00214] Provided are heteromultimers which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, $NaBH_4$; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

[00215] Additional post-translational modifications encompassed herein include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The heteromultimer proteins are modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

[00216] Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include iodine, carbon, sulfur, tritium, indium, technetium, thallium, gallium, palladium, molybdenum, xenon, fluorine.

[00217] In specific embodiments, heteromultimer proteins or fragments or variants thereof are attached to macrocyclic chelators that associate with radiometal ions.

[00218] As mentioned, the heteromultimer described herein is modified by either natural processes, such as post-translational processing, or by chemical modification techniques which are well known in the art. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Polypeptides of the invention may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS--STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POST-TRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth. Enzymol. 182:626-646 (1990); Rattan et al., Ann. N.Y. Acad. Sci. 663:48-62 (1992)).

[00219] In certain embodiments, heteromultimeric proteins may also be attached to solid supports, which are particularly useful for immunoassays or purification of polypeptides that are bound by, that bind to, or associate with albumin fusion proteins of the invention. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

[00220] In embodiments where the heteromultimeric protein comprises only the VH domain of an antibody, it may be necessary and/or desirable to coexpress the protein with the VL domain of the same antibody, such that the VH-albumin fusion protein and VL protein will associate (either covalently or non-covalently) post-translationally.

[00221] In embodiments where the heteromultimeric protein comprises only the VL domain of an antibody, it may be necessary and/or desirable to coexpress the fusion protein with the

VH domain of the same antibody, such that the VL-albumin fusion protein and VH protein will associate (either covalently or non-covalently) post-translationally.

[00222] Also provided herein are chemically modified derivatives of the heteromultimeric proteins which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Pat. No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The proteins may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

[00223] The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a Therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 105,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 45,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 kDa.

[00224] The presence and quantity of heteromultimer proteins described herein may be determined using ELISA, a well known immunoassay known in the art. In one ELISA protocol that would be useful for detecting/quantifying heteromultimers described herein, comprises the steps of coating an ELISA plate with an anti-human serum albumin antibody, blocking the plate to prevent non-specific binding, washing the ELISA plate, adding a solution containing the protein described herein (at one or more different concentrations), adding a secondary anti-cargo polypeptide specific antibody coupled to a detectable label (as described herein or otherwise known in the art), and detecting the presence of the secondary antibody. In an alternate version of this protocol, the ELISA

plate might be coated with the anti-cargo polypeptide specific antibody and the labeled secondary reagent might be the anti-human albumin specific antibody.

[00225] Provided herein are multifunctional heteromultimers that comprise: at least two monomers, wherein at least one monomer comprises at least one cargo molecule attached to a transporter polypeptide, such that said monomers associate to form the heteromultimer; wherein at least one transporter polypeptide is derived from a monomeric protein and wherein said transporter polypeptides self-assemble to form a quasi-native structure of said monomeric protein or analog thereof. In certain embodiments, the cargo molecule is a biomolecule. In specific embodiments is a heteromultimer that comprises: at least two monomeric proteins, wherein each monomeric protein comprises at least one cargo polypeptide, attached to a transporter polypeptide, such that said monomeric proteins self-assemble to form the heteromultimer. In certain embodiments, the heteromultimer is a heterodimer. In an embodiment, the heteromultimer is bispecific. In an embodiment, the heteromultimer is multispecific. In certain embodiments, at least one transporter polypeptide is not derived from an antibody. In certain embodiments, the transporter polypeptides are not derived from an antibody. In an embodiment, the heteromultimer is multifunctional. In certain embodiments, the transporter polypeptides are derivatives of albumin. In certain embodiments of the heteromultimer described herein, the transporter polypeptides are derived from human serum albumin of SEQ ID No. 1. In certain embodiments of the heteromultimer described herein, the transporter polypeptides are derived from alloalbumins. In certain embodiments, the cargo polypeptides are therapeutic proteins described herein, or fragments or variants thereof. In some embodiments, at least one cargo polypeptide is fused to the transporter polypeptide. In certain embodiments, at least one cargo polypeptide is attached to the N-terminus of the transporter polypeptide. In some embodiments, at least one cargo polypeptide is attached to the C-terminus of the transporter polypeptide.

[00226] Provided herein are heteromultimers, each heteromultimer comprising: at least a first monomer that comprises at least one cargo molecule, and a first transporter polypeptide; and at least a second monomer that comprises at least one cargo molecule and a second transporter polypeptide wherein at least one transporter polypeptide is derived from a monomeric protein and wherein said transporter polypeptides self-assemble to form a quasi-native structure of said monomeric protein or analog thereof. In certain embodiments, at least one cargo molecule is a therapeutic agent described herein. In certain embodiments, at least one cargo molecule is a biomolecule described herein.

Provided herein are heteromultimers, each heteromultimer comprising: at least a first monomeric protein that comprises at least one cargo polypeptide and a first transporter polypeptide; and at least a second monomeric protein that comprises at least one cargo polypeptide and a second transporter polypeptide. In certain embodiments, the heteromultimer is a heterodimer. In certain embodiments, the heteromultimer is multivalent. In an embodiment, the heteromultimer is bivalent. In some embodiments, the heteromultimer is multispecific. In an embodiment, the heteromultimer is bispecific. In certain embodiments, the transporter polypeptides are derivatives of albumin. In certain embodiments of the heteromultimer described herein, the transporter polypeptides are derived from human serum albumin of SEQ ID No. 1.

[00227] In certain embodiments, are heteromultimers, each heteromultimer comprising: at least a first monomeric protein that comprises at least one cargo polypeptide and a first transporter polypeptide comprising a sequence of SEQ ID NO:2; and at least a second monomeric protein that comprises at least one cargo polypeptide and a second transporter polypeptide comprising a sequence of SEQ ID NO: 3. In certain embodiments of the heteromultimer described herein, at least one transporter polypeptide is derived from alloalbumins. In certain embodiments, both transporter polypeptides are derived from alloalbumins. In certain embodiments, all transporter polypeptides are derivatives of the same alloalbumin. In some other embodiments, the transporter polypeptides are derivatives of different alloalbumins. In some embodiments, each transporter polypeptide is an alloalbumin derivative based on an alloalbumin selected from Table 2. In certain embodiments, the first monomeric protein comprises two cargo polypeptides. In some embodiments, the second monomeric protein comprises two cargo polypeptides.

[00228] In some embodiments of the heteromultimer described herein, the transporter polypeptides are derivatives of an annexin protein. In an embodiment, the transporter polypeptides are derived from different annexin proteins. In certain embodiments, the transporter polypeptides are derived from the same annexin protein. In an embodiment, at least one transporter polypeptide is derived from Annexin A1 or lipocortin I. In certain embodiments of the heteromultimer, all transporter polypeptides are derived from Annexin A1 of SEQ ID NO: 14. In certain embodiments of the heteromultimer, at least one transporter polypeptides is derived from a sequence homologous to SEQ ID NO: 14. In an embodiment, at least one transporter polypeptide is derived from Annexin A2 or annexin II. In certain embodiments of the heteromultimer, all transporter polypeptides are derived from Annexin A2 or lipocortin II. In an embodiment, at least one transporter

polypeptide is derived from Annexin like protein. In certain embodiments of the heteromultimer, all transporter polypeptides are derived from Annexin like protein. In an embodiment, at least one transporter polypeptide is derived from the group comprising Annexin A1-Annexin A7. In an embodiment of the heteromultimer described herein, all transporter polypeptides are derived from the group comprising Annexin A1-Annexin A7.

14. In certain embodiments, the first annexin based transporter polypeptide has a sequence comprising SEQ ID NO:15, and the second annexin based transporter polypeptide has a sequence comprising SEQ ID NO: 16.

[00229] In some embodiments of the heteromultimer described herein, the transporter polypeptides are derivatives of transferrin. In an embodiment, at least one transporter polypeptide is derived from transferrin. In certain embodiments of the heteromultimer, at least one transporter polypeptides are derived from transferrin of SEQ ID NO: 19 or analog thereof. In certain embodiments of the heteromultimer, at least one transporter polypeptide is derived from a polypeptide seuquence homologous to the transferrin. In certain embodiments of the heteromultimer described herein, at least one transporter polypeptide is derived from apo-transferrin. In certain embodiments, the first transferrin based transporter polypeptide has a sequence comprising SEQ ID NO:15 and the second transferrin based transporter polypeptide has a sequence comprising SEQ ID NO: 16.Provided herein are heteromultimers, each heteromultimer comprising: at least a first monomeric protein that comprises at least one cargo polypeptide and a first transporter polypeptide; and at least a second monomeric protein that comprises at least one cargo polypeptide and a second transporter polypeptide, wherein said cargo polypeptides are selected from the proteins listed in Table 2, and wherein at least one transporter polypeptide is derived from a monomeric protein and wherein said transporter polypeptides self-assemble to form a quasi-native structure of said monomeric protein or analog thereof. In certain embodiments, are heteromultimers, each heteromultimer comprising: at least a first monomeric protein that comprises at least one cargo polypeptide and a first transporter polypeptide; and at least a second monomeric protein that comprises at least one cargo polypeptide and a second transporter polypeptide, wherein at least one at least one cargo polypeptide is an antibody, or fragment or variant thereof. In certain embodiments, all cargo polypeptides are antibodies or fragments or variants thereof. In certain embodiments, at least one cargo molecule attached to the first transporter polypeptide is the same as at least one cargo molecule attached to the second transporter polypeptide. In certain embodiments, the cargo molecules attached to the first

transporter polypeptide are different from the cargo molecule on the second transporter polypeptide. In certain embodiments, there are at least two cargo molecules attached to the first transporter polypeptide and at least two cargo molecule attached to the second transporter polypeptide. In certain embodiments the cargo molecules attached to the first transporter polypeptide are the same. In certain embodiments at least two cargo molecules attached to the first transporter polypeptide are different from each other. In certain embodiments at least two cargo molecules attached to the second transporter polypeptide are the same. In certain embodiments at least two cargo molecules attached to the second transporter polypeptide are different. In some embodiments, the antibody fragment comprises antibody Fc region. In some embodiments, the antibody is an immunoglobulin selected from the group consisting of IgG, IgA, IgD, IgE, and IgM. In certain embodiments, the IgG is of subtype selected from IgG1, IgG2a, IgG2b, IgG3 and IgG4. In certain embodiments, the antibody is a multispecific antibody. In some embodiments, the multispecific antibody is a bispecific antibody.

[00230] Provided herein are heteromultimers, each heteromultimer comprising: at least a first monomeric protein that comprises at least one cargo polypeptide and a first transporter polypeptide; and at least a second monomeric protein that comprises at least one cargo polypeptide and a second transporter polypeptide, wherein at least one cargo polypeptide is a therapeutic antibody. In some embodiments of the heteromultimers described herein, at least one cargo polypeptide is a therapeutic antibody or fragment or variant thereof, wherein the antibody is selected from antibody is selected from abagovomab, adalimumab, alemtuzumab, aurograb, bapineuzumab, basiliximab, belimumab, bevacizumab, briakinumab, canakinumab, catumaxomab, certolizumab pegol, certuximab, daclizumab, denosumab, efalizumab, galiximab, gemtuzumab ozagamicin, golimumab, ibritumomab tiuxetan, infliximab, ipilimumab, lumiliximab, mepolizumab, motavizumab, muromonab, mycograb, natalizumab, nimotuzumab, ocrelizumab, ofatumumab, omalizumab, palivizumab, panitumumab, pertuzumab, ranizumab, reslizumab, rituximab, teplizumab, toclizumab, tositumomab, trastuzumab, Proxinium, Rencarex, ustekinumab, and zalutumumab. In certain embodiments, the therapeutic antibody binds a cancer antigen.

[00231] Provided herein are heteromultimers, each heteromultimer comprising: at least a first monomeric protein that comprises at least one cargo polypeptide and a first transporter polypeptide; and at least a second monomeric protein that comprises at least one cargo polypeptide and a second transporter polypeptide, wherein at least one cargo

polypeptide is an enzyme, hormone, therapeutic polypeptide, antigen, chemotoxin, radiotoxin, cytokine or variant or fragment thereof.

[00232] Provided herein are heteromultimers, each heteromultimer comprising: at least a first monomeric protein that comprises at least one cargo polypeptide and a first transporter polypeptide; and at least a second monomeric protein that comprises at least one cargo polypeptide and a second transporter polypeptide, wherein the cargo polypeptide is attached to the transporter polypeptide by chemical conjugation, native ligation, chemical ligation, a disulfide bond or fusion.

[00233] Provided herein are host cells comprising nucleic acid encoding a heteromultimer described herein. In certain embodiments, the nucleic acid encoding the first monomeric protein and the nucleic acid encoding the second monomeric protein are present in a single vector. In certain embodiments, the nucleic acid encoding the first monomeric protein and the nucleic acid encoding the second monomeric protein are present in separate vectors.

[00234] Provided herein is a method of making a heteromultimer, wherein said method comprises: culturing a host cell described herein such that the nucleic acid encoding a heteromultimer described herein is expressed; and recovering the heteromultimer from the cell culture. In some embodiments, the host cell is a prokaryotic cell or a eukaryotic cell. In certain embodiments, the host cell is yeast cell. In some embodiments, the yeast is S. cerevisiae. In some embodiments, the yeast is glycosylation deficient, and/or protease deficient. In some embodiments, the host cell is a bacterial cell. In some embodiments, the host cell expressing a heteromultimer descried herein is a mammalian cell. In certain embodiments, the mammalian cell is a CHO cell, a BHK cell, NSO cell, COS cell or a human cell.

[00235] Provided is a pharmaceutical composition that comprises a heteromultimer described herein and a pharmaceutically acceptable adjuvant. Also provided are methods of treating an individual suffering from a disease or disorder, said method comprising administering to the individual an effective amount of a formulation or pharmaceutical composition described herein. In certain embodiments is a method of treating cancer in a patient, said method comprising administering to the patient a therapeutically effective amount of a heteromultimer described herein. In some embodiments is a method of treating an immune disorder in a patient, said method comprising administering to the patient a therapeutically effective amount of a heteromultimer described herein. Also provided is a method of treating an infectious disease in a patient, said method

comprising administering to the patient a therapeutically effective amount of a heteromultimer described herein. In certain embodiments is a method of treating a cardiovascular disorder in a patient, said method comprising administering to the patient a therapeutically effective amount of a heteromultimer described herein. In certain embodiments is a method of treating a respiratory disorder in a patient, said method comprising administering to the patient a therapeutically effective amount of a heteromultimer described herein.

[00236] Provided is a kit for detecting the presence of a biomarker of interest in an individual, said kit comprising (a) an amount of a heteromultimer described herein, wherein said heteromultimer comprises at least one cargo polypeptide such that said cargo polypeptide is capable of binding to the biomarker of interest; and (b) instructions for use.

[00237] Provided herein are heteromultimer proteins that comprise at least two monomeric proteins, wherein each monomeric protein comprises at least one cargo polypeptide, and an albumin based polypeptide, such that said monomeric proteins self-assemble to form the heteromultimer.

[00238] In certain embodiments, the cargo polypeptide is fused to the albumin or alloalbumin based polypeptide. In some embodiments, the cargo polypeptide is chemically conjugated to the albumin or alloalbumin based polypeptide. In certain embodiments, the cargo polypeptide is attached to the albumin or alloalbumin based polypeptide by means of chemical ligation or a disulfide bond.

[00239] Provided herein are heteromultimer proteins that comprise at least two monomeric proteins, wherein each monomeric protein comprises at least one cargo polypeptide, and an alloalbumin based polypeptide, such that said alloalbumin based polypeptides self-assemble to form the heteromultimer with a quasi-native structure of said alloalbumin or analog thereof.. In some embodiments, a heteromultimer described herein is a heterodimer. In some embodiments cargo polypeptide is an antibody, enzyme, hormone, therapeutic polypeptide, antigen, chemotoxin, radiotoxin, cytokine or variant or fragment thereof. In some embodiments, the cargo polypeptide of one monomeric protein functions in synergy with the cargo polypeptide of another monomeric protein.

[00240] In an aspect described herein is a method to derive protein segments from a protein of interest that can efficiently fold and selectively associate together to form an active quasi-native protein like structure.

[00241] Provided herein is a strategy for creating polypeptides based on a monomeric protein such as but not restricted to human serum albumin (HSA) that yield a quasi-native monomeric protein like structure and function when associated with each other. In embodiments described herein, this strategy is also used to design heteromultimers comprising monomeric polypeptides that comprise transporter polypeptides that are derivatives of HSA variants, alloalbumins other homologous albumin molecules from other species and also Annexin and Transferrin. The monomers described herein can be engineered using a variety of strategies to improve biophysical characteristics such as the stability of the individual transporter polypeptides or their associated complex.

[00242] In an embodiment is a scaffold for the development of bispecific or other multispecific or multifunctional protein molecules based on fragments derived from HSA.

[00243] Provided is a transporter polypeptide which is a HAS, HAA, Annexin or Transferrin derived scaffold that can be conjugated or fused with cargo polypeptides such as other functional domains such as antigen binding protein units, target substrates or inhibitors or payloads such as chemotoxins, radiotoxins, cytokines, etc. to achieve a multispecific or multifunctional therapeutic protein.

[00244] Described herein are fusions of heterodimeric Fc with transporter polypeptides based on HSA to yield bispecific antibody based therapeutics with sufficient purity and stability for pharmaceutical applications.

[00245] In an aspect, desribed herein is a method of deriving a multispecific or multifunctional protein comprising self-assembling monomers that comprise transporter polypeptides based on HSA, such that, the protein has a number of favorable pharmacokinetic properties including improved half-life, improved stability, low immunogenicity, etc.

[00246] Provided herein are heterodimer proteins that comprise at least two monomeric fusion proteins, wherein each monomeric fusion proteins comprises at least one cargo polypeptide fused to an albumin derived polypeptide, such that said albumin derived polypeptides self-assemble to form the multifunctional heterodimer with a quasi-native structure of albumin or an analog thereof.

[00247] In certain embodiments are heterodimer proteins that comprise at least two monomeric fusion proteins, wherein each monomeric fusion proteins comprises at least one cargo polypeptide fused to an alloalbumin derived polypeptide, such that said alloalbumin derived polypeptides self-assemble to form the multifunctional heterodimer.

[00248] In certain embodiments described herein are heteromultimer proteins that comprise at least two monomeric fusion proteins, wherein each monomeric fusion proteins comprises at least one cargo polypeptide fused to an alloalbumin derived polypeptide, such that said alloalbumin derived polypeptides self-assemble to form the multifunctional heterodimer. In certain embodiments are heterodimeric proteins comprising a first monomer which comprises at least one cargo polypeptide fused to an alloalbumin derived polypeptide; and a second monomer that comprises at least one cargo polypeptide fused to an alloalbumin derived polypeptide. In certain embodiments, the at least one cargo polypeptide of the first monomer is different from the at least one cargo polypeptide of the second monomer.

[00249] Provided herein is a heteromultimer that comprises: at least two monomers, each comprising a transporter polypeptide and optionally at least one cargo molecule attached to said transporter polypeptide, wherein each transporter polypeptide is obtained by segmentation of a whole protein such that said transporter polypeptides self-assemble to form quasi-native whole protein. In certain embodiments,the heteromultimer is multispecific. In certain embodiments, the transporter polypeptides are not derived from an antibody. In some embodiments, each monomer preferentially forms the heteromultimer as compared to a monomer or a homomultimer. In an embodiment of the heteromultimer, at least one cargo molecule is a theraputic agent, or a biomolecule. In some embodiments, at least one cargo molecule is a biomolecule which is selected from a polypeptide, DNA, PNA, or RNA.In some embodiments, each transporter polypeptide is a derivate of albumin or alloalbumin. In an embodiment, each transporter polypeptide is a derivate of annexin. In certain embodiments, each transporter polypeptide is a derivate of transferrin.

[00250] In certain embodiments are pharmaceutical formulations that comprise an albumin-based and/or alloalbumin-based heteromultimeric protein described herein and a pharmaceutically acceptable diluent or carrier. In certain embodiments, a formulation described herein is provided as part of a kit or container. In certain embodiments, the kit or container is packaged with instructions pertaining to extended shelf life of the therapeutic protein. In some embodiments, a heteromultimer described herein is used in a method of treating (e.g., ameliorating) preventing, or diagnosing a disease or disease symptom in an individual, comprising the step of administering said formulation to the individual.

[00251] Also provided are transgenic organisms modified to contain nucleic acid molecules described herein to encode and express monomeric fusion proteins described herein.

[00252] Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

EXAMPLES

Example 1: The Protein Splitting Method

[00253] Specific protein-protein association is driven by strong surface complementarity between interacting partners and the accompanying structural and thermodynamic changes. The surface complementarity provides an opportunity to form contacts that support the creation of favorable electrostatic and hydrophobic interactions. Electrostatic interactions involve the formation of salt bridges, hydrogen bonds and the pervasive dispersion interactions. Solvent exclusion and reorganization around non-polar atomic groups at the interface and its associated entropic effects play a role in the hydrophobic component of the binding thermodynamics. Residues with geometries that are optimized for hydrophobic interaction with one another will form contacts (i.e. stacking, pi-pi, cation-pi contacts favorable for stabilizing a protein-protein interface. Similar thermodynamic effects control multi-step protein folding processes that involve the pre-organization of secondary structural units and tertiary domains, which is followed by their association to form the folded quaternary state of the protein. An alternate mechanism to protein folding and binding involves a coupled protein folding and binding process that ultimately results in the quaternary state of the protein. In the context of protein association, the individual protein components need to be co-expressed or be present in the same medium and each of the components or monomers will stably fold into its final structural state only on association with its obligate partner. (Fig. 6)

[00254] Generation of a split protein involves recognizing a segmentation site in the native protein, using information from sequence, secondary structure and fold that will yield at least two transporter polypeptides that efficiently form the quasi-native protein structure by self-assembling to form a heteromultimer together. For example, these split protein transporter polypeptides selectively self-assemble and form the quasi-native state when co-expressed. While generating a split protein complementary pair of transporter polypeptides, in a way, the attempt is to emulate a number of naturally occurring obligate

protein-protein complexes that exhibit their functionality as a complex while being non-functional in their uncomplexed state. A successful implementation of the strategy results in polypeptides that selectively self-assemble to form heteromultimers with each other, are soluble as individual entities and for functional relevance, do not impair the folding, binding and activity of other components in the environment. The intrinsic nature of the polypeptides to reconstitute with each other has applications in area of creating heteromultimeric fusion entities out of cargo molecules that are not efficient at forming multimers by themselves. The functional role of the split protein segments is to act as transporter polypeptides that drive heteromultimerization.

Example 2:Preparation of HA/Alloalbumin based heteromultimer proteins

[00255] Shown is a method to determine the segmentation site along the HSA sequence and structure that will yield monomeric polypeptide chains that stably fold and fuse to form a quasi-native quaternary structure of the original protein. One of the critical requirements for such stable association is the formation of a large buried area of surface complementarity at the interface between the polypeptide chains. The native fold of the original protein provides indication of the natural complementarity of regions within the protein.

[00256] Figure 2 shows the solvent accessible surface area buried at the interface of two albumin-based polypeptides that would ideally fold into the quasi-native structure of HSA, when the segmentation point is moved along the protein sequence. The analysis indicates that a large surface area, of the order of about 5000 $Å^2$ is buried when the split segmentation is introduced anywhere between residues 30 and 520 with a few exceptions. Albumin has an exceptionally large number of disulphides bridges that contributes to the stability of the native protein structure. Section of the protein near residues 110, 190, 300, 390 and 500 provide sites for segmentation that do not split the residues involved in a disulphide link across the two transporter polypeptides. Segmentation in other regions would result in heterodimers with a cross linking disulphide bond between the two transporter polypeptide pairs. Figure 3 presents a model representation of one such quasi-native albumin structure derived by removal of loop from residues 293 to 313 in the HSA sequence. The total buried surface area for the two albumin based polypeptides of SEQ ID No. 2, and SEQ ID No: 3 shown herein is approximately 5500 $Å^2$. This is considerably larger than the average of 1910 - 3880 $Å^2$ observed in a number of protein-protein heterodimeric and homodimeric co-complex structures [Bahadhur R.P. & Zacharias M.

(2008) *Cell Mol Life Sci* **65**, 1059-1072]. This suggests that there is a strong likelihood for the two polypeptides to selectively associate with each other if the folding pathway of the two polypeptides is fairly independent of each other.

[00257] In an aspect of this invention, selective formation of a stable quasi-native structure with the two polypeptides (the pair formed by SEQ ID No. 2 and SEQ ID No. 3 or the transporter pair formed by SEQ ID No. 8 and SEQ ID No. 10) gives us the opportunity to employ these polypeptides to drive the formation of bispecific or other multifunctional molecules after fusing the appropriate cargo proteins of interest to the N or C terminus of the albumin based polypeptides employed as transporter polypeptides. A number of other alternate segmentation patterns resulting in transportation polypeptide pair heterodimer can be designed. The fused cargo proteins can be antigen binding domains or other payloads such as chemotoxins, radiotoxins or cytokines (as represented in Figure 4). The resulting heterodimers have many of the favorable properties intrinsic to HSA including properties like improved half-life, stability and low immunogenicity. Traditional linkers such as $(Gly_4Ser)_x$ can be used for the association of the cargo protein with the transporter polypeptide.

[00258] In another aspect of this invention, each of the HSA based transporter polypeptides is fused independently to the C-terminus of two heavy chains in a bispecific Fc molecule (as represented in Figure 5). The strong and selective pairing of the two transporter polypeptides (such as SEQ ID No. 2, and SEQ ID No. 3) drives the selectively heterodimerization of the Fc and also contribute to its stability and other valuable pharmacokinetic properties.

[00259] Serum albumin preprotein NP_000468.1 GI 4502027 mRNA sequence from NM_000477.5, Consensus CDS (CCDS) ID 3555.1

[00260] SEQ ID No. 4: Residue 1-24 (EFATMAVMAPRTLVLLLSGALALTQTWAG) is the N-terminal export signal sequence region that gets cleaved. This sequence fulfills the same role as the natural signal sequence but it's optimized for mammalian and CHO cell lines.

[00261] SEQ ID No. 1: gi|4502027|ref|NP_000468.1| serum albumin preproprotein [Homo sapiens]

EFATMAVMAPRTLVLLLSGALALTQTWAGDAHKSEVAHRFKDLGEENFKAL
VLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDK
LCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMC
TAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKA

ACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKA
EFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKE
CCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLG
MFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKP
LVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNL
GKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESL
VNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVEL
VKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETC**F**AEEGKKLVAASQAAL
GL

[00262] SEQ ID No. 5: Human serum albumin nucleotide CCDS Sequence (1852 nt)

[00263] GAATTCGCCACTATGGCTGTGATGGCCCCTAGGACCCTGGTGCTGCT
GCTGTCCGGAGCTCTGGCTCTGACTCAGACCTGGGCTGGAGATGCACACAA
GAGTGAGGTTGCTCATCGGTTTAAAGATTTGGGAGAAGAAAATTTCAAAGC
CTTGGTGTTGATTGCCTTTGCTCAGTATCTTCAGCAGTGTCCATTTGAAGA
TCATGTAAAATTAGTGAATGAAGTAACTGAATTTGCAAAAACATGTGTTGC
TGATGAGTCAGCTGAAAATTGTGACAAATCACTTCATACCCTTTTTGGAGA
CAAATTATGCACAGTTGCAACTCTTCGTGAAACCTATGGTGAAATGGCTGA
CTGCTGTGCAAAACAAGAACCTGAGAGAAATGAATGCTTCTTGCAACACAA
AGATGACAACCCAAACCTCCCCCGATTGGTGAGACCAGAGGTTGATGTGAT
GTGCACTGCTTTTCATGACAATGAAGAGACATTTTTGAAAAAATACTTATA
TGAAATTGCCAGAAGACATCCTTACTTTTATGCCCCGGAACTCCTTTTCTT
TGCTAAAAGGTATAAAGCTGCTTTTACAGAATGTTGCCAAGCTGCTGATAA
AGCTGCCTGCCTGTTGCCAAAGCTCGATGAACTTCGGGATGAAGGGAAGGC
TTCGTCTGCCAAACAGAGACTCAAGTGTGCCAGTCTCCAAAAATTTGGAGA
AAGAGCTTTCAAAGCATGGGCAGTAGCTCGCCTGAGCCAGAGATTTCCCAA
AGCTGAGTTTGCAGAAGTTTCCAAGTTAGTGACAGATCTTACCAAAGTCCA
CACGGAATGCTGCCATGGAGATCTGCTTGAATGTGCTGATGACAGGGCGGA
CCTTGCCAAGTATATCTGTGAAAATCAAGATTCGATCTCCAGTAAACTGAA
GGAATGCTGTGAAAAACCTCTGTTGGAAAAATCCCACTGCATTGCCGAAGT
GGAAAATGATGAGATGCCTGCTGACTTGCCTTCATTAGCTGCTGATTTTGT
TGAAAGTAAGGATGTTTGCAAAAACTATGCTGAGGCAAAGGATGTCTTCCT
GGGCATGTTTTTGTATGAATATGCAAGAAGGCATCCTGATTACTCTGTCGT
GCTGCTGCTGAGACTTGCCAAGACATATGAAACCACTCTAGAGAAGTGCTG
TGCCGCTGCAGATCCTCATGAATGCTATGCCAAAGTGTTCGATGAATTTAA

ACCTCTTGTGGAAGAGCCTCAGAATTTAATCAAACAAAATTGTGAGCTTTT
TGAGCAGCTTGGAGAGTACAAATTCCAGAATGCGCTATTAGTTCGTTACAC
CAAGAAAGTACCCCAAGTGTCAACTCCAACTCTTGTAGAGGTCTCAAGAAA
CCTAGGAAAAGTGGGCAGCAAATGTTGTAAACATCCTGAAGCAAAAAGAAT
GCCCTGTGCAGAAGACTATCTATCCGTGGTCCTGAACCAGTTATGTGTGTT
GCATGAGAAAACGCCAGTAAGTGACAGAGTCACCAAATGCTGCACAGAATC
CTTGGTGAACAGGCGACCATGCTTTTCAGCTCTGGAAGTCGATGAAACATA
CGTTCCCAAAGAGTTTAATGCTGAAACATTCACCTTCCATGCAGATATATG
CACACTTTCTGAGAAGGAGAGACAAATCAAGAAACAAACTGCACTTGTTGA
GCTCGTGAAACACAAGCCCAAGGCAACAAAAGAGCAACTGAAAGCTGTTAT
GGATGATTTCGCAGCTTTTGTAGAGAAGTGCTGCAAGGCTGACGATAAGGA
GACCTGCTTTGCCGAGGAGGGTAAAAAACTTGTTGCTGCAAGTCAAGCTGC
CTTAGGCTTATGA

[00264] The protein and nucleotide sequence of albumin based polypeptides useful as transporter polypeptides are as follows:

[00265] **Albumin based heteromultimer 1:**

[00266] Albumin based Transporter polypeptide 1-Ver 1: SEQ ID No. 2:

[00267] DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVT
EFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPER
NECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYF
YAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKC
ASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLL
ECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEV

[00268] Nucleotide sequence encoding Albumin based Transporter polypeptide 1-Ver 1: SEQ ID No. 6:

[00269] GATGCACACAAGAGTGAGGTTGCTCATCGGTTTAAAGATTTGGGAGA
AGAAAATTTCAAAGCCTTGGTGTTGATTGCCTTTGCTCAGTATCTTCAGCA
GTGTCCATTTGAAGATCATGTAAAATTAGTGAATGAAGTAACTGAATTTGC
AAAAACATGTGTTGCTGATGAGTCAGCTGAAAATTGTGACAAATCACTTCA
TACCCTTTTTGGAGACAAATTATGCACAGTTGCAACTCTTCGTGAAACCTA
TGGTGAAATGGCTGACTGCTGTGCAAAACAAGAACCTGAGAGAAATGAATG
CTTCTTGCAACACAAAGATGACAACCCAAACCTCCCCCGATTGGTGAGACC
AGAGGTTGATGTGATGTGCACTGCTTTTCATGACAATGAAGAGACATTTTT

GAAA**AAA**TACTTATATGAAATTGCCAGAAGACATCCTTACTTTTATGCCCC
GGAACTCCTTTTCTTTGCTAAAAGGTATAAAGCTGCTTTTACAGAATGTTG
CCAAGCTGCTGATAAAGCTGCCTGCCTGTTGCCAAAGCTCGATGAACTTCG
GGATGAAGGGAAGGCTTCGTCTGCCAAACAGAGACTCAAGTGTGCCAGTCT
CCAAAAATTTGGAGAAAGAGCTTTCAAAGCATGGGCAGTAGCTCGCCTGAG
CCAGAGATTTCCCAAAGCTGAGTTTGCAGAAGTTTCCAAGTTAGTGACAGA
TCTTACCAAAGTCCACACGGAATGCTGCCATGGAGATCTGCTTGAATGTGC
TGATGACAGGGCGGACCTTGCCAAGTATATCTGTGAAAATCAAGATTCGAT
CTCCAGTAAACTGAAGGAATGCTGTGAAAAACCTCTGTTGGAAAAATCCCA
CTGCATTGCCGAAGTGTGA

[00270] Albumin based Transporter polypeptide 2-Ver1: SEQ ID No. 3:

[00271] SLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLA
KTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEY
KFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDY
LSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFN
AETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAF
VEKCCKADDKETCFAEEGKKLVAASQAALGL

[00272] Nucleotide sequence encoding Albumin based Transporter polypeptide 2-Ver1: SEQ ID No. 7:

[00273] TCATTAGCTGCTGATTTTGTTGAAAGTAAGGATGTTTGCAAAAACTA
TGCTGAGGCAAAGGATGTCTTCCTGGGCATGTTTTTGTATGAATATGCAAG
AAGGCATCCTGATTACTCTGTCGTGCTGCTGCTGAGACTTGCCAAGACATA
TGAAACCACTCTAGAGAAGTGCTGTGCCGCTGCAGATCCTCATGAATGCTA
TGCCAAAGTGTTCGATGAATTTAAACCTCTTGTGGAAGAGCCTCAGAATTT
AATCAAACAAAATTGTGAGCTTTTTGAGCAGCTTGGAGAGTACAAATTCCA
GAATGCGCTATTAGTTCGTTACACCAAGAAAGTACCCCAAGTGTCAACTCC
AACTCTTGTAGAGGTCTCAAGAAACCTAGGAAAAGTGGGCAGCAAATGTTG
TAAACATCCTGAAGCAAAAAGAATGCCCTGTGCAGAAGACTATCTATCCGT
GGTCCTGAACCAGTTATGTGTGTTGCATGAGAAAACGCCAGTAAGTGACAG
AGTCACCAAATGCTGCACAGAATCCTTGGTGAACAGGCGACCATGCTTTTC
AGCTCTGGAAGTCGATGAAACATACGTT**CCC**AAAGAGTTTAATGCTGAAAC
ATTCACCTTCCATGCAGATATATGCACACTTTCTGAGAAGGAGAGACAAAT
CAAGAAACAAACTGCACTTGTTGAGCTCGTGAAACACAAGCCCAAGGCAAC
AAAAGAGCAACTGAAAGCTGTTATGGATGATTTCGCAGCTTTTGTAGAGAA

GTGCTGCAAGGCTGACGATAAGGAGACCTGCTTTGCCGAGGAGGGTAAAAA
ACTTGTTGCTGCAAGTCAAGCTGCCTTAGGCTTATGA

[00274] **Albumin based heteromultimer 2:**

[00275] Albumin based Transporter polypeptide 1-Ver 2: SEQ ID No. 8:

[00276] DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVT
EFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPER
NECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYF
YAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKC
ASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLL
ECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADL
PSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARA

[00277] Nucleotide sequence encoding Albumin based Transporter polypeptide 1-Ver 2: SEQ ID No. 9:

[00278] GATGCACACAAGAGTGAGGTTGCTCATCGGTTTAAAGATTTGGGAGA
AGAAAATTTCAAAGCCTTGGTGTTGATTGCCTTTGCTCAGTATCTTCAGCA
GTGTCCATTTGAAGATCATGTAAAATTAGTGAATGAAGTAACTGAATTTGC
AAAAACATGTGTTGCTGATGAGTCAGCTGAAAATTGTGACAAATCACTTCA
TACCCTTTTTGGAGACAAATTATGCACAGTTGCAACTCTTCGTGAAACCTA
TGGTGAAATGGCTGACTGCTGTGCAAAACAAGAACCTGAGAGAAATGAATG
CTTCTTGCAACACAAAGATGACAACCCAAACCTCCCCCGATTGGTGAGACC
AGAGGTTGATGTGATGTGCACTGCTTTTCATGACAATGAAGAGACATTTTT
GAAAAAATACTTATATGAAATTGCCAGAAGACATCCTTACTTTTATGCCCC
GGAACTCCTTTTCTTTGCTAAAAGGTATAAAGCT**GCT**TTTACAGAATGTTG
CCAAGCTGCTGATAAAGCTGCCTGCCTGTTGCCAAAGCTCGATGAACTTCG
GGATGAAGGGAAGGCTTCGTCTGCCAAACAGAGACTCAAGTGTGCCAGTCT
CCAAAAATTTGGAGAAAGAGCTTTCAAAGCATGGGCAGTAGCTCGCCTGAG
CCAGAGATTTCCCAAAGCTGAGTTTGCAGAAGTTTCCAAGTTAGTGACAGA
TCTTACCAAAGTCCACACGGAATGCTGCCATGGAGATCTGCTTGAATGTGC
TGATGACAGGGCGGACCTTGCCAAGTATATCTGTGAAAATCAAGATTCGAT
CTCCAGTAAACTGAAGGAATGCTGTGAAAAACCTCTGTTGGAAAAATCCCA
CTGCATTGCCGAAGTGGAAAATGATGAGATGCCTGCTGACTTGCCTTCATT
AGCTGCTGATTTTGTTGAAAGTAAGGATGTTTGCAAAAACTATGCTGAGGC
AAAGGATGTCTTCCTGGGCATGTTTTTGTATGAATATGCAAGAGCATGA

[00279] Albumin based Transporter polypeptide 2-Ver 2: SEQ ID No. 10:

[00280] SVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLI KQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCK HPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSA LEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATK EQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQAALGL

[00281] Nucleotide sequence encoding Albumin based Transporter polypeptide 2-Ver 2: SEQ ID No. 11:

[00282] TCTGTCGTGCTGCTGCTGAGACTTGCCAAGACATATGAAACCACTCT AGAGAAGTGCTGTGCCGCTGCAGATCCTCATGAATGCTATGCCAAAGTGTT CGATGAATTTAAACCTCTTGTGGAAGAGCCTCAGAATTTAATCAAACAAAA TTGTGAGCTTTTTGAGCAGCTTGGAGAGTACAAATTCCAGAATGCGCTATT AGTTCGTTACACCAAGAAAGTACCCCAAGTGTCAACTCCAACTCTTGTAGA GGTCTCAAGAAACCTAGGAAAAGTGGGCAGCAAA**TGT**TGTAAACATCCTGA AGCAAAAAGAATGCCCTGTGCAGAAGACTATCTATCCGTGGTCCTGAACCA GTTATGTGTGTTGCATGAGAAAACGCCAGTAAGTGACAGAGTCACCAAATG CTGCACAGAATCCTTGGTGAACAGGCGACCATGCTTTTCAGCTCTGGAAGT CGATGAAACATACGTTCCCAAAGAGTTTAATGCTGAAACATTCACCTTCCA TGCAGATATATGCACACTTTCTGAGAAGGAGAGACAAATCAAGAAACAAAC TGCACTTGTTGAGCTCGTGAAACACAAGCCCAAGGCAACAAAAGAGCAACT GAAAGCTGTTATGGATGATTTCGCAGCTTTTGTAGAGAAGTGCTGCAAGGC TGACGATAAGGAGACCTGCTTTGCCGAGGAGGGTAAAAAACTTGTTGCTGC AAGTCAAGCTGCCTTAGGCTTATGA

[00283] **Generation and Expression of HA or HAA based heteromultimers**

[00284] The genes encoding the full length WT HA and the HA based transporter polypeptide monomers were constructed via gene synthesis using codons optimized for human/mammalian expression. The constructs were designed from known full-length Human Serum Albumin Preprotein (GENEBANK: NP_000468.1), after exclusion of the signal sequence EFATMAVMAPRTLVLLLSGALALTQTWAG. The final gene products were subcloned into the mammalian expression vector pTT5 (NRC-BRI, Canada) (Durocher et al). High level and high-throughput recombinant protein production by transient transfection of suspension-growing human CHO-3E7 was performed. See *Table 3* for construct boundaries of the two scaffolds described here: Albumin based heteromultimer 1 (ABH1) and Albumin based heteromultimer 2 (ABH2). Albumin based heteromultimer 2 comprises one disulfide bond between the two transporter polypeptides,

while Albumin based heteromultimer 1 is formed entirely by non-covalent interactions. Figure 6A provides SDS-PAGE (non-reducing) gel analysis of the two heteromultimer constructs (ABH1 and ABH2), after co-expression (different DNA transfection ratios are shown). WT full-length HSA is shown as control. As expected, ABH2 retains the disulfide linkage in non-reducing SDS-PAGE, with a MW roughly double the non-disulfide linked ABH1. Figure 6B provides Native gel analysis of the two Albumin based heteromultimer constructs (ABH1 and ABH2), after co-expression (1:1 DNA level). WT full-length HSA is shown as control. ABH1 and ABH2 both form a complex of expected mass, comparable to the full-length WT HSA. Furthermore, upon expression, neither the transporter polypeptides forming ABH1 nor the ones forming ABH2 homodimerize; rather they preferably form a stable hetercomplex. See *Table 3* below for details.

[00285] **Table 3: Albumin based heteromultimer constructs**

Construct	Segment Boundaries*	MW (KDa)
Wild Type HA	1:585 (SEQ ID NO: 1)	64.3
ABH1	1:293 (SEQ ID NO: 2)	32.2
	304:585 (SEQ ID NO: 3)	30.9
ABH2	1:337 (SEQ ID NO: 8)	37
	342:585 (SEQ ID NO: 10)	26.7

[00286] WT-HSA and the two Albumin based heteromultimers (ABH1 and ABH2) were expressed in CHO-3E7 cell line grown in suspension in FreeStyle F17 medium (Invitrogen) supplemented with 0.1% w/v pluronic and 4 mM glutamine. The day of transfection cell density should be around 1.5-2 million cells/ml and viability must be greater than 97%. Transfection is done according to patent application WO 2009/137911 using a mixture of plasmid DNA made of 5% pTTo-GFP plasmid (green fluorescent protein to determine transfection efficiency, *Table 4*), 15% pTT22-AKT plasmid, 21 % HSA plasmids (10.63% of each), 68.37% of Salmon Sperm DNA. Following transfection, the shake flask containing cells is then placed on an orbital shaker set to 120 rpm in a humidified incubator with 5% CO2 at 37°C. Twenty-four hours post-transfection, 1 % w/v TN1 and 0.5 mM VPA (Valproic acid) are added to the cultures. The cultures are then transferred on an orbital shaker (120 rpm) placed in a humidified incubator with 5% CO2 set at 32°C. At 24-48 hours, GFP positive cells should be between 30-60% as determined by flow cytometry. Cells were harvested 7 days post-transfection and spun at 4,000 rpm for 20 minutes. The supernatant was filter-sterlized (clarified) using a 0.45 μm

filter (Millipore). Keep the supernatant at 4 °C for short period storage and at -80 °C for long period storage. Prior to purification, the frozen supernatant was thawed at 37 °C, re-filtered and degassed through a 0. 45 μm membrane filter under vacuum for 5 – 10 minutes.

[00287] **Table4:** Cell viability at different stages of expression for WT and ABH1 construct.

HSA scaffold	% GFP 48 hrs post-transfection	% viability 48 hrs post-transfection	% viability 48 hrs post-transfection
Wild Type HSA	67	94.6	72.3
ABH2	66.3	93.6	77.1

[00288] **Purification of HSA and heteromultimers ABH1 and ABH2**

[00289] Purification was performed by gravity flow using a bench-top QIAGEN-tip 500 column packed with a Blue Sepharose matrix (GE Healthcare). The Blue Sepharose matrix was equilibrated with 20 ml of PBS pH 7.2. The sample was loaded at a flow rate of 5 ml/min and subsequently washed with with 20 ml of PBS. The protein was eluted with 0.1 M Na2HPO4 pH 7.2 supplemented with 1 M NaCl and collected in 1 ml fractions (20 ml total). Fractions containing HSA (as per Bradford protein assay) were pooled, and applied on a HiLoad 16/60 Superdex 200 prep grade gel filtration column coupled to an AKTA Express system (GE Healthcare) using a flow rate of 1 ml/ml. Protein with a purity of >85% was collected; fractions containing pure sample were pooled and concentrated by centrifugation using an Amicon Ultra membrane with a cutoff weight of 10 000 MWCO. Figure 6C shows SDS-PAGE (non-reducing) analysis of the ABH2 heteromultimer and WT HSA, both after the final stage of purification. Both constructs show the expected MW.

[00290] **Stability determination of Albumin based Heteromultimers using Differential Scanning Calorimetry (DSC)**

[00291] All DSC experiments were carried out using a GE or MicroCal VP-Capillary instrument. The proteins were buffer-exchanged into PBS (pH 7.4) and diluted to 0.3 to 0.7mg/mL with 0.137mL loaded into the sample cell and measured with a scan rate of 1°C/min from 20 to 100°C. Data was analyzed using the Origin software (GE Healthcare) with the PBS buffer background subtracted. See Table 5 and Figure 7 for resulting melting temperature determined.

Table 5: Melting temperature for Albumin based heteromultimers

Molecule	Measured Mass (Da)	Theoretical MW (Da)	Tm° C
HSA Wild Type	66620	66470	75
ABH2	66100	65880	63

[00292] Evaluation of FcRn Binding of HSA and ABH2 using Surface Plasmon Resonance

[00293] As seen in Figures 8A-B, when HSA and a HSA-based heteromultimer are immobilized on the SPR surface, affinity towards FcRn appears to be comparable between the full length WT HSA and ABH2, indicating FcRn binding functionality of albumin is retained by the heteromultimer formed by the self-assembly of albumin based transporter polypeptides. The following Table 6 illustrates FcRn binding data. Values in parenthesis refer to standard deviation.

[00294] **Table 6:** Kinetic and Equilibrium fit of FcRn Binding of HSA and ABH2 using Surface Plasmon Resonance

	Ka (1/Ms) Grouped Fitted	Kd (1/s) Grouped Fitted	KD (M) Grouped Fitted	
HAS	5.3E+04 (7E+03)	7.0E-02 (2.0E-02)	1.4E-06 (6.0E-07)	Kinetic fit
ABH2	5.0E+04 (4E+03)	4.2E-02 (8.0E-03)	8.0E-07 (2.0E-07)	Kinetic fit
HAS			9.0E-07 (1.0E-07)	Equilibrium Fit
ABH2			9.0E-07 (1.0E-07)	Equilibrium Fit

[00295]

[00296] Example 3 Generation and Expression of Albumin based heteromultimers with mono- and tetravalency comprising anti-Her2/neu and anti-CD16 scFv bioactive fusions.

[00297] Multivalent heteromultimer ABH2 was generated by expressing its single monomeric transporter polypeptides, SEQ ID NO: 8 and SEQ ID NO: 10, fused at one or both termini to cargo polypeptides that are either antiHer2scFv (4D5) and/or anti-CD16 scFv (NM3E). These form a set of 8 base construct monomers based off transporter polypeptide 1 and 8 base construct monomers based off transporter polypeptide 2. Different combinations of these base constructs were combined upon co-expression to form heteromultimers displaying all combination of the two cargo polypeptides at any of

the four terminal positions of the two transporter polypeptides, ranging from monovalent to tetravalent.

[00298] As shown in Figure 9, the bioactive cargo polypeptides were fused to the heteromultimer transporter polypeptides via a GGSG linker, for the N terminus of one monomer and a longer (GGS)4GG linker for all other termini in the other monomer.

[00299] **Table 7** illustrates the 16 base constructs (Base construct #1-Base construct #16) that were generated by fusing the 4D5 and NM3 cargo polypeptides to either N or C terminus of transporter polypeptide 1 (F1) or transporter polypeptide 2 (F2). F1: corresponds to SEQ ID 8 and F2 corresponds to SEQ ID 10.

Single fusions

#	Fusion 1	Fusion 2
1	NM3E2	F1
2	F1	NM3E2
3	NM3E2	F2
4	F2	NM3E2
5	4D5	F1
6	F1	4D5
7	4D5	F2
8	F2	4D5

Double fusions

#	Fusion 1	Fusion 2	Fusion 3
9	NM3E2	F1	NM3E2
10	NM3E2	F2	NM3E2
11	4D5	F1	4D5
12	4D5	F2	4D5
13	NM3E2	F1	4D5
14	4D5	F1	NM3E2
15	NM3E2	F2	4D5
16	4D5	F2	NM3E2

[00300]

[00301] Multivalent constructs were generated as outlined in Example 2 using heteromultimer ABH2. The final gene products were subcloned into the mammalian expression vector pTT5 (NRC-BRI, Canada) (Durocher et al). High level and high-throughput recombinant protein production by transient transfection of suspension-growing human CHO-3E7 was performed.

Purification was performed by application of the cellular supernatant with exopressed protein to a QIAGEN-tip 500 column packed with Blue Sepharose matrix (GE Healthcare) coupled to an AKTA Express system (GE Healthcare) using a flow rate of 1

ml/ml. The column was equilibrated with equilibrated with sample buffer composed of 20 ml of PBS pH 7.2, 300 mM NaCl. The sample was loaded at a flow rate of 5 ml/min and subsequently washed with sample buffer. The protein was eluted by applicatuion of NaCl gradient ranging from 300 mM to 2000 mM. Fractions eluting in higher salt concentration were the purest and were pooled, concentrated and subsequently applied to a HiLoad 16/60 Superdex 200 prep grade gel filtration column coupled to an AKTA Express system (GE Healthcare) using a flow rate of 1 ml/ml. Protein with a purity of >85% was collected; fractions containing pure sample were pooled and concentrated by centrifugation using an Amicon Ultra membrane with a cutoff weight of 10 000 MWCO. Figures 10A-10B shows SDS-PAGE (non-reducing) analysis of the ABH2 heteromultimer fused to different cargo polypeptides. The position of those polypeptides in the heteromultimer relative to the transporter polypeptides is outlined in table 8 below. All constructs showed the expected molecular weight.

[00302] **Table 8:** Monovalent, multivalent, and multispecific constructs that were generated by fusing the 4D5 and NM3 cargo polypeptides to either N or C terminus of transporter polypeptide 1 or transporter polypeptide 2 of ABH2.

[00303]

Variant	**N terminus-transporter polypeptide 1 (SEQ ID No: 8)**	**C terminus-transporter polypeptide 1 (SEQ ID No: 8)**	**N terminus-transporter polypeptide 2 (SEQ ID No: 10)**	**C terminus-transporter polypeptide 2 (SEQ ID No: 10)**	**Valency**
513	NM3E				monovalent
514		NM3E			monovalent
515			**NM3E**		**monovalent**
516				NM3E	monovalent
517	4D5				monovalent
518		4D5			monovalent
519			4D5		monovalent
520				4D5	monovalent
521	NM3E		NM3E		bivalent
522	NM3E			NM3E	bivalent
523		NM3E	NM3E		bivalent
524		NM3E		NM3E	bivalent
525	4D5		4D5		bivalent
526	4D5			4D5	bivalent
527		4D5	4D5		bivalent

528		4D5		4D5	bivalent
529	NM3E	NM3E			bivalent
530			NM3E	NM3E	bivalent
531	4D5	4D5			bivalent
532			4D5	4D5	bivalent
543	NM3E		4D5		bispecific
544	NM3E			4D5	bispecific
545		NM3E	4D5		bispecific
546		NM3E		4D5	bispecific
547	4D5		NM3E		bispecific
548		4D5	NM3E		bispecific
549	4D5			NM3E	bispecific
550		4D5		NM3E	bispecific
551	NM3E	4D5			bispecific
552	4D5	NM3E			bispecific
553			NM3E	4D5	bispecific
554			4D5	NM3E	bispecific
593	4D5			NM3E	bispecific
594	NM3E			4D5	bispecific

[00304]

[00305] **SPR binding of monovalent ABH2 fused to a single antiCD16scFv**

[00306] Purified heteromultimer ABH2 fused to a single antiCD16scFv to the N terminus of transporter polypeptide SEQ ID 2 (construct v515) was used in a binding experiment using Surface Plasmon Resonance (SPR). Soluble CD16 was covalently immobilized onto a CM5 surface and ABH2 fused to antiCD16scFv was captured and binding kinetics were determined.

[00307] *SPR supplies.* GLM sensorchips, the Biorad ProteOn amine coupling kit (EDC, sNHS and ethanolamine), and 10mM sodium acetate buffers were purchased from Bio-Rad Laboratories (Canada) Ltd. (Mississauga, ON). Recombinant Her-2 protein was purchased from eBioscience (San Diego, CA). HEPES buffer, EDTA, and NaCl were purchased from from Sigma-Aldrich (Oakville, ON). 10% Tween 20 solution was purchased from Teknova (Hollister, CA).

[00308] *SPR biosensor assays.* All surface plasmon resonance assays were carried out using a BioRad ProteOn XPR36 instrument (Bio-Rad Laboratories (Canada) Ltd. (Mississauga, ON)) with HBST running buffer (10mM HEPES, 150 mM NaCl, 3.4 mM EDTA, and 0.05% Tween 20 pH 7.4) at a temperature of 25°C. The CD16 capture surface was generated using a GLM sensorchip activated by a 1:5 dilution of the standard

BioRad sNHS/EDC solutions injected for 300 s at 30 μL/min in the analyte (horizontal) direction. Immediately after the activation, a 4.0 μg/mL solution of CD16 in 10 mM NaOAc pH 4.5 was injected in the ligand (vertical) direction at a flow rate of 25 μL/min until approximately 3000 resonance units (RUs) were immobilized. Remaining active groups were quenched by a 300 s injection of 1M ethanolamine at 30 μL/min in the analyte direction, and this also ensures mock-activated interspots are created for blank referencing.

[00309] A 500nM 3-fold dilution series of V515 was injected over 3000 RUs CD16aWT (L6) compared to blank (L5). Flow rate 50 uL / min for 120s, with a 240s disassociation phase. Injections were repeated in standard running buffer (DPBS/3.4mM EDTA/0.05% Tween20) and running buffer with an additional 350mM NaCl. Sensorgrams were aligned and double-referenced using the buffer blank injection and interspots, and the resulting sensorgrams were analyzed using ProteOn Manager software v3.0. Typically, K_D values were determined from binding isotherms using the Equilibrium Fit model. For high affinity interactions with slow off-rates, kinetic and affinity values were additionally determined by fitting the referenced sensorgrams to the 1:1 Langmuir binding model using local R_{max}, and affinity constants (K_D M) were derived from the resulting rate constants (k_d s^{-1}/ k_a $M^{-1}s^{-1}$). All K_D values are reported as the mean and standard deviation from three independent runs.

[00310] As shown in Table 9, ABH2 heteromultimer fused to a single antiCD16scFv has full activity and binds its target with good reproducibility and KD similar to the free anti CD16 scFv (NM3E).

[00311] Table 9: SPR data for monovalent ABH2 fused to a single antiCD16scFv.

	Injection #1			Injection #2				
	ka	kd	KD	ka	kd	KD	KD (M) Ave	KD SD
	1/Ms	1/s	M	1/Ms	1/s	M		
NM3E	5.37E+04	5.76E-03	1.07E-07	5.89E+04	6.03E-03	1.02E-07	1.05E-07	4.E-09
V515 Dec	6.11E+04	6.71E-03	1.10E-07					
V515 Jan	5.56E+04	7.30E-03	1.31E-07					

Example 4 Preparation of HA or HAA based heteromultimer proteins wherein cargo protein(s) comprise one or more EGF-A like domain.

[00312] The peptide sequence of the EGF-A domain in PCSK9 protein or another polypeptide sequence homologous to the EGF-A domain, capable of specifically binding the low density lipoprotein receptor (LDL-R) is derived by sequencing or from a database such as GenBank. The cDNA for the cargo polypeptide comprising EGF-A like domain is isolated by a variety of means including but not exclusively, from cDNA libraries, by RT-

PCR and by PCR using a series of overlapping synthetic oligonucleotide primers, all using standard methods. In certain examples, the cargo protein is engineered to improve stability, target binding features or other biophysical or therapeutically relevant properties. The polypeptide is employed as the cargo protein in the creation of a heteromultimer with application in the treatment of hypercholesterolemia. The first and second monomeric fusion polypeptide sequence is derived by fusing the cargo protein sequence directly or with an intermediate linker peptide to the N-terminus and/or C-terminus of HA or HAA based transporter polypeptide such as SEQ ID No: 2, SEQ ID NO: 3, SEQ ID NO: 8 or SEQ ID NO: 10. This monomeric fusion protein sequence is reverse translated to its corresponding DNA sequence to be introduced in an expression vector, sequence optimized for expression in a particular cell line of interest. The first and second monomeric fusion proteins are transfected and coexpressed in the cell line of interest. In certain cases, the transfection is in 1:1 ratio for the two vectors. In some examples, the ratio is selected from 1.5:1, 2:1, 1:1.5, 1:2 etc.

Example 5 Preparation of HA or HAA based heteromultimeric proteins wherein cargo protein(s) are the GLP-1 and/or Glucagon.

[00313] The peptide sequence of GLP-1 or another polypeptide sequence homologous to this peptide, capable of specifically binding the GLP-1 receptor or acting as a GLP-1 agonist is derived by sequencing or from a database such as GenBank. Alternately, the peptide sequence of Glucagon or another polypeptide sequence homologous to this peptide, capable of specifically binding the Glucagon receptor or acting as a Glucagon receptor agonist is derived by sequencing or from a database such as GenBank. The cDNA for each cargo polypeptide comprising GLP-1 or Glucagon is isolated by a variety of means including but not exclusively, from cDNA libraries, by RT-PCR and by PCR using a series of overlapping synthetic oligonucleotide primers, all using standard methods. In certain examples, these GLP-1 or Glucagon based cargo polypeptides are engineered to improve stability, target binding features or other biophysical or therapeutically relevant properties. These GLP-1 and Glucagon based polypeptides are employed as one or more cargo molecules in the creation of a heteromultimer with application in the treatment of type-2 diabetes or another disease related to glucose metabolism. The first and second monomeric fusion polypeptide sequence is derived by fusing the cargo protein sequence directly or with an intermediate linker peptide to the N-terminus and/or C-terminus of HA or HAA based transporter polypeptide such as SEQ ID

No: 2, SEQ ID NO: 3, SEQ ID NO: 8 or SEQ ID NO: 10. The fusion proteins can be monospecific with either GLP-1 or Glucagon like polypeptides or be bispecific (coagonist) with both the GLP-1 and Glucagon like polypeptides. Each monomeric fusion protein sequence is reverse translated to its corresponding DNA sequence to be introduced in an expression vector, sequence optimized for expression in a particular cell line of interest. The first and second monomeric fusion proteins are transfected and coexpressed in the cell line of interest. In certain cases, the transfection is in 1:1 ratio for the two vectors. In some examples, the ratio is selected from 1.5:1, 2:1, 1:1.5, 1:2 etc

[00314] Sequence of Cargo molecule GLP-1

[00315] SEQ ID No: 12: HAEGTFTSDVSSYLEGQAAKEFIAWLVKGRG

[00316] Sequence of Cargo molecule Glucagon

[00317] SEQ ID NO: 13: HSQGTFTSDYSKYLDSRRAQDFVQWLMNT

Example 6: Annexin protein repeat as membrane-sensing multivalent scaffold

[00318] Annexin is split with an extensive interface to generate a multivalent heteromultimer scaffold comprising two transporter polypeptides. Annexin is a 346 residue protein (PDB ID 1MCX). Heteromultimer comprising two transporter polypeptides based on annexin split in the region between residue 186 and 194 is show in Figure 9. When co-expressed in solution, the large interfacial area between the two transporter polypeptides leads to self-assembly of the heterodimer. The self-assembly of the two units allows for the design of multivalent construct with transporter polypeptides based on the annexin core. Two structures are available, Pig and Human. The two structures are superimposable with an rmsd of 0.6 A. The following stretch of sequence can be removed from the human Annexin sequence DRSEDF (residues 160 through 165). The truncation does not break any secondary structure element and does not involve introducing or removing any Proline residue.

[00319] Human annexin WT Sequence

[00320] SEQ ID NO: 14:

GSAVSPYPTFNPSSDVAALHKAIMVKGVDEATIIDILTKRNNAQRQQIKAAYLQE
TGKPLDETLKKALTGHLEEVVLALLKTPAQFDADELRAAMKGLGTDEDTLIEILA
SRTNKEIRDINRVYREELKRDLAKDITSDTSGDFRNALLSLAKGDRSEDFGVNED
LADSDARALYEAGERRKGTDVNVFNTILTTRSYPQLRRVFQKYTKYSKHDMNK
VLDLELKGDIEKCLTAIVKCATSKPAFFAEKLHQAMKGVGTRHKALIRIMVSRSEI
DMNDIKAFYQKMYGISLCQAILDETKGDYEKILVALCGGN

[00321] Sequence of Annexin based transporter polypeptide-1:

[00322] SEQ ID NO: 15:

SAVSPYPTFNPSSDVAALHKAIMVKGVDEATIIDILTKRNNAQRQQIKAAYLQET GKPLDETLKKALTGHLEEVVLALLKTPAQFDADELRAAMKGLGTDEDTLIEILAS RTNKEIRDINRVYREELKRDLAKDITSDTSGDFRNALLSLAKG

[00323] Sequence of Annexin based transporter polypeptide-2:

[00324] SEQ ID NO: 16:

GVNEDLADSDARALYEAGERRKGTDVNVFNTILTTRSYPQLRRVFQKYTKYSKH DMNKVLDLELKGDIEKCLTAIVKCATSKPAFFAEKLHQAMKGVGTRHKALIRIM VSRSEIDMNDIKAFYQKMYGISLCQAILDETKGDYEKILVALCGGN

[00325] Figure 10 shows a plot of the buried solvent accessible surface area at the interface of Annexin based tranporter polypeptide-1 (ABT-1), and Annexin based tranporter polypeptide-2 (ABT-2). A split annexin near residue position 186 forms a heterodimer with about 3200 Å2 of buried surface area. The transporter polypeptides such as ABT-1 and ABT-2 based on Annexin can be used to attach cargo biomolecules using the same methods as described above for albumin based transporter polypeptides.

Example 7: Transferrin as a multivalent scaffold

[00326] Based on the large number of therapeutically relevant properties of transferrin, this protein presents itself as an interesting scaffold molecule for the design of multivalent protein fusion drugs following the creation of a self-assembling protein and its split component parts. The structure of transferrin is shown in Figure 11 based on the crystal structure (1H76) available in the protein data bank [Hall DR et al. Acta Crystallogr D 2002, 58, 70-80]. The transferrin molecule is composed of two structurally similar lobes, the N and C terminal lobes, connected by a short peptide linker between residues 333 and 342.

[00327] A heterodimer is designed based on transferrin protein, said heterodimer comprising a first transporter polypeptide involving residues 1-333 of transferrin and a second transporter polypeptide composed of residues from 342 to the C-terminus of the original transferrin sequence. When coexpressed, the two transporter polypeptides fold independently and pair to form a quasi-transferrin scaffold capable of maintaining its therapeutically relevant properties. Furthermore, such a Transferrin scaffold allows for the production of multivalent fusion molecules, e.g. a multivalent GLP-1 fusion with

trasnsporter polypeptides based on transferring. These fusions can be similar to the GLP-1-fusion polypeptides with Albumin based transporter polypeptides.

[00328] Figure 11 provides structure of transferrin molecule based on the PDB structure 1H76. The two monomering transporter polypeptides derived by splitting the transferrin molecule are color coded as light and dark grey units. The sites of fusion for the cargo molecules are represented as spheres. Figure 12 shows a plot of the buried solvent accessible surface area at the interface of two transferrin based polypeptides. A split transferrin near residue position 330 such as the two transporter polypeptides shown below, forms a heterodimer with about 1800 Å2 of buried surface area.

[00329] Sequence of Transferrin based transporter polypeptide-1:

[00330] SEQ ID NO: 17:

MRLAVGALLV CAVLGLCLAV PDKTVRWCAV SEHEATKCQS FRDHMKSVIP SDGPSVACVK KASYLDCIRA IAANEADAVT LDAGLVYDAY LAPNNLKPVV AEFYGSKEDP QTFYYAVAVV KKDSGFQMNQ LRGKKSCHTG LGRSAGWNIP IGLLYCDLPE PRKPLEKAVA NFFSGSCAPC ADGTDFPQLC QLCPGCGCST LNQYFGYSGA FKCLKDGAGD VAFVKHSTIF ENLANKADRD QYELLCLDNT RKPVDEYKDC HLAQVPSHTV VARSMGGKED LIWELLNQAQ EHFGKDKSKE FQLFSSPHGK DLLFKDSAHG FLKVPPRMDA KMYLGYEYVT AIRNLREG.

[00331] Sequence of Transferrin based transporter polypeptide-2:

[00332] SEQ ID NO: 18:

ECKPVKWCALSHHE RLKCDEWSVN SVGKIECVSA ETTEDCIAKI MNGEADAMSL DGGFVYIAGK CGLVPVLAEN YNKSDNCEDT PEAGYFAVAV VKKSASDLTW DNLKGKKSCH TAVGRTAGWN IPMGLLYNKI NHCRFDEFFS EGCAPGSKKD SSLCKLCMGS GLNLCEPNNK EGYYGYTGAF RCLVEKGDVA FVKHQTVPQN TGGKNPDPWA KNLNEKDYEL LCLDGTRKPV EEYANCHLAR APNHAVVTRK DKEACVHKIL RQQQHLFGSN VTDCSGNFCL FRSETKDLLF RDDTVCLAKL HDRNTYEKYL GEEYVKAVGN LRKCSTSSLL EACTFRRP.

Example 9: Multiple Cargo Proteins

[00333] The heteromultimer proteins described herein (e.g, containing a cargo polypeptide (or fragment or variant thereof) fused to transporter albumin segment or variant thereof) may additionally be fused to other proteins to generate "multifusion proteins". These multifusion proteins can be used for a variety of applications. For example, fusion of the proteins described herein to His-tag IgG domains, and maltose binding protein facilitates

purification. (See e.g EP A 394,827; Traunecker et al., Nature 331:84-86 (1988)). Nuclear localization signals fused to the polypeptides can target the protein to a specific subcellular localization. Furthermore, the fusion of additional protein sequences to proteins described herein may further increase the solubility and/or stability of the heteromultimer. The heteromultimer proteins described above can be made using or routinely modifying techniques known in the art and/or by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule.

[00334] Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian or yeast expression vector.

[00335] For example, if pC4 (ATCC Accession No. 209646) is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the vector, and a polynucleotide encoding a heteromultimeric protein described herein (generated and isolated using techniques known in the art), is ligated into this BamHI site. Note that the polynucleotide encoding the fusion protein of the invention is cloned without a stop codon; otherwise an Fc containing fusion protein will not be produced.

[00336] If the naturally occurring signal sequence is used to produce the heteromultimeric protein described herein, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., International Publication No. WO 96/34891.)

CLAIMS

What is claimed:

1. A heteromultimer comprising:

at least a first monomeric protein that comprises (i) a first transporter polypeptide; and (ii) at least one cargo polypeptide and

at least a second monomeric protein that comprises (iii) a second transporter polypeptide and (iv) at least one cargo polypeptide;

wherein at least one transporter polypeptide is derived from a monomeric protein and wherein said transporter polypeptides self-assemble to form a quasi-native structure of said monomeric protein or analog thereof.

2. The heteromultimer of claim 1, wherein said heteromultimer is a heterodimer.

3. The heteromultimer of claim 1 wherein at least one transporter polypeptide is not derived from an antibody.

4. The heteromultimer of any one of claims 1-2, wherein each transporter polypeptide is an albumin derivative.

5. The heteromultimer of claim 3, wherein said albumin is human serum albumin having a sequence of SEQ ID NO: 1.

6. The heteromultimer of any one of claims 1-4, wherein said first transporter polypeptide has a sequence comprising SEQ ID NO:2 and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:3.

7. The heteromultimer of any one of claims 1-4, wherein said first transporter polypeptide has a sequence comprising SEQ ID NO:8, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:10.

8. The heteromultimer of any of claims 1-2, wherein at least one transporter polypeptide is an allo-albumin derivative.

9. The heteromultimer of claim 7, wherein at least one transporter polypeptide is an alloalbumin derivatives, and at least one transporter polypeptide is an albumin derivative.

10. The heteromultimer of claim 7, wherein each transporter polypeptide is derived from a different alloalbumin.

11. The heteromultimer of claim 7, wherein each transporter polypeptide is an alloalbumin derivative based on an alloalbumin selected from Table 2.

12. The heteromultimer of any one of claims 1-2, wherein each transporter polypeptide is an annexin derivative.

13. The heteromultimer of claim 12, wherein said annexin is annexin A1 having a sequence of SEQ ID NO: 14.

14. The heteromultimer according to claim 12, wherein said first transporter polypeptide has a sequence comprising SEQ ID NO:15, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO: 16.

15. The heteromultimer of any one of claims 1-2, wherein each transporter polypeptide is an transferrin derivative.

16. The heteromultimer according to claim 15, wherein said first transporter polypeptide has a sequence comprising SEQ ID NO: 17, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO: 18.

17. The heteromultimer according to any of claims 1-8 and 11-16, wherein the transporter polypeptides are derived from the same protein.

18. The heteromultimer of any of claims 1-17, wherein said cargo polypeptides are selected from the proteins provided in Table 2 or a fragment, or variant thereof or a receptor, agonist, antagonist or antibody to a protein provided in Table 2 or fragment or variant thereof.

19. The heteromultimer of any of claims 1-16, wherein at least one cargo polypeptide comprises GLP-1 or fragment or variant thereof.

20. The heteromultimer of any of claims 1-17 and 19, wherein at least one cargo polypeptide comprises glucagon or fragment or variant thereof.

21. The heteromultimer of any of claims 1-17, wherein at least one cargo polypeptide comprises an EGF-A like domain.

22. The heteromultimer of any of claims 1-17, wherein at least one cargo polypeptide is an antibody, or fragment or variant thereof

23. The heteromultimer of claim 22, wherein said antibody fragment comprises antibody Fc region.

24. The heteromultimer of any of claims 22-23 wherein the antibody is an immunoglobulin selected from the group consisting of IgG, IgA, IgD, IgE, and IgM.

25. The heteromultimer of claim 24 wherein the immunoglobin is IgG of subtype selected from IgG1, IgG2a, IgG2b, IgG3 and IgG4.

26. The heteromultimer of any of claims 22-25, wherein the antibody is a bispecific antibody.

27. The heteromultimer of any of claims 22-25, wherein the antibody is a multispecific antibody.

28. The heteromultimer of any of claims 22-27 wherein the antibody is a therapeutic antibody.

29. The heteromultimer of claim 28 wherein the therapeutic antibody binds a cancer antigen.

30. The heteromultimer of claim 22 wherein at least one antibody is selected from abagovomab, adalimumab, alemtuzumab, aurograb, bapineuzumab, basiliximab, belimumab, bevacizumab, briakinumab, canakinumab, catumaxomab, certolizumab

pegol, certuximab, daclizumab, denosumab, efalizumab, galiximab, gemtuzumab ozagamicin, golimumab, ibritumomab tiuxetan, infliximab, ipilimumab, lumiliximab, mepolizumab, motavizumab, muromonab, mycograb, natalizumab, nimotuzumab, ocrelizumab, ofatumumab, omalizumab, palivizumab, panitumumab, pertuzumab, ranizumab, reslizumab, rituximab, teplizumab, toclizumab, tositumomab, trastuzumab, Proxinium, Rencarex, ustekinumab, and zalutumumab.

31. The heteromultimer of one of claims 1-30 wherein said first monomeric protein binds a target antigen, and said second monomeric protein comprises a toxin moiety.

32. The heteromultimer of claim 31, wherein said target antigen is at least one of a-chain (CD25) of IL-2R, Amyloid beta, anti-EpCAM, amti-CD3, CD1 ia, CD20, CD22, CD23, CD3, CD4, CD52, CD80, CTLA-4, EGFR, EpCAM, F protein of RSV, G250, glycoprotein IIB/IIIa R, HER2, HER2/neu R, HSP90, IgE antibody, IL-12, IL-23, IL-1b, IL-5, IL-6, RANKL, TNF alpha, VEGF-A, and other therapeutically advantages targets.

33. The heteromultimer of one of claims 1-32 wherein at least one cargo polypeptide is an enzyme, hormone, therapeutic polypeptide, antigen, chemotoxin, radiotoxin, cytokine or variant or fragment thereof.

34. The heteromultimer of any of claims 1-16, wherein said first monomeric protein and said second monomeric protein comprise the same cargo polypeptide.

35. A heteromultimer that comprises: at least two monomers, each comprising a transporter polypeptide and optionally at least one cargo molecule attached to said transporter polypeptide, wherein each transporter polypeptide is obtained by segmentation of a whole protein such that said transporter polypeptides self-assemble to form quasi-native whole protein.

36. The heteromultimer of claim 35, wherein said heteromultimer is multispecific.

37. The heteromultimer of any of claims 35-36 wherein said transporter polypeptide is not derived from an antibody.

38. The heteromultimer of any of claims 35-37, wherein each monomer preferentially forms the heteromultimer as compared to a monomer or a homomultimer.

39. The heteromultimer of any of claims 35-38, wherein said at least one cargo molecule is a theraputic agent, or a biomolecule.

40. The heteromultimer of any of claims 35-39, wherein said at least one cargo molecule is a biomolecule which is selected from a polypeptide, DNA, PNA, or RNA.

41. The heteromultimer of any of claims 35-40, wherein each transporter polypeptide is a derivate of albumin or alloalbumin.

42. The heteromultimer of any of claims 35-40, wherein each transporter polypeptide is a derivate of annexin.

43. The heteromultimer of any of claims 35-40, wherein each transporter polypeptide is a derivate of transferrin.

44. A host cell comprising nucleic acid encoding heteromultimer of any of claim 1-44.

45. The host cell of claim 44, wherein the nucleic acid encoding the first monomeric protein and the nucleic acid encoding the second monomeric protein are present in a single vector.

46. The host cell of claim 44, wherein the nucleic acid encoding the first monomeric protein and the nucleic acid encoding the second monomeric protein are present in separate vectors.

47. A method of making heteromultimers from a protein of interest by segmentation of said protein of interest to obtain polypeptides such that said polypeptides self-assemble to form said heteromultimer.

48. The method of claim 47, wherein the protein of interest is albumin.

49. The method of claim 47, wherein the protein of interest is annexin.

50. The method of claim 47, wherein the protein of interest is transferrin.

51. The method of claim 47, wherein the segmentation is performed such that the heteromultimer comprises no covalent bond between the polypeptides.

52. The method of claim 47, wherein the segmentation is performed such that the heteromultimer comprises at least one covalent bond between the polypeptides.

53. A heteromultimer designed by the method of any of claims 47-52.

54. A therapeutic scaffold comprising a heteromultimer designed by the method of any of claims 47-52.

Figure 1

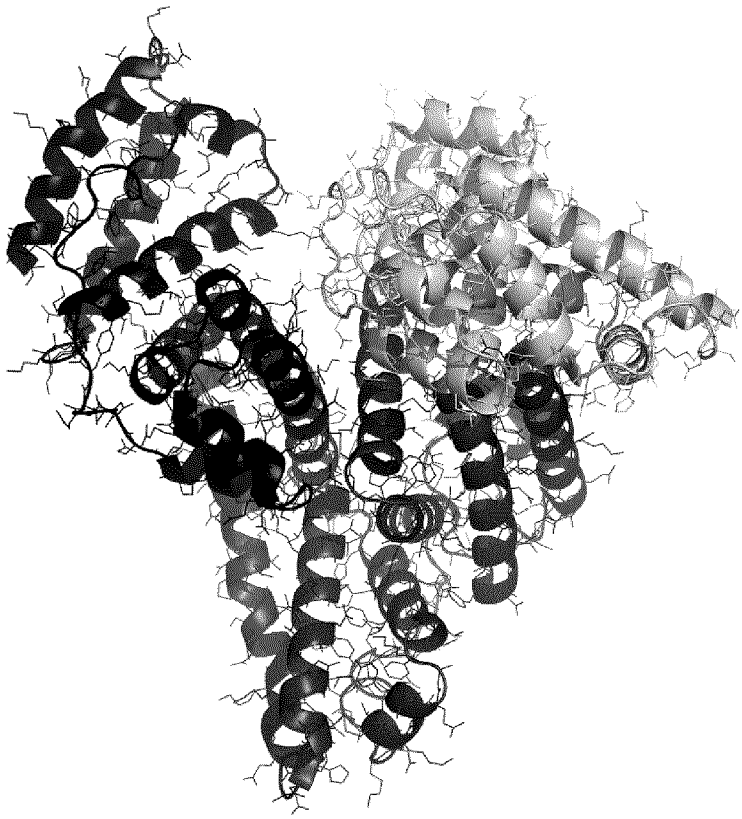


Figure 2

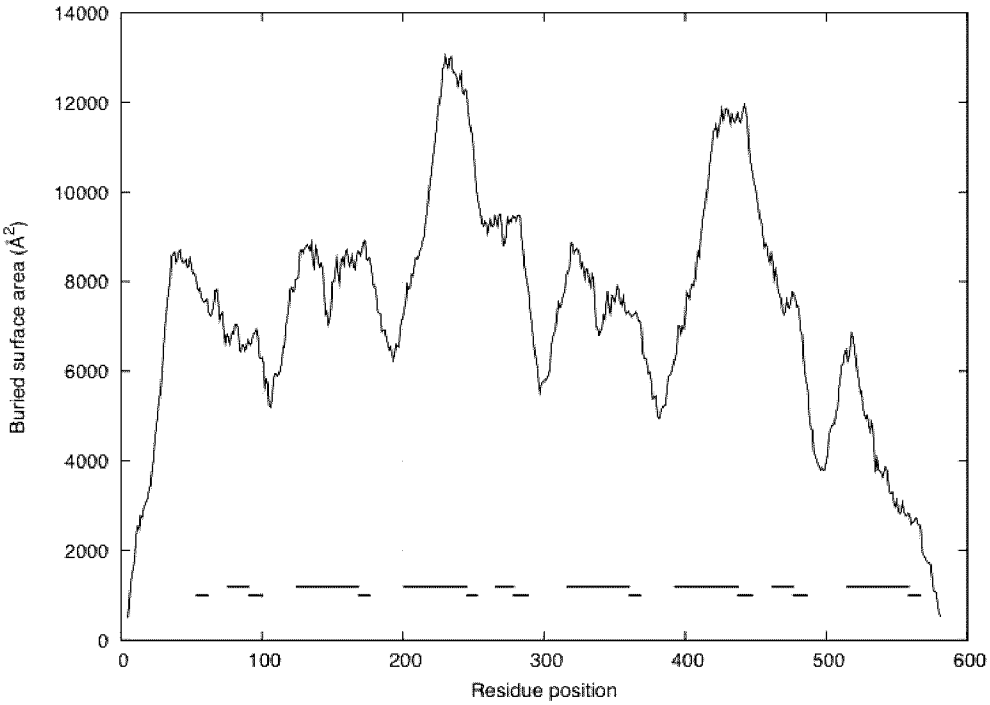

14000
12000
10000
8000
6000
4000
2000
0
Buried surface area (Å2)
0
100
200
300
400
500
600
Residue position

Figure 3



Figure 4

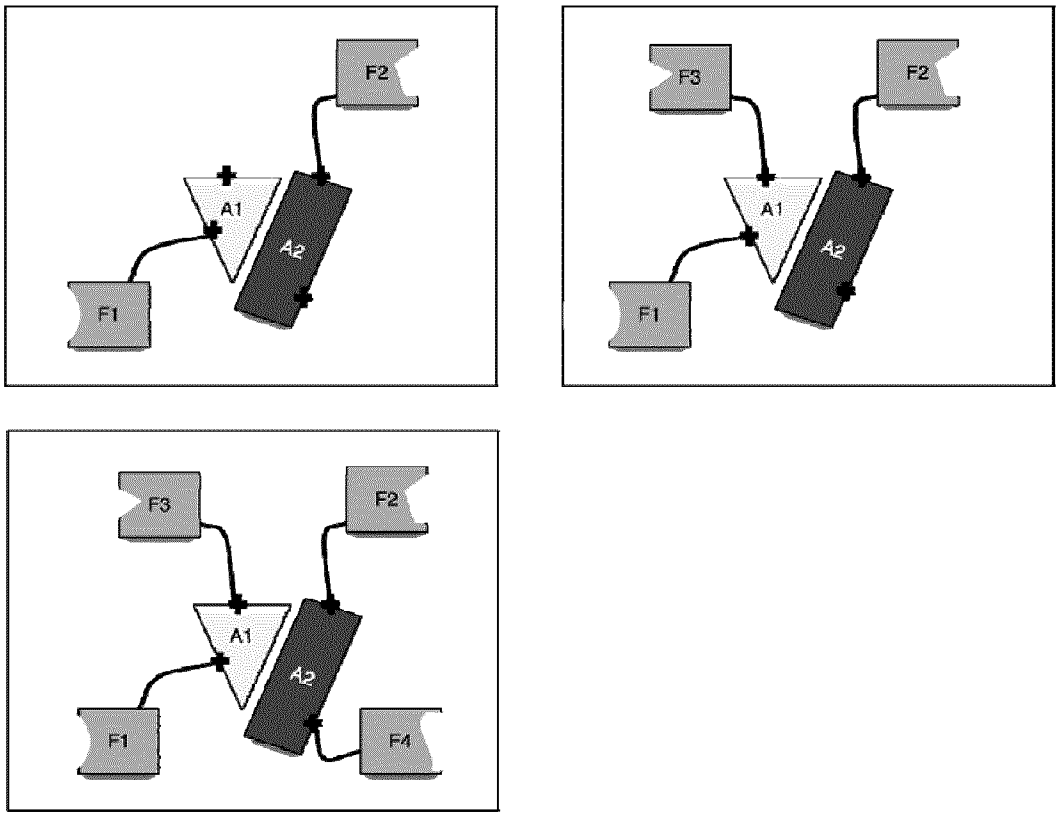


Figure 5

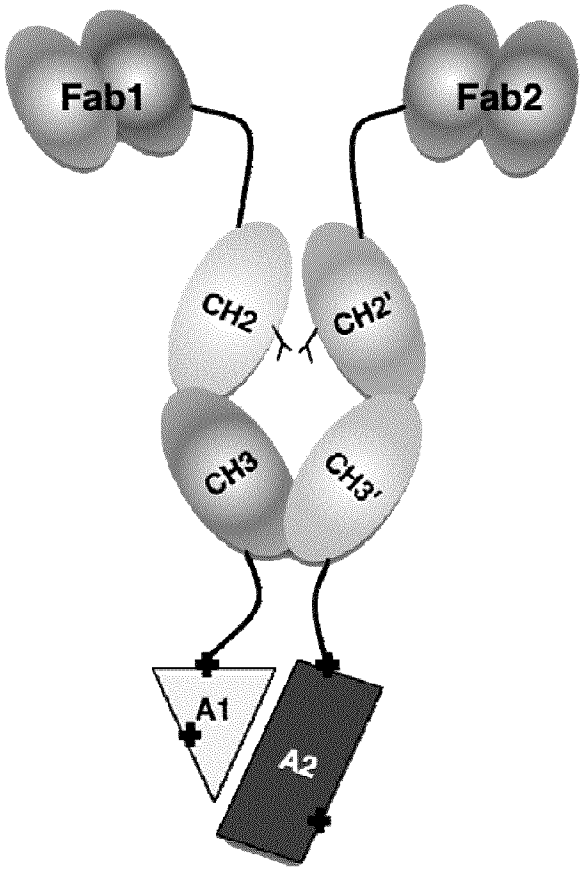


Figure 6A

- v221: ABH2
- v224: ABH1 v225: Wild type Albumin

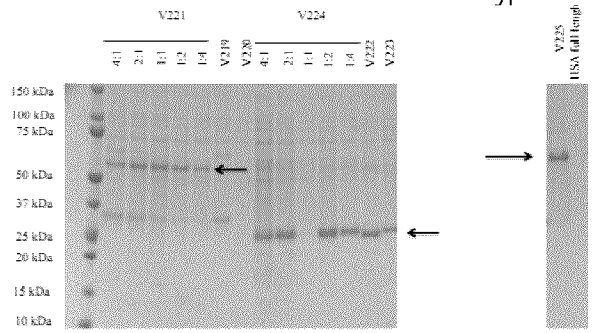


Figure 6B

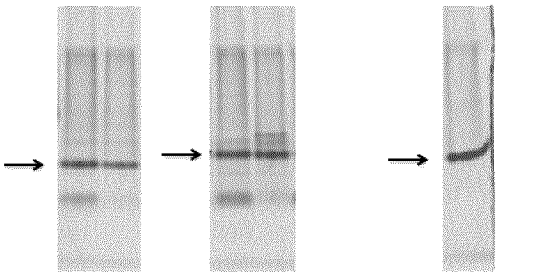
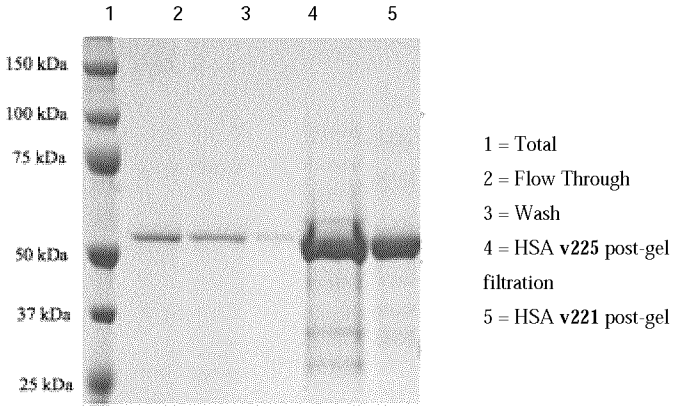


Figure 6C



1 = Total
2 = Flow Through
3 = Wash
4 = HSA **v225** post-gel filtration
5 = HSA **v221** post-gel

Figure 7

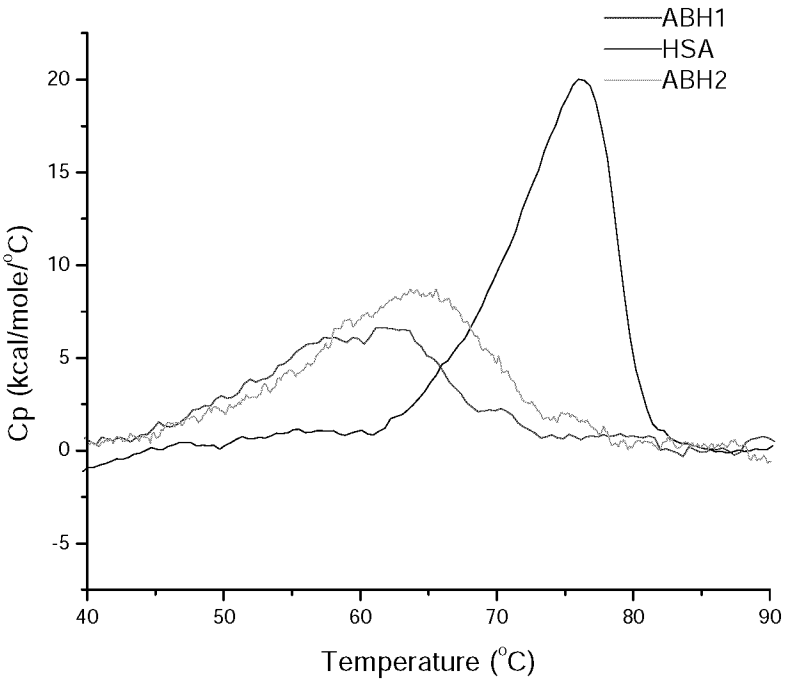

ABH1
HSA
ABH2
Cp (kcal/mole/°C)
Temperature (°C)
20
15
10
5
0
-5
40
50
60
70
80
90

Figure 8A

ABH2

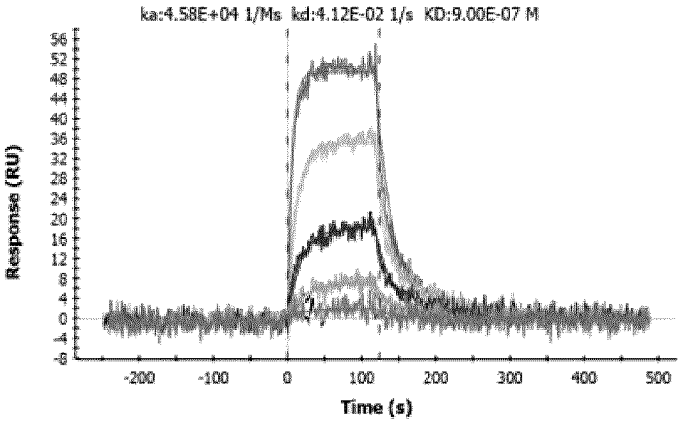


Figure 8B

Wildtype Albumin

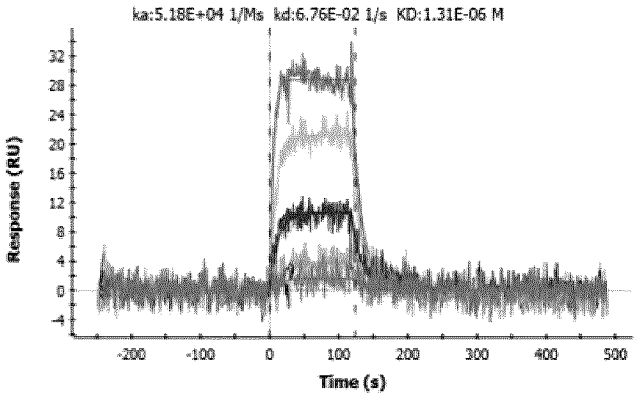


Figure 9

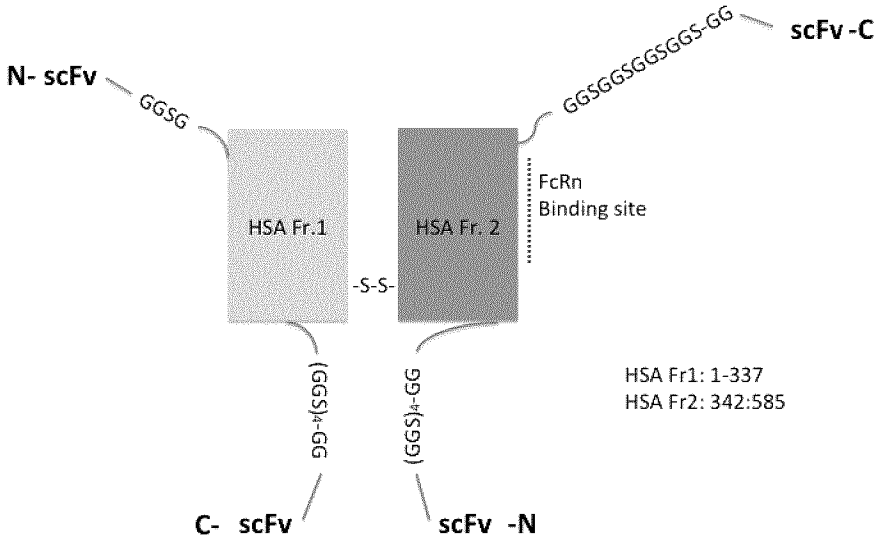

N- scFv
GGSG
GGSGGSGGSGGS-GG
scFv -C
HSA Fr.1
HSA Fr. 2
FcRn
Binding site
-S-S-
(GGS)4-GG
(GGS)4-GG
HSA Fr1: 1-337
HSA Fr2: 342:585
C- scFv
scFv -N

Figure 10A

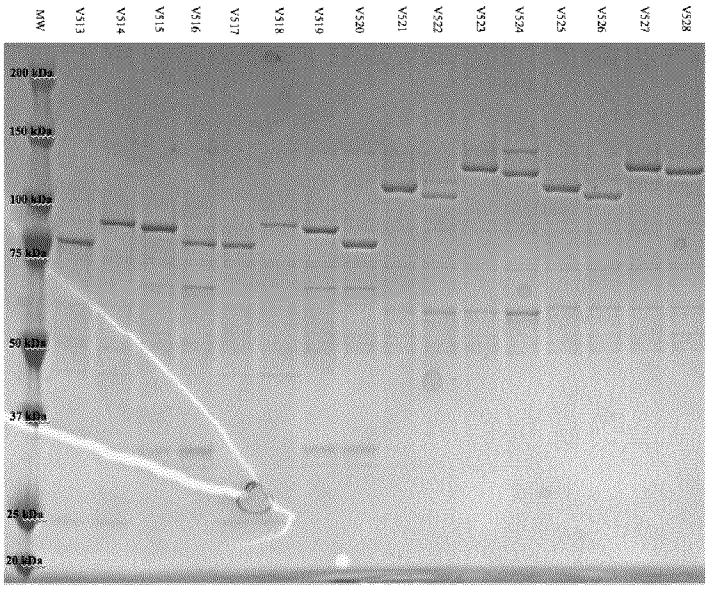


Figure 10B

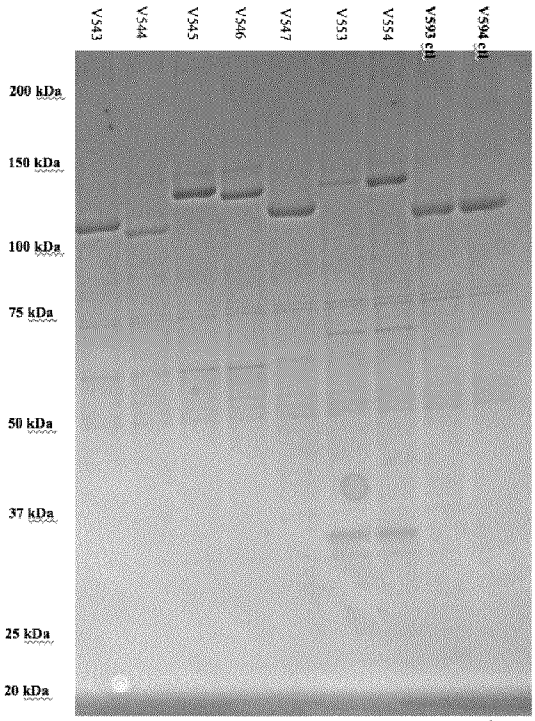
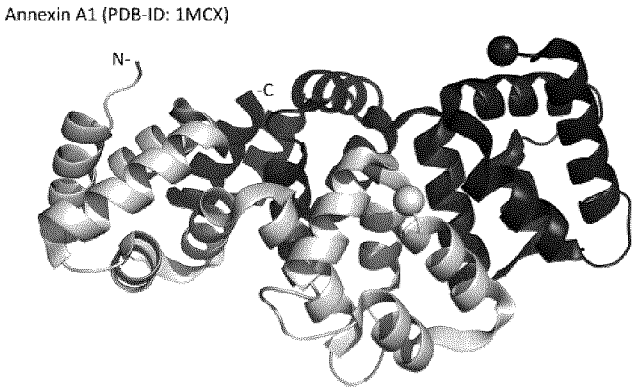


Figure 11



Annexin based Transporter Polypeptide 1: residues 41-186 (gray)

Annexin based Transporter Polypeptide 2: residues 194-344 (black)

Figure 12

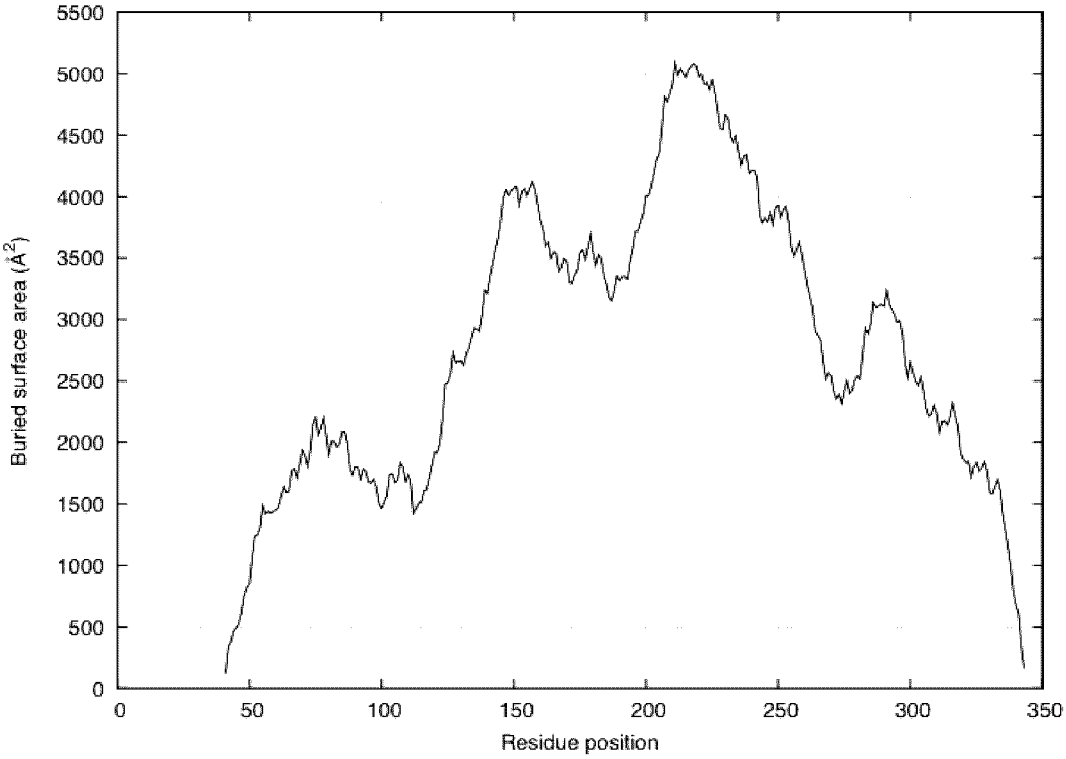

Buried surface area (Å^2)
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
50
100
150
200
250
300
350
Residue position

Figure 13

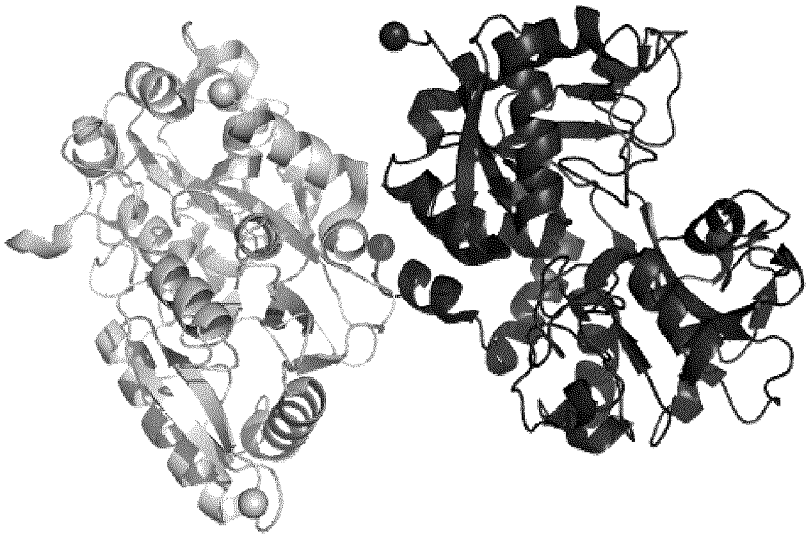


Figure 14

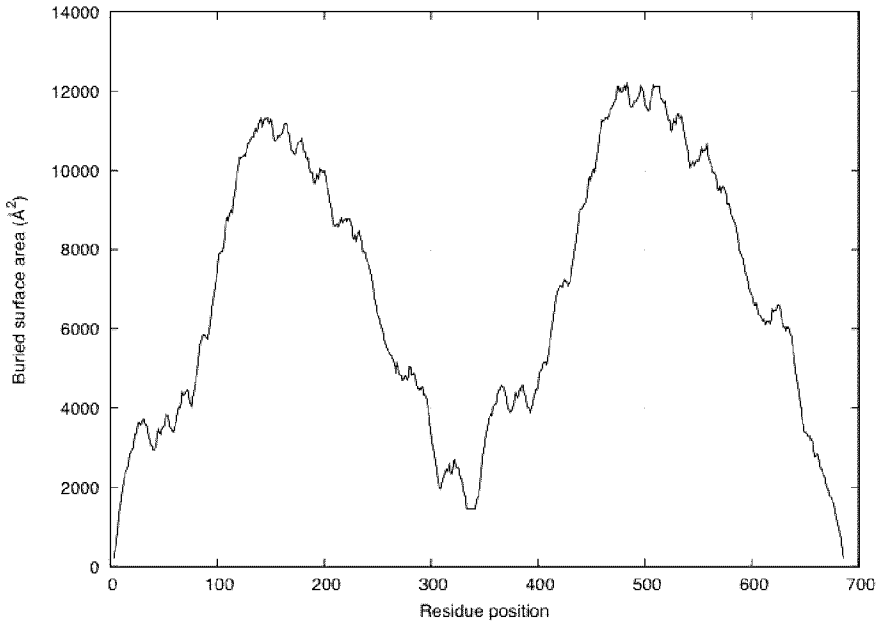

14000
12000
10000
8000
6000
4000
2000
0
Buried surface area (Å^2)
0
100
200
300
400
500
600
700
Residue position

Figure 15

v438: NM32 scFv

TCAAGCGAGCTGACCCAGGACCCCGCCGTGAGCGTCGCACTGGGGCAGACCGTGC
GCATCACATGCCAGGGAGATAGCCTGCGATCCTACTATGCATCTTGGTACCAGCA
GAAGCCAGGACAGGCACCTGTGCTGGTCATCTATGGGAAAAACAATAGACCATCA
GGCATCCCCGACAGGTTCAGCGGAAGCTCCTCTGGCAACACAGCTTCTCTGACCA
TTACAGGCGCACAGGCCGAGGACGAAGCAGATTACTATTGCAACAGTCGGGATAG
TTCAGGGAATCACGTGGTCTTTGGAGGAGGAACTAAGCTGACCGTGGGA**GGA**GGA
TCAGGAGGAGGAAGCGGAGGAGGCAGCGGAGGAGGATCTGGAGGAGGAAGTGGAG
AGGTGCAGCTGGTCGAAAGCGGAGGAGGAGTGGTCCGACCTGGAGGGTCACTGCG
ACTGAGCTGTGCAGCTTCCGGCTTCACATTTGACGATTACGGGATGTCATGGGTG
AGACAGGCCCCAGGGAAAGGACTGGAATGGGTCTCCGGCATCAACTGGAATGGAG
GCTCTACTGGATACGCCGACAGTGTGAAGGGCAGGTTCACCATTTCCCGCGATAA
CGCTAAAAATTCTCTGTATCTGCAGATGAACAGTCTGAGGGCCGAGGACACTGCC
GTGTACTATTGTGCCCGGGGCAGATCCCTGCTGTTTGATTACTGGGGCCAGGGGA
CACTGGTGACTGTCTCTCGCGGCAGTGAAAATCTGTATTTTCAG

SSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPS
GIPDRFSGSSSGNTASLTI**T**GAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGGG
SGGGSGGGSGGGSGGGSGEVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWV
RQAPGKGLEWVSGINWNGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTA
VYYCARGRSLLFDYWGQGTLVTVSRGSENLYFQ

V218: 4D5 scFv

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAG
TCACCATCACTTGCCGGGCAAGTCAGGACGTTAACACCGCTGTAGCTTGGTATCA
GCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATTCTGCATCCTTTTTGTAC
AGTGGGGTCCCATCA**AGG**TTCAGTGGCAGTCGATCTGGGACAGATTTCACTCTCA
CCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAACAGCATTA
CACTACCCCACCCACTTTCGGCCAAGGGACCAAAGTGGAGATCAAAGGTGGTTCT
GGTGGTGGTTCTGGTGGTGGTTCTGGTGGTGGTTCTGGTGGTGGTTCTGGTGAAG
TGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCGGGTCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTCAACATTAAAGATACTTATATCCACTGGGTCCGG
CAAGCTCCAGGGAAGGGCCTGGAGTGGGTCGCACGTATTTATCCCACAAATGGTT
ACACACGGTATGCGGACTCTGTGAAGGGCCGATTCACCATCTCCGCAGACACTTC
CAAGAACACCGCGTATCTGCAAATGAACAGTCTGAGAGCTGAGGACACGGCCGTT
TATTACTGTTCAAGATGGGGCGGAGACGGTTTCTACGCTATGGACTACTGGGGCC
AAGGGACCCTGGTCACCGTCTCCTCAGGCAGCGAGAACCTGTATTTTCAG

Protein Sequence

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLY
SGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKGGS
GGGSGGGSGGGSGGGSGEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVR
QAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAV
YYCSRWGGDGFYAMDYWGQGTLVTVSSGSENLYFQ

Base construct # 1:

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GAATCACTTGCCAGGGGGACTCACTGCGGAGCTACTATGCCTCCTGGTACCAGCA
GAAACCAGGCCAGGCTCCCGTGCTGGTCATCTATGGCAAGAACAATAGGCCTAGT
GGGATTCCAGATCGCTTTTCAGGGAGCTCCTCTGGAAACACTGCAAGTCTGACCA
TTACAGGCGCTCAGGCAGAGGACGAAGCCGATTACTATTGCAACAGCAGGGACAG
TTCAGGGAATCACGTGGTCTTCGGAGGAGGAACTAAGCTGACCGTGGGAGGAGGC
AGCGGAGGAGGATCTGGAGGAGGAAGTGGAGGAGGATCAGGAGGAGGAAGCGGAG
AGGTGCAGCTGGTCGAAAGCGGAGGAGGAGTGGTCCGGCCAGGAGGGTCCCTGAG
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CGGCAGGCACCTGGCAAGGGACTGGAGTGGGTGAGCGGCATCAACTGGAATGGAG
GCTCCACAGGGTACGCTGATTCTGTGAAAGGACGCTTTACTATTAGCCGAGACAA
CGCCAAGAACAGCCTGTATCTGCAGATGAACTCTCTGAGAGCTGAGGATACCGCA
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CACTGGTGACTGTCTCACGCGGGGGAAGCGGGGATGCTCACAAGTCCGAGGTCGC
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GAGACATACGGCGAAATGGCAGACTGCTGTGCCAAACAGGAGCCTGAAAGAAACG
AGTGCTTCCTGCAGCACAAGGACGATAACCCCAATCTGCCTCGACTGGTGCGGCC
AGAAGTGGACGTCATGTGTACTGCTTTCCACGATAATGAGGAAACCTTTCTGAAG
AAATACCTGTATGAGATTGCCCGGAGACATCCATACTTTTATGCCCCCGAACTGC
TGTTCTTTGCTAAGCGCTATAAAGCAGCCTTCACCGAGTGCTGTCAGGCTGCAGA
TAAGGCCGCTTGCCTGCTGCCAAAACTGGACGAGCTGAGAGATGAAGGCAAAGCA
AGCTCCGCCAAGCAGAGGCTGAAATGTGCAAGCCTGCAGAAGTTCGGCGAGAGGG
CCTTTAAAGCATGGGCCGTGGCTAGACTGTCTCAGAGGTTCCCCAAGGCTGAGTT
TGCAGAAGTCAGTAAGCTGGTGACTGACCTGACCAAAGTGCACACAGAGTGCTGT
CATGGCGACCTGCTGGAATGCGCCGACGATCGCGCCGATCTGGCTAAGTACATCT
GTGAGAACCAGGACTCCATTTCTAGTAAGCTGAAAGAGTGCTGTGAAAAGCCACT
GCTGGAGAAATCTCATTGCATCGCTGAGGTGGAAAATGACGAAATGCCCGCAGAT
CTGCCTAGCCTGGCAGCCGACTTCGTCGAGTCCAAGGATGTGTGTAAAAACTATG

CCGAGGCTAAAGATGTGTTTCTGGGAATGTTTCTGTATGAGTATGCAAGAGCATG
AGGATCC

Base construct # 1 Protein:

SSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPS
GIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGGG
SGGGSGGGSGGGSGGGSGEVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWV
RQAPGKGLEWVSGINWNGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTA
VYYCARGRSLLFDYWGQGTLVTVSRGGSGDAHKSEVAHRFKDLGEENFKALVLIA
FAQYLQQCPFEDHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLR
ETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLK
KYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKA
SSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECC
HGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPAD
LPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARA

Base construct # 2:

GATGCTCATAAATCTGAGGTCGCTCACCGGTTCAAGGATCTGGGCGAGGAAAACT
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GGACCACGTGAAGCTGGTCAACGAGGTGACAGAGTTCGCCAAAACTTGCGTCGCC
GACGAGTCTGCTGAAAATTGTGATAAGAGTCTGCATACACTGTTTGGAGATAAAC
TGTGTACTGTGGCCACCCTGAGAGAGACTTATGGCGAAATGGCAGACTGCTGTGC
CAAGCAGGAGCCTGAAAGGAACGAGTGCTTCCTGCAGCATAAAGACGATAACCCC
AATCTGCCTAGGCTGGTGCGCCCAGAAGTGGACGTCATGTGTACCGCCTTCCACG
ATAATGAGGAAACATTTCTGAAGAAATACCTGTATGAGATTGCCCGGAGACATCC
ATACTTTTATGCACCCGAACTGCTGTTCTTTGCCAAGAGATACAAAGCCGCTTTC
ACCGAGTGCTGTCAGGCAGCCGATAAGGCTGCATGCCTGCTGCCAAAACTGGACG
AGCTGCGAGATGAAGGGAAGGCCAGCTCCGCTAAGCAGCGGCTGAAATGTGCTAG
CCTGCAGAAGTTCGGAGAGCGAGCCTTCAAGGCATGGGCTGTGGCACGACTGTCC
CAGCGGTTCCCCAAAGCAGAGTTTGCCGAAGTCTCTAAGCTGGTGACAGACCTGA
CTAAAGTGCACACCGAGTGCTGTCATGGCGACCTGCTGGAATGCGCCGACGATCG
AGCTGATCTGGCAAAGTACATCTGTGAGAATCAGGACAGCATTTCTAGTAAGCTG
AAAGAGTGCTGTGAAAAGCCTCTGCTGGAGAAATCCCACTGCATCGCCGAGGTGG

AAAACGACGAAATGCCAGCTGATCTGCCCTCACTGGCCGCTGACTTTGTCGAGAG
CAAGGATGTGTGTAAAAATTATGCCGAAGCTAAGGATGTGTTCCTGGGCATGTTT
CTGTACGAGTATGCAAGGGCAGGAGGGTCCGGAGGCTCTGGAGGAAGTGGAGGGT
CAGGAGGCTCAAGCGAACTGACTCAGGACCCCGCTGTGAGCGTCGCACTGGGACA
GACTGTGAGGATCACCTGCCAGGGGGACAGCCTGCGCTCCTACTATGCATCCTGG
TACCAGCAGAAGCCTGGCCAGGCCCCAGTGCTGGTCATCTATGGCAAAAACAATC
GGCCCTCAGGGATTCCTGATCGGTTCAGCGGGTCCTCTAGTGGAAACACAGCTTC
TCTGACCATTACAGGCGCTCAGGCAGAGGACGAAGCCGATTACTATTGCAACAGC
CGCGACTCAAGCGGGAATCATGTGGTCTTCGGAGGAGGAACCAAGCTGACAGTGG
GAGGAGGCTCTGGAGGAGGCAGTGGGGGAGGCTCAGGAGGAGGCAGCGGAGGAGG
CTCCGGAGAGGTCCAGCTGGTGGAAAGCGGAGGAGGAGTGGTCCGCCCAGGAGGA
TCTCTGCGACTGAGTTGTGCAGCCTCAGGATTCACCTTTGACGATTACGGAATGA
GTTGGGTCCGGCAGGCACCTGGAAAGGGACTGGAGTGGGTGAGCGGCATCAACTG
GAATGGCGGGAGCACTGGGTACGCTGATTCCGTGAAGGGAAGATTCACCATTTCC
AGGGACAACGCCAAAAATTCTCTGTATCTGCAGATGAATAGTCTGAGAGCCGAGG
ACACAGCTGTGTACTATTGCGCCAGGGGGAGGTCTCTGCTGTTCGACTACTGGGG
GCAGGGCACTCTGGTCACTGTGTCAAGATGAGGATCC

Base construct # 2 Protein:

DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVA
DESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNP
NLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAF
TECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLS
QRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKL
KECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMF
LYEYARAGGSGGSGGSGGSGGSSELTQDPAVSVALGQTVRITCQGDSLRSYYASW
YQQKPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNS
RDSSGNHVVFGGGTKLTVGGGSGGGSGGGSGGGSGGGSGEVQLVESGGGVVRPGG
SLRLSCAASGFTFDDYGMSWVRQAPGKGLEWVSGINWNGGSTGYADSVKGRFTIS
RDNAKNSLYLQMNSLRAEDTAVYYCARGRSLLFDYWGQGTLVTVSR

Base construct #3:

TCCTCCGAGCTGACCCAGGACCCTGCCGTGTCCGTCGCTCTGGGACAGACCGTGC
GGATCACATGCCAGGGAGATAGCCTGAGATCCTACTATGCTAGCTGGTACCAGCA

GAAACCCGGCCAGGCACCTGTGCTGGTCATCTATGGGAAGAACAATCGCCCATCT
GGCATCCCCGACCGATTCAGTGGAAGCTCCTCTGGCAACACAGCCTCTCTGACTA
TTACCGGCGCTCAGGCAGAGGACGAAGCTGATTACTATTGCAACAGCAGGGATAG
TTCAGGGAATCACGTGGTCTTTGGAGGAGGAACTAAGCTGACCGTGGGAGGAGGA
TCTGGAGGAGGAAGTGGCGGGGGATCAGGAGGAGGAAGCGGAGGAGGCAGCGGAG
AGGTGCAGCTGGTCGAAAGCGGAGGAGGAGTGGTCAGACCAGGAGGGTCTCTGAG
GCTGAGTTGTGCCGCTTCAGGCTTCACCTTTGACGATTACGGAATGTCTTGGGTG
CGGCAGGCACCTGGAAAGGGACTGGAGTGGGTGAGTGGCATCAACTGGAATGGAG
GCAGCACAGGATACGCAGACTCCGTGAAAGGCCGATTCACTATTTCACGGGATAA
CGCCAAGAATAGCCTGTATCTGCAGATGAACAGCCTGAGAGCAGAGGACACAGCC
GTGTACTATTGTGCCAGGGGCCGCTCTCTGCTGTTTGATTACTGGGGGCAGGGAA
CACTGGTGACTGTCAGCCGAGGAGGATCTGGAGGGAGTGGAGGCTCAGGAGGAAG
CGGAGGGTCCGTGGTCCTGCTGCTGCGACTGGCTAAAACTTACGAGACCACACTG
GAAAAGTGCTGTGCAGCCGCTGACCCCCATGAGTGCTATGCAAAAGTGTTCGATG
AGTTCAAGCCTCTGGTCGAGGAACCACAGAACCTGATCAAACAGAATTGTGAGCT
GTTCGAACAGCTGGGCGAGTACAAGTTTCAGAACGCCCTGCTGGTGAGATATACC
AAGAAAGTGCCCCAGGTCTCTACACCTACTCTGGTGGAGGTCAGTAGGAATCTGG
GCAAAGTGGGGTCAAAATGCTGTAAGCACCCAGAGGCTAAGCGCATGCCCTGCGC
AGAAGACTACCTGAGCGTGGTCCTGAACCAGCTGTGTGTGCTGCATGAGAAAACT
CCAGTGTCCGATAGGGTCACTAAGTGCTGTACCGAAAGCCTGGTGAACCGGAGAC
CTTGCTTCTCCGCCCTGGAGGTGGACGAAACCTATGTCCCAAAAGAGTTTAATGC
CGAAACCTTCACATTTCACGCTGATATCTGTACCCTGTCCGAGAAGGAACGCCAG
ATTAAGAAACAGACAGCTCTGGTGGAGCTGGTCAAGCATAAACCCAAGGCAACAA
AAGAACAGCTGAAGGCCGTGATGGACGATTTCGCAGCCTTTGTGGAGAAATGCTG
TAAGGCCGACGATAAGGAAACTTGCTTTGCTGAAGAA**GGG**AAGAAACTGGTCGCC
GCATCACAGGCTGCTCTGGGACTGTGAGGATCC

Base Construct #3 Protein:

SSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPS
GIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGGG
SGGGSGGGSGGGSGGGSGEVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWV
RQAPGKGLEWVSGINWNGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTA
VYYCARGRSLLFDYWGQGTLVTVSRGGSGGSGGSGGSGGSVVLLLRLAKTYETTL
EKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYT

KKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKT
PVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQ
IKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFA**E**EGKKLVA
ASQAALGL

Base construct # 4

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CAGCTGGGCGAGTACAAGTTTCAGAACGCCCTGCTGGTGAGGTATACTAAGAAAG
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GCGATAGGGTCACAAAGTGCTGTACTGAATCCCTGGTGAACCGGAGACCTTGCTT
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AACAGACTGCCCTGGTGGAGCTGGTCAAGCATAAACCCAAGGCCACCAAAGAACA
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GACGATAAGGAAACATGCTTCGCCGAGGAAGGGAAGAAACTGGTGGCAGCAAGCC
AGGCTGCACTGGGACTGGGAGGGTCTGGAGGCAGTGGAGGATCAGGAGGGAGCGG
AGGCAGCTCCGAGCTGACCCAGGACCCCGCTGTGAGCGTCGCACTGGGACAGACC
GTGCGCATCACATGTCAGGGCGATTCCCTGCGATCTTACTATGCTTCCTGGTACC
AGCAGAAACCCGGCCAGGCACCTGTGCTGGTCATCTATGGAAAGAACAATAGACC
AAGTGGCATTCCCGACAGGTTCTCAGGCTCTAGTTCAGGGAACACCGCCTCCCTG
ACCATTACAGGCGCACAGGCCGAGGACGAAGCTGATTACTATTGCAACTCTCGGG
ATAGCTCCGGCAATCATGTGGTCTTTGGGGGAGGCACTAAGCTGACCGTGGGGGG
AGGCAGTGGGGGAGGCTCAGGAGGAGGCAGCGGAGGAGGCTCCGGAGGAGGCTCT
GGCGAGGTGCAGCTGGTCGAATCCGGAGGAGGAGTGGTCCGACCAGGAGGAAGTC
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GGTGCGACAGGCACCTGGAAAGGGACTGGAGTGGGTGTCTGGCATCAACTGGAAT
GGCGGGTCCACTGGCTACGCAGACTCTGTGAAAGGGAGGTTTACCATTAGCCGCG
ATAACGCCAAGAACAGCCTGTATCTGCAGATGAACAGCCTGCGCGCCGAGGACAC

AGCTGTGTACTATTGCGCCAGGGGGAGGTCACTGCTGTTTGATTACTGGGGGCAG
GGGACTCTGGTCACTGTGTCACGGTGAGGATCC

Protein Base Construct #4

SVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFE
QLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAED
YLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAET
FTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKA
DDKETCFAEEGKKLVAASQAALGLGGSGGSGGSGGSGGSSELTQDPAVSVALGQT
VRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNTASL
TITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGGGSGGGSGGGSGGGSGGGS
GEVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWVSGINWN
GGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGRSLLFDYWGQ
GTLVTVSR

Base construct 5

GACATTCAGATGACACAGTCCCCAAGCTCCCTGAGCGCTTCCGTCGGCGATCGAG
TGACTATCACCTGCCGAGCCTCTCAGGACGTCAACACTGCTGTGGCATGGTACCA
GCAGAAGCCTGGGAAAGCACCAAAGCTGCTGATCTACTCTGCCAGTTTTCTGTAT
TCTGGAGTGCCCAGTAGATTCTCAGGAAGCAGGTCCGGCACCGATTTTACACTGA
CTATCTCTAGTCTGCAGCCTGAGGACTTCGCCACATACTATTGCCAGCAGCACTA
TACCACACCCCCTACATTTGGACAGGGCACTAAAGTGGAAATTAAGGGCGGGTCA
GGCGGAGGGAGCGGAGGAGGGTCCGGAGGAGGGTCTGGAGGAGGGAGTGGAGAGG
TGCAGCTGGTCGAATCCGGAGGAGGACTGGTGCAGCCTGGAGGCTCACTGAGGCT
GAGCTGTGCCGCTTCCGGCTTCAACATCAAGGATACCTACATTCATTGGGTCAGA
CAGGCTCCTGGGAAAGGACTGGAGTGGGTGGCAAGGATCTATCCAACCAATGGGT
ACACACGGTATGCCGATAGCGTGAAGGGAAGATTCACTATTTCTGCTGACACTAG
TAAAAACACCGCATACCTGCAGATGAATAGCCTGAGGGCAGAGGACACCGCCGTG
TACTATTGCTCCCGCTGGGGGGGAGACGGCTTTTACGCCATGGATTATTGGGGCC
AGGGGACCCTGGTGACAGTCTCAAGCGGCGGGTCAGGAGATGCACACAAAAGCGA
GGTCGCCCATCGCTTCAAGGACCTGGGCGAGGAAAATTTTAAAGCCCTGGTGCTG
ATTGCCTTCGCTCAGTACCTGCAGCAGTGCCCATTCGAAGACCACGTGAAGCTGG
TCAACGAGGTGACCGAATTTGCCAAAACATGCGTCGCTGACGAGTCCGCAGAAAA
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CTGAAGAAATACCTGTATGAGATCGCCCGGAGACATCCATACTTCTATGCCCCCG
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GCCGATCTGCCTAGTCTGGCAGCCGACTTCGTCGAGTCAAAAGATGTGTGTAAGA
ACTACGCCGAAGCAAAAGATGTGTTTCTGGGAATGTTTCTGTATGAGTATGCCCG
AGCCTGAGGATCC

Base construct 5 protein

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLY
SGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKGGS
GGGSGGGSGGGSGGGSGEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVR
QAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAV
YYCSRWGGDGFYAMDYWGQGTLVTVSSGGSGDAHKSEVAHRFKDLGEENFKALVL
IAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVAT
LRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETF
LKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEG
KASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTE
CCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMP
ADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARA

Base construct # 6:

GACGCACATAAGTCCGAGGTCGCTCACAGGTTCAAAGATCTGGGCGAGGAAAACT
TTAAGGCCCTGGTGCTGATCGCTTTCGCACAGTACCTGCAGCAGTGCCCATTCGA
AGACCACGTGAAACTGGTCAACGAAGTGACTGAATTTGCCAAGACCTGCGTCGCC
GACGAGTCCGCTGAAAATTGTGATAAATCTCTGCATACTCTGTTCGGGGATAAGC
TGTGTACCGTGGCCACACTGCGCGAGACCTATGGAGAAATGGCAGACTGCTGTGC
CAAACAGGAGCCAGAACGAAACGAGTGCTTTCTGCAGCATAAGGACGATAACCCA
AATCTGCCAAGGCTGGTGCGCCCAGAAGTGGACGTCATGTGTACCGCCTTCCACG
ATAATGAGGAAACATTTCTGAAGAAATACCTGTATGAGATTGCCCGGAGACATCC
ATACTTCTATGCCCCCGAACTGCTGTTCTTTGCTAAGCGCTACAAAGCCGCTTTT
ACCGAGTGCTGTCAGGCAGCCGATAAAGCTGCATGCCTGCTGCCTAAGCTGGACG
AGCTGAGGGATGAAGGAAAGGCCAGCTCCGCTAAACAGCGCCTGAAGTGTGCCTC
TCTGCAGAAATTCGGCGAGCGGGCTTTTAAGGCATGGGCTGTCGCACGACTGAGC
CAGCGGTTCCCAAAGGCAGAGTTTGCCGAAGTCTCCAAACTGGTGACTGACCTGA
CCAAGGTGCACACCGAGTGCTGTCATGGCGACCTGCTGGAATGCGCCGACGATAG
AGCTGATCTGGCAAAGTACATCTGTGAGAACCAGGACAGCATTTCTAGTAAGCTG
AAAGAGTGCTGTGAAAAACCCCTGCTGGAGAAGAGCCACTGCATCGCAGAGGTGG
AAAACGACGAAATGCCTGCCGATCTGCCAAGTCTGGCCGCTGACTTCGTCGAGTC
AAAAGATGTGTGTAAGAATTATGCCGAAGCTAAGGATGTGTTCCTGGGCATGTTT
CTGTACGAGTATGCACGAGCAGGAGGGAGCGGAGGCTCCGGAGGATCTGGCGGGA
GTGGAGGCGACATCCAGATGACTCAGTCCCCTTCAAGCCTGAGTGCTTCAGTCGG
CGATCGCGTGACTATTACCTGCCGAGCCTCTCAGGACGTCAATACAGCTGTGGCA
TGGTACCAGCAGAAGCCCGGCAAAGCTCCTAAGCTGCTGATCTACAGCGCATCCT
TTCTGTATTCAGGGGTGCCCAGCAGATTCTCTGGCAGTAGATCAGGGACAGATTT
TACACTGACTATTTCCTCTCTGCAGCCTGAGGACTTCGCCACTTACTATTGCCAG
CAGCACTATACCACACCCCCTACATTTGGACAGGGCACTAAAGTGGAAATCAAGG
GAGGCAGCGGAGGAGGATCTGGAGGAGGAAGTGGAGGAGGATCAGGAGGAGGAAG
CGGAGAGGTCCAGCTGGTGGAAAGCGGAGGAGGACTGGTGCAGCCTGGAGGGTCC
CTGAGACTGTCTTGTGCAGCCAGTGGCTTCAACATCAAAGATACCTACATTCATT
GGGTCAGACAGGCTCCTGGGAAGGGACTGGAGTGGGTGGCAAGGATCTATCCAAC
AAATGGATACACTCGGTATGCCGATAGCGTGAAAGGCCGGTTCACCATTTCAGCA
GACACCAGCAAGAACACAGCCTACCTGCAGATGAACAGCCTGCGAGCTGAGGACA
CAGCAGTGTACTATTGCAGTCGGTGGGGCGGCGATGGCTTTTACGCTATGGACTA
TTGGGGGCAGGGGACACTGGTGACTGTGAGTTCTTGAGGATCC

Protein Base construct # 6:

DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVA
DESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNP
NLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAF
TECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLS
QRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKL
KECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMF
LYEYARAGGSGGSGGSGGSGGDIQMTQSPSSLSASVGDRVTITCRASQDVNTAVA
WYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQ
QHYTTPPTFGQGTKVEIKGGSGGGSGGGSGGGSGGGSGEVQLVESGGGLVQPGGS
LRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISA
DTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDY**W**GQGTLVTVSS

Base construct # 7:

GACATTCAGATGACACAGAGCCCAAGCTCCCTGTCCGCATCTGTGGGCGACCGAG
TCACAATCACTTGCCGGGCCTCCCAGGATGTGAACACTGCTGTCGCATGGTACCA
GCAGAAACCAGGGAAGGCTCCCAAACTGCTGATCTACAGTGCATCATTCCTGTAT
AGTGGCGTGCCATCAAGGTTTAGCGGCTCCCGATCTGGAACCGACTTCACCCTGA
CAATCTCTAGTCTGCAGCCCGAGGATTTTGCCACATACTATTGCCAGCAGCACTA
TACCACACCCCCTACTTTCGGGCAGGGAACCAAGGTGGAGATCAAGGGAGGGAGC
GGAGGAGGGTCCGGAGGAGGGTCTGGAGGCGGGAGTGGAGGAGGGTCAGGAGAGG
TGCAGCTGGTCGAAAGCGGAGGAGGACTGGTGCAGCCTGGAGGCAGCCTGCGACT
GTCCTGTGCCGCTTCTGGCTTTAACATCAAGGACACCTACATTCATTGGGTGCGG
CAGGCACCTGGCAAAGGACTGGAGTGGGTGGCTAGAATCTATCCAACTAATGGAT
ACACCAGATATGCTGACAGCGTGAAGGGCAGGTTTACTATCAGTGCTGATACATC
AAAGAACACTGCATACCTGCAGATGAATAGCCTGCGCGCCGAGGATACCGCTGTG
TACTATTGTAGCCGATGGGGGGGAGACGGCTTCTACGCCATGGATTATTGGGGAC
AGGGCACCCTGGTGACAGTCTCAAGCGGAGGGAGTGGAGGCTCAGGAGGAAGCGG
AGGGTCCGGAGGCTCTGTGGTCCTGCTGCTGAGACTGGCTAAGACCTACGAGACT
ACCCTGGAAAAATGCTGTGCAGCCGCTGACCCCCACGAGTGCTATGCAAAGGTGT
TCGATGAGTTCAAGCCTCTGGTCGAGGAACCACAGAACCTGATCAAGCAGAATTG
TGAGCTGTTCGAACAGCTGGGCGAGTACAAGTTTCAGAACGCCCTGCTGGTGAGG
TATACAAAGAAAGTGCCCCAGGTCAGCACTCCTACCCTGGTGGAGGTCTCCAGGA

ATCTGGGGAAGGTCGGATCTAAGTGCTGTAAACACCCAGAGGCAAAACGCATGCC
CTGCGCCGAAGACTACCTGTCCGTGGTCCTGAATCAGCTGTGTGTGCTGCATGAG
AAGACCCCTGTGTCTGATCGAGTCACCAAATGCTGTACAGAAAGTCTGGTGAACC
GGAGACCCTGCTTTTCTGCCCTGGAGGTGGACGAAACATATGTCCCTAAGGAGTT
CAATGCCGAAACATTCACTTTTCACGCTGATATCTGTACACTGTCCGAGAAGGAA
CGCCAGATTAAGAAACAGACTGCTCTGGTGGAGCTGGTCAAGCATAAACCAAAGG
CAACCAAGGAACAGCTGAAAGCCGTGATGGACGATTTCGCAGCCTTTGTCGAGAA
GTGCTGTAAAGCCGACGATAAGGAAACTTGTTTCGCCGAGGAAGGCAAAAAACTG
GTCGCAGCATCACAGGCAGCACTGGGACTGTGAGGATCC

Base construct # 7 Protein:

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLY
SGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKGGS
GGGSGGGSGGGSGGGSGEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVR
QAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAV
YYCSRWGGDGFYAMDYWGQGTLVTVSSGGSGGSGGSGGSGGSVVLLLRLAKTYET
TLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVR
YTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHE
KTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKE
RQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKL
VAASQAALGL

Base construct # 8

TCCGTCGTCCTGCTGCTGAGACTGGCTAAGACCTACGAGACCACACTGGAAAAAT
GCTGTGCCGCTGCAGACCCCCACGAGTGCTATGCCAAGGTGTTCGATGAGTTCAA
GCCTCTGGTCGAGGAACCACAGAACCTGATCAAGCAGAATTGTGAGCTGTTCGAA
CAGCTGGGCGAGTACAAATTTCAGAACGCCCTGCTGGTGAGGTATACAAAGAAAG
TGCCCCAGGTCTCTACACCTACTCTGGTGGAGGTCAGTAGGAATCTGGGCAAGGT
CGGGTCAAAATGCTGTAAGCACCCAGAGGCCAAACGCATGCCCTGCGCTGAAGAC
TACCTGTCTGTGGTCCTGAACCAGCTGTGTGTGCTGCATGAGAAGACCCCTGTGA
GCGATCGAGTCACCAAATGCTGTACAGAAAGCCTGGTGAATCGGAGACCCTGCTT
TTCCGCTCTGGAGGTGGACGAAACATATGTCCCTAAGGAGTTCAATGCAGAAACC
TTCACATTTCACGCCGATATCTGTACTCTGTCCGAGAAGGAACGCCAGATTAAGA
AACAGACCGCCCTGGTGGAGCTGGTCAAGCATAAACCAAAGGCTACTAAGGAACA

GCTGAAAGCAGTGATGGACGATTTCGCCGCTTTTGTCGAGAAATGCTGTAAGGCA
GACGATAAGGAAACCTGCTTTGCCGAGGAAGGCAAGAAACTGGTGGCAGCCAGCC
AGGCTGCACTGGGACTGGGAGGGTCCGGAGGCTCTGGAGGAAGTGGAGGGTCAGG
AGGCGACATCCAGATGACACAGAGCCCAAGCTCCCTGTCAGCAAGCGTGGGCGAC
CGAGTCACTATTACCTGTCGGGCCTCCCAGGATGTGAATACTGCAGTCGCCTGGT
ACCAGCAGAAACCAGGAAAGGCTCCCAAACTGCTGATCTACTCCGCATCTTTCCT
GTATAGCGGCGTGCCATCCAGGTTTAGTGGATCACGCAGCGGCACAGACTTCACA
CTGACTATTTCTAGTCTGCAGCCCGAGGATTTTGCCACTTACTATTGCCAGCAGC
ACTATACTACCCCCCCTACCTTCGGACAGGGCACAAAGGTGGAGATCAAGGGAGG
ATCTGGAGGAGGAAGTGGAGGAGGATCAGGAGGAGGAAGCGGAGGAGGCAGCGGA
GAGGTGCAGCTGGTCGAATCTGGAGGAGGACTGGTGCAGCCTGGAGGGTCTCTGC
GACTGAGTTGTGCCGCTTCAGGCTTTAACATCAAGGACACCTACATTCATTGGGT
GCGGCAGGCACCTGGGAAGGGACTGGAGTGGGTCGCTAGAATCTATCCAACTAAT
GGGTACACCAGATATGCCGACAGCGTGAAGGGA**AGG**TTCACCATTAGCGCCGATA
CATCCAAAAACACTGCTTACCTGCAGATGAACAGCCTGCGCGCTGAGGATACAGC
AGTGTACTATTGCAGTCGATGGGGCGGCGATGGGTTCTACGCAATGGACTACTGG
GGACAGGGGACTCTGGTCACCGTCAGCAGCTGAGGATCC

Base construct # 8 Protein

SVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFE
QLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAED
YLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAET
FTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKA
DDKETCFAEEGKKLVAASQAALGLGGSGGSGGSGGSGGDIQMTQSPSSLSASVGD
RVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSG**S**RSGTDFT
LTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKGGSGGGSGGGSGGGSGGGSG
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTN
GYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYW
GQGTLVTVSS

Base construct # 9:

TCAAGCGAACTGACTCAGGACCCCGCTGTGAGCGTCGCACTGGGACAGACTGTGC
GGATCACCTGCCAGGGGGACTCCCTGAGATCTTACTATGCCTCCTGGTACCAGCA
GAAACCAGGCCAGGCTCCCGTGCTGGTCATCTATGGCAAGAACAATAGACCTTCC

GGGATTCCAGATAGGTTTTCTGGAAGCTCCTCTGGCAACACAGCTAGCCTGACCA
TTACAGGAGCCCAGGCTGAGGACGAAGCAGATTACTATTGCAACTCCAGGGACAG
TTCAGGCAATCACGTGGTCTTCGGCGGGGGAACAAAGCTGACTGTGGGAGGAGGA
TCAGGAGGAGGAAGCGGAGGAGGCAGCGGAGGAGGATCTGGAGGAGGAAGTGGAG
AGGTGCAGCTGGTCGAAAGCGGAGGAGGAGTGGTCAGGCCTGGAGGGTCACTGCG
ACTGAGCTGTGCCGCTTCCGGATTCACATTTGACGATTACGGAATGTCTTGGGTC
CGGCAGGCACCAGGAAAGGGACTGGAGTGGGTGAGTGGCATCAACTGGAATGGAG
GCTCTACAGGGTACGCTGATAGTGTGAAAGGACGCTTTACTATTAGTCGAGACAA
CGCCAAGAACAGCCTGTATCTGCAGATGAACAGCCTGAGAGCCGAGGATACTGCT
GTGTACTATTGTGCCAGGGGCCGCTCCCTGCTGTTCGACTACTGGGGGCAGGGAA
CCCTGGTGACAGTCTCTAGGGGGGGAAGTGGCGATGCTCACAAGAGCGAGGTCGC
ACATCGCTTCAAAGACCTGGGGGAGGAAAATTTTAAGGCCCTGGTGCTGATCGCA
TTCGCCCAGTATCTGCAGCAGTGCCCTTTTGAAGACCACGTGAAACTGGTCAACG
AGGTGACCGAGTTCGCCAAGACATGCGTGGCAGACGAGTCCGCCGAAAATTGTGA
TAAATCTCTGCATACTCTGTTTGGGGATAAGCTGTGTACTGTGGCCACCCTGCGG
GAGACCTACGGAGAAATGGCTGACTGCTGTGCAAAACAGGAGCCAGAAAGAAACG
AGTGCTTCCTGCAGCACAAGGACGATAACCCCAATCTGCCTCGACTGGTGCGGCC
CGAAGTGGACGTCATGTGTACTGCCTTCCACGATAATGAGGAAACCTTTCTGAAG
AAATACCTGTATGAGATTGCCCGGAGACATCCCTACTTTTATGCCCCTGAACTGC
TGTTCTTTGCTAAGCGGTACAAAGCAGCCTTCACCGAGTGCTGTCAGGCTGCAGA
TAAGGCCGCTTGCCTGCTGCCAAAACTGGACGAGCTGCGAGATGAAGGGAAAGCT
AGCTCCGCAAAGCAGAGACTGAAATGTGCAAGCCTGCAGAAGTTCGGCGAGAGGG
CCTTTAAAGCTTGGGCAGTGGCCAGACTGAGCCAGAGGTTCCCCAAGGCCGAGTT
TGCTGAAGTCTCCAAGCTGGTGACAGACCTGACTAAAGTGCACACCGAGTGCTGT
CATGGCGACCTGCTGGAATGCGCCGACGATCGCGCAGATCTGGCCAAATACATCT
GTGAGAACCAGGACTCTATTTCTAGTAAGCTGAAAGAGTGCTGTGAAAAGCCTCT
GCTGGAGAAAAGCCACTGCATCGCTGAGGTGGAAAACGACGAAATGCCCGCAGAT
CTGCCTAGTCTGGCAGCCGACTTTGTCGAGTCAAAGGATGTGTGTAAAAATTATG
CTGAAGCAAAGGATGTGTTCCTGGGCATGTTTCTGTACGAGTATGCACGAGCTGG
AGGGAGTGGAGGCTCAGGAGGAAGCGGCGGGTCCGGAGGCTCAAGCGAACTGACC
CAGGACCCCGCCGTGTCTGTCGCTCTGGGACAGACAGTGAGGATCACTTGCCAGG
GCGACTCTCTGCGCAGTTACTATGCAAGTTGGTATCAGCAGAAGCCTGGCCAGGC
CCCTGTCCTGGTCATCTATGGCAAGAATAATCGCCCTAGTGGGATTCCAGATCGA
TTTTCAGGGTCCTCTAGTGGAAACACAGCTTCTCTGACTATTACCGGCGCACAGG

CCGAGGACGAAGCCGATTACTATTGCAACAGCAGAGACTCAAGCGGCAATCATGT
GGTCTTCGGAGGAGGAACCAAGCTGACAGTGGGAGGAGGCTCAGGCGGCGGCAGC
GGAGGAGGCTCCGGGGGAGGCTCTGGAGGAGGCAGTGGAGAGGTCCAGCTGGTGG
AATCCGGAGGAGGAGTGGTCCGACCAGGAGGATCACTGAGACTGTCCTGTGCTGC
ATCCGGATTCACCTTCGATGATTACGGAATGAGCTGGGTCAGGCAGGCACCTGGC
AAGGGCCTGGAATGGGTGTCCGGCATCAACTGGAATGGCGGGTCAACCGGGTACG
CTGATAGCGTGAAAGGACGGTTCACAATTAGCAGGGATAATGCTAAGAACAGCTT
ATATCTGCAAATGAACAGCCTG**CGC**GCAGAGGACACAGCCGTGTACTATTGCGCC
CGGGGGCGGAGCCTGCTGTTTGATTACTGGGGGCAGGGCACACTGGTGACCGTCT
CTCGGTGAGGATCC

Base construct # 9 protein:

SSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPS
GIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGGG
SGGGSGGGSGGGSGGGSGEVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWV
RQAPGKGLEWVSGINWNGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTA
VYYCARGRSLLFDYWGQGTLVTVSRGGSGDAHKSEVAHRFKDLGEENFKALVLIA
FAQYLQQCPFEDHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLR
ETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLK
KYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKA
SSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECC
HGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPAD
LPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARAGGSGGSGGSGGSGGSSELT
QDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPSGIPDR
FSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGGGSGGGS
GGGSGGGSGGGSGEVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWVRQAPG
KGLEWVSGINWNGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCA
RGRSLLFDYWGQGTLVTVSR

Base construct # 10:

AGTAGCGAACTGACCCAGGACCCCGCAGTGAGCGTCGCACTGGGGCAGACAGTGA
GAATCACTTGCCAGGGAGATTCTCTGAGGAGTTACTATGCCTCCTGGTACCAGCA
GAAACCCGGCCAGGCTCCTGTGCTGGTCATCTATGGGAAGAACAATAGGCCAAGC

GGCATCCCCGACCGCTTCTCCGGCAGCTCCTCTGGGAACACAGCTAGCCTGACTA
TTACCGGCGCTCAGGCAGAGGACGAAGCAGATTACTATTGCAACTCCAGGGATAG
TTCAGGCAATCACGTGGTCTTTGGCGGGGGAACAAAGCTGACTGTGGGAGGAGGA
AGCGGAGGAGGCAGCGGAGGGGGATCTGGAGGAGGAAGTGGAGGAGGATCAGGAG
AGGTGCAGCTGGTCGAAAGCGGAGGAGGAGTGGTCCGCCCTGGAGGGAGCCTGCG
ACTGTCCTGTGCCGCTTCTGGCTTCACCTTTGACGATTACGGAATGAGCTGGGTG
CGGCAGGCACCAGGGAAGGGACTGGAGTGGGTGTCCGGCATCAACTGGAATGGAG
GCTCCACAGGATACGCAGACTCTGTGAAAGGCCGATTCACTATTTCTCGGGATAA
CGCCAAGAATAGTCTGTATCTGCAGATGAACAGCCTGAGAGCTGAGGACACTGCA
GTGTACTATTGTGCCAGGGGCCGCAGCCTGCTGTTTGATTACTGGGGCCAGGGAA
CCCTGGTGACAGTCTCCAGGGGAGGATCAGGAGGGAGCGGAGGCTCCGGAGGATC
TGGAGGGAGTGTGGTCCTGCTGCTGCGACTGGCTAAAACCTACGAGACCACACTG
GAAAAGTGCTGTGCAGCCGCTGACCCTCATGAGTGCTATGCCAAAGTGTTCGATG
AGTTCAAGCCACTGGTCGAGGAACCCCAGAACCTGATCAAACAGAATTGTGAGCT
GTTCGAACAGCTGGGCGAGTACAAGTTTCAGAACGCCCTGCTGGTGCGCTATACC
AAGAAAGTGCCTCAGGTCAGCACACCAACTCTGGTGGAAGTCTCCCGGAATCTGG
GGAAAGTGGGATCTAAATGCTGTAAGCACCCCGAGGCTAAGAGAATGCCTTGCGC
AGAAGACTACCTGTCTGTGGTCCTGAACCAGCTGTGTGTGCTGCATGAGAAAACC
CCAGTGAGCGATAGGGTCACCAAGTGCTGTACAGAAAGTCTGGTGAACCGGAGAC
CATGCTTCTCAGCCCTGGAGGTGGACGAAACATATGTCCCCAAAGAGTTTAATGC
CGAAACCTTCACATTTCACGCTGATATCTGTACTCTGTCCGAGAAGGAACGCCAG
ATTAAGAAACAGACCGCCCTGGTGGAGCTGGTCAAGCATAAACCCAAGGCAACAA
AAGAACAGCTGAAGGCCGTGATGGACGATTTCGCAGCCTTTGTCGAGAAATGCTG
TAAGGCTGACGATAAGGAAACTTGCTTCGCAGAGGAAGGAAAGAAACTGGTGGCT
GCAAGCCAGGCAGCTCTGGGACTGGGAGGCTCAGGAGGAAGCGGCGGGTCCGGAG
GCTCTGGGGGAAGCTCCGAGCTGACCCAGGACCCAGCCGTGTCTGTCGCTCTGGG
CCAGACTGTGCGCATCACCTGTCAGGGGGATAGTCTGCGATCATACTATGCAAGT
TGGTATCAGCAGAAACCTGGCCAGGCCCCAGTCCTGGTCATCTATGGGAAGAATA
ATCGACCTTCCGGCATCCCCGACCGGTTCTCCGGATCTAGTTCAGGCAACACAGC
CTCTCTGACTATTACCGGCGCCCAGGCTGAGGACGAAGCTGATTACTATTGCAAC
AGCAGGGATAGCTCCGGAAACCACGTGGTCTTTGGAGGAGGAACTAAGCTGACCG
TGGGAGGAGGAAGTGGCGGGGGATCAGGCGGCGGAAGCGGCGGCGGCAGCGGAGG
AGGATCTGGCGAAGTGCAGCTGGTCGAATCTGGCGGAGGAGTGGTCCGGCCAGGA
GGGAGTCTGAGACTGTCATGTGCAGCCAGCGGCTTCACATTCGATGATTACGGAA

TGTCTTGGGTGCGGCAGGCACCTGGAAAGGGCCTGGAATGGGTGAGTGGCATCAA
CTGGAACGGCGGCAGTACCGGATACGCTGACTCAGTGAAAGGCAGATTCACAATT
TCTAGAGACAATGCTAAGAATAGTTTATATCTGCAAATGAACAGCCTGAGAGCAG
AGGACACTGCCGTGTACTATTGCGCCCGGGGGAGGTCACTGCTGTTCGATTACTG
GGGGCAGGGCACTCTGGTCACTGTGTCAAGGTGAGGATCC

Base construct # 10 protein:

SSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPS
GIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGGG
SGGGSGGGSGGGSGGGSGEVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWV
RQAPGKGLEWVSGINWNGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTA
VYYCARGRSLLFDYWGQGTLVTVSRGGSGGSGGSGGSGGSVVLLLRLAKTYETTL
EKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYT
KKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKT
PVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQ
IKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVA
ASQAALGLGGSGGSGGSGGSGGSSELTQDPAVSVALGQTVRITCQGDSLRSYYAS
WYQQKPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCN
SRDSSGNHVVFGGGTKLTVGGGSGGGSGGGSGGGSGGGSGEVQLVESGGGVVRPG
GSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWVSGINWNGGSTGYADSVKGRFTI
SRDNAKNSLYLQMNSLRAEDTAVYYCARGRS**L**LFDYWGQGTLVTVSR

Base construct # 11:

GATATTCAGATGACTCAGTCTCCTAGCTCCCTGTCAGCTAGCGTCGGCGATCGGG
TGACAATCACTTGCAGAGCCAGCCAGGACGTCAACACAGCCGTGGCTTGGTACCA
GCAGAAGCCCGGAAAAGCACCTAAGCTGCTGATCTACTCCGCCTCTTTTCTGTAT
TCTGGCGTGCCCAGTAGATTCAGTGGATCAAGGAGCGGCACCGATTTTACCCTGA
CAATCTCTAGTCTGCAGCCTGAGGACTTTGCCACATACTATTGCCAGCAGCACTA
TACCACACCCCCTACTTTCGGGCAGGGAACCAAGGTGGAAATCAAAGGCGGGTCA
GGCGGAGGGAGCGGAGGAGGGTCCGGAGGAGGGTCTGGAGGAGGGAGTGGAGAGG
TGCAGCTGGTCGAATCTGGAGGAGGACTGGTGCAGCCAGGAGGCTCACTGCGGCT
GAGCTGTGCCGCTTCCGGCTTCAACATCAAAGATACCTACATTCATTGGGTCCGA
CAGGCACCAGGCAAGGGACTGGAGTGGGTGGCTAGAATCTATCCCACCAATGGCT
ACACACGATATGCCGATAGCGTGAAAGGGCGGTTTACAATTTCTGCAGACACTAG

TAAGAACACCGCCTACCTGCAGATGAACAGCCTGCGCGCTGAGGACACTGCAGTG
TACTATTGTAGTCGATGGGGGGGAGACGGCTTCTACGCCATGGATTATTGGGGAC
AGGGCACCCTGGTGACAGTCTCAAGCGGAGGGTCCGGCGATGCACACAAGTCTGA
GGTCGCTCATAGATTCAAAGACCTGGGGGAGGAAAATTTTAAGGCCCTGGTGCTG
ATTGCATTCGCCCAGTACCTGCAGCAGTGCCCCTTTGAAGACCACGTGAAACTGG
TCAACGAGGTGACAGAGTTCGCCAAGACTTGCGTCGCCGACGAGAGTGCTGAAAA
TTGTGATAAATCACTGCATACACTGTTTGGGGATAAGCTGTGTACTGTGGCCACC
CTGCGGGAGACTTATGGAGAAATGGCAGACTGCTGTGCCAAACAGGAGCCTGAAA
GAAACGAGTGCTTCCTGCAGCACAAGGACGATAACCCTAATCTGCCAAGGCTGGT
GCGCCCAGAAGTGGACGTCATGTGTACTGCCTTCCACGATAATGAGGAAACCTTT
CTGAAGAAATACCTGTATGAGATCGCCCGGAGACATCCCTACTTTTATGCTCCTG
AACTGCTGTTCTTTGCAAAACGGTACAAGGCAGCCTTCACCGAGTGCTGTCAGGC
TGCAGATAAGGCCGCTTGCCTGCTGCCCAAACTGGACGAGCTGCGGGATGAAGGC
AAGGCTTCCTCTGCAAAGCAGAGACTGAAATGTGCAAGCCTGCAGAAGTTCGGGG
AGAGGGCCTTTAAAGCTTGGGCAGTCGCACGACTGAGCCAGCGATTCCCTAAGGC
CGAGTTTGCTGAAGTCTCCAAGCTGGTGACAGACCTGACTAAAGTGCACACCGAG
TGCTGTCATGGCGACCTGCTGGAATGCGCCGACGATCGCGCAGATCTGGCCAAGT
ACATCTGTGAGAACCAGGACAGCATTAGTTCAAAGCTGAAAGAGTGCTGTGAAAA
GCCACTGCTGGAGAAATCCCACTGCATTGCTGAGGTGGAAAACGACGAAATGCCA
GCAGATCTGCCCAGCCTGGCAGCCGACTTCGTCGAGTCCAAGGATGTGTGTAAAA
ATTATGCTGAAGCAAAGGATGTGTTCCTGGGCATGTTTCTGTACGAGTATGCCAG
GGCTGGAGGCAGTGGAGGATCAGGAGGGAGCGGAGGCTCCGGAGGAGACATCCAG
ATGACCCAGAGCCCAAGCTCCCTGTCCGCTTCTGTCGGCGATAGGGTGACTATTA
CCTGCCGCGCCTCCCAGGACGTCAATACAGCAGTGGCCTGGTACCAGCAGAAACC
TGGGAAGGCTCCAAAACTGCTGATCTACAGTGCATCATTCCTGTATTCAGGAGTG
CCAAGCCGCTTTAGCGGGTCCCGATCTGGAACTGATTTCACACTGACTATCTCTA
GTCTGCAGCCCGAGGACTTTGCCACCTATTACTGCCAGCAGCACTACACTACCCC
ACCCACCTTCGGGCAGGGAACAAAGGTGGAAATCAAAGGGGGGTCCGGCGGCGGG
TCTGGCGGAGGGAGTGGAGGAGGGTCAGGCGGCGGGAGCGGCGAGGTCCAGCTGG
TGGAATCCGGCGGCGGCCTGGTGCAGCCTGGAGGCTCCCTGCGACTGTCTTGTGC
TGCAAGTGGCTTTAACATCAAGGACACTTACATTCATTGGGTCAGGCAGGCTCCT
GGCAAGGGCCTGGAATGGGTGGCACGAATCTATCCAACAAATGGATACACTAGGT
ACGCCGATAGCGTGAAAGGCAGGTTCACCATTTCAGCCGACACCAGCAAGAACAC
AGCTTACCTGCAAATGAACAGCCTGAGGGCTGAGGACACAGCAGTGTACTATTGC

AGCCGCTGGGGCGGGGACGGGTTCTATGCTATGGACTATTGGGGGCAGGGCACTC
TGGTCACTGTGTCAAGCTGAGGATCC

Base construct # 11 protein:

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLY
SGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKGGS
GGGSGGGSGGGSGGGSGEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVR
QAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAV
YYCSRWGGDGFYAMDYWGQGTLVTVSSGGSGDAHKSEVAHRFKDLGEENFKALVL
IAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVAT
LRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETF
LKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEG
KASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTE
CCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMP
ADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARAGGSGGSGGSGGSGGDIQ
MTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGV
PSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKGGSGGG
SGGGSGGGSGGGSGEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAP
GKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYC
SRWGGDGFYAMDYWGQGTLVTVSS

Base construct # 12:

GACATTCAGATGACTCAGAGCCCAAGCTCCCTGAGCGCATCCGTGGGCGACAGAG
TCACCATCACATGCAGGGCCTCCCAGGATGTGAACACCGCTGTCGCATGGTACCA
GCAGAAACCTGGGAAGGCTCCAAAACTGCTGATCTACTCTGCAAGTTTCCTGTAT
AGTGGAGTGCCATCAAGGTTTTCAGGCAGCCGCTCCGGGACCGACTTCACTCTGA
CCATCTCTAGTCTGCAGCCCGAGGATTTCGCCACATACTATTGCCAGCAGCACTA
TACCACACCCCCTACCTTTGGCCAGGGGACAAAAGTGGAAATTAAGGGAGGGAGC
GGAGGAGGGTCCGGAGGAGGGTCTGGAGGCGGGAGTGGAGGAGGGTCAGGAGAGG
TGCAGCTGGTCGAATCCGGAGGAGGACTGGTGCAGCCAGGAGGCAGCCTGCGGCT
GTCCTGTGCCGCTTCTGGCTTCAACATCAAAGACACCTACATTCATTGGGTGCGC
CAGGCTCCAGGAAAGGGACTGGAGTGGGTCGCACGAATCTATCCCACTAATGGGT
ACACCCGGTATGCCGATTCCGTGAAAGGAAGATTCACAATTAGTGCAGATACATC
AAAGAACACTGCCTACCTGCAGATGAACAGCCTGCGAGCAGAGGATACTGCCGTG

TACTATTGTAGTCGGTGGGGGGGAGACGGCTTTTACGCCATGGATTATTGGGGGC
AGGGAACCCTGGTGACAGTCTCAAGCGGAGGGTCAGGAGGCAGCGGAGGCAGCGG
AGGGTCTGGAGGCAGTGTGGTCCTGCTGCTGAGGCTGGCTAAAACCTACGAGACT
ACCCTGGAAAAGTGCTGTGCAGCCGCTGACCCCCACGAGTGCTATGCCAAAGTGT
TCGATGAGTTCAAGCCACTGGTCGAGGAACCCCAGAACCTGATCAAACAGAATTG
TGAGCTGTTCGAACAGCTGGGCGAGTACAAGTTTCAGAACGCCCTGCTGGTGCGC
TATACCAAGAAAGTGCCTCAGGTCTCTACACCAACTCTGGTGGAGGTCAGTAGGA
ATCTGGGGAAAGTGGGATCAAAGTGCTGTAAACACCCCGAGGCCAAGCGCATGCC
TTGCGCTGAAGACTACCTGTCTGTGGTCCTGAACCAGCTGTGTGTGCTGCATGAG
AAAACCCCCGTGAGCGATCGGGTCACCAAGTGCTGTACAGAAAGCCTGGTGAACC
GGAGACCCTGCTTCTCCGCTCTGGAGGTGGACGAAACATATGTCCCTAAGGAGTT
TAATGCTGAAACCTTCACATTTCACGCAGATATCTGTACACTGTCCGAGAAGGAA
AGACAGATTAAGAAACAGACTGCCCTGGTGGAGCTGGTCAAGCATAAACCTAAGG
CCACAAAAGAACAGCTGAAGGCTGTGATGGACGATTTCGCAGCCTTTGTCGAGAA
GTGCTGTAAAGCCGACGATAAGGAAACTTGCTTCGCTGAGGAAGGAAAGAAACTG
GTGGCTGCAAGCCAGGCAGCTCTGGGCCTGGGAGGATCAGGAGGGAGCGGAGGCT
CCGGAGGATCTGGAGGGGACATCCAGATGACCCAGTCTCCTTCCTCTCTGTCTGC
TAGTGTGGGCGACCGCGTCACTATTACCTGTCGAGCCAGCCAGGATGTGAATACA
GCCGTCGCTTGGTACCAGCAGAAGCCCGGCAAAGCACCTAAGCTGCTGATCTACT
CAGCCAGCTTTCTGTATAGCGGGGTGCCTTCCCGATTCTCCGGATCTCGGAGTGG
CACT**GAC**TTTACACTGACTATCAGTTCACTGCAGCCAGAGGATTTCGCCACCTAT
TACTGCCAGCAGCACTACACAACTCCACCCACTTTTGGCCAGGGGACCAAAGTGG
AAATCAAGGGAGGCTCTGGAGGAGGCAGTGGAGGAGGCTCAGGAGGAGGCAGCGG
AGGAGGCTCCGGCGAAGTGCAGCTGGTCGAATCTGGCGGCGGCCTGGTCCAGCCA
GGAGGATCTCTGAGGCTGAGTTGTGCAGCCTCAGGCTTCAACATCAAGGATACTT
ACATTCATTGGGTGCGGCAGGCACCTGGAAAGGGCCTGGAATGGGTCGCTAGAAT
CTATCCAACTAATGGCTACACCAGATATGCCGACAGCGTGAAAGGGCGCTTTACC
ATTAGCGCAGATACATCCAAAAATACCGCTTACCTGCAGATGAATAGCCTGAGAG
CTGAGGATACAGCAGTGTACTATTGCTCCAGATGGGGCGGCGATGGGTTTTACGC
AATGGACTACTGGGGACAGGGAACACTGGTCACCGTCTCTTCTTGAGGATCC

Base construct # 12 protein:

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLY
SGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKGGS

GGGSGGGSGGGSGGGSGEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVR
QAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAV
YYCSRWGGDGFYAMDYWGQGTLVTVSSGGSGGSGGSGGSGGSVVLLLRLAKTYET
TLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVR
YTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHE
KTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKE
RQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKL
VAASQAALGLGGSGGSGGSGGSGGDIQMTQSPSSLSASVGDRVTITCRASQDVNT
AVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATY
YCQQHYTTPPTFGQGTKVEIKGGSGGGSGGGSGGGSGGGSGEVQLVESGGGLVQP
GGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFT
ISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS

Base construct # 13:

AGTTCTGAGCTGACCCAGGACCCCGCTGTGAGCGTCGCACTGGGACAGACAGTGC
GGATCACTTGCCAGGGCGACAGCCTGAGATCCTACTATGCTAGCTGGTACCAGCA
GAAGCCTGGCCAGGCACCAGTGCTGGTCATCTATGGAAAAAACAATAGACCCAGC
GGCATTCCTGATAGGTTCTCCGGGAGCTCCTCTGGAAACACAGCTAGCCTGACTA
TTACCGGCGCCCAGGCTGAGGACGAAGCCGATTACTATTGCAACAGCAGGGACAG
TTCAGGGAATCACGTGGTCTTTGGAGGAGGAACTAAGCTGACCGTGGGAGGAGGC
AGCGGAGGAGGATCTGGAGGAGGAAGTGGAGGAGGATCAGGAGGAGGAAGCGGAG
AGGTGCAGCTGGTCGAAAGCGGAGGAGGAGTGGTCAGGCCAGGAGGGTCCCTGCG
ACTGTCTTGTGCCGCTAGTGGGTTCACTTTTGACGATTACGGAATGAGTTGGGTC
AGGCAGGCACCAGGAAAGGGACTGGAGTGGGTGAGCGGCATCAACTGGAATGGAG
GCAGTACAGGCTACGCTGATTCAGTGAAGGGGCGCTTCACTATTTCTCGAGACAA
CGCCAAAAATAGTCTGTATCTGCAGATGAACTCACTGCGCGCCGAGGATACAGCT
GTGTACTATTGCGCCAGGGGCCGCTCCCTGCTGTTTGACTACTGGGGGCAGGGAA
CACTGGTGACTGTCTCACGGGGGGGAAGCGGAGATGCACACAAATCTGAGGTCGC
CCATAGATTCAAGGACCTGGGCGAGGAAAATTTTAAAGCCCTGGTGCTGATCGCA
TTCGCCCAGTATCTGCAGCAGTGCCCTTTCGAAGACCACGTGAAGCTGGTCAACG
AGGTGACAGAATTTGCCAAAACTTGCGTCGCAGACGAGAGCGCCGAAAATTGTGA
TAAGTCCCTGCATACCCTGTTCGGCGATAAACTGTGTACCGTGGCCACACTGAGG
GAGACATACGGGGAAATGGCTGACTGCTGTGCAAAGCAGGAGCCCGAACGCAACG
AGTGCTTTCTGCAGCACAAAGACGATAACCCAAATCTGCCCCGACTGGTGCGGCC

TGAAGTGGACGTCATGTGTACTGCCTTCCACGATAATGAGGAAACCTTTCTGAAG
AAATACCTGTATGAGATTGCCCGGAGACATCCCTACTTCTATGCTCCTGAACTGC
TGTTCTTTGCAAAGCGGTACAAAGCAGCCTTTACCGAGTGCTGTCAGGCTGCAGA
TAAAGCCGCTTGCCTGCTGCCTAAGCTGGACGAGCTGAGGGATGAAGGCAAGGCT
AGCTCCGCAAAACAGCGCCTGAAGTGTGCTAGCCTGCAGAAATTCGGCGAGCGGG
CCTTCAAGGCTTGGGCAGTGGCCAGACTGTCACAGAGGTTCCCAAAGGCCGAGTT
TGCTGAAGTCAGCAAACTGGTGACTGACCTGACCAAGGTGCACACCGAGTGCTGT
CATGGCGACCTGCTGGAATGCGCCGACGATAGAGCAGATCTGGCCAAGTACATCT
GTGAGAACCAGGACTCCATTTCTAGTAAGCTGAAAGAGTGCTGTGAAAAACCCCT
GCTGGAGAAGTCTCATTGCATCGCCGAGGTGGAAAACGACGAAATGCCAGCTGAT
CTGCCCTCTCTGGCAGCCGACTTCGTCGAGAGTAAAGATGTGTGTAAGAATTATG
CTGAAGCAAAGGATGTGTTCCTGGGCATGTTTCTGTACGAGTATGCACGAGCTGG
AGGGTCTGGAGGCAGTGGAGGATCAGGAGGGAGCGGAGGCGACATCCAGATGACC
CAGTCCCCTTCAAGCCTGAGTGCTTCAGTCGGCGATCGAGTGACAATTACTTGCC
GGGCCTCTCAGGACGTCAATACAGCAGTGGCTTGGTATCAGCAGAAGCCTGGGAA
AGCACCAAAGCTGCTGATCTACAGCGCCTCCTTTCTGTATTCCGGAGTGCCTTCT
CGGTTCTCTGGCAGTAGATCAGGGACTGATTTTACCCTGACAATTTCCTCTCTGC
AGCCAGAGGACTTCGCCACCTACTATTGCCAGCAGCACTATACCACACCCCCTAC
CTTTGGCCAGGGGACAAAAGTGGAAATCAAGGGGGGAAGTGGCGGGGGATCAGGC
GGCGGAAGCGGCGGCGGCAGCGGCGGCGGATCTGGAGAGGTCCAGCTGGTGGAAA
GCGGAGGAGGACTGGTGCAGCCAGGAGGGAGTCTGAGACTGTCATGTGCTGCAAG
CGGCTTCAACATCAAGGATACCTACATTCACTGGGTCAGGCAGGCCCCAGGAAAA
GGCCTGGAGTGGGTGGCCCGCATCTATCCCACCAATGGGTACACACGCTATGCCG
ATTCCGTGAAGGGACGATTCACAATTTCCGCCGACACTTCTAAAAACACCGCTTA
CCTGCAGATGAACAGCCTGCGAGCCGAGGACACTGCTGTGTACTATTGTTCTAGA
TGGGGCGGGGACGGGTTTTACGCAATGGACTACTGGGGGCAGGGGACTCTGGTCA
CTGTCAGCAGCTGAGGA**TCC**

Base construct # 13 protein:

SSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPS
GIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGGG
SGGGSGGGSGGGSGGGSGEVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWV
RQAPGKGLEWVSGINWNGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTA

VYYCARGRSLLFDYWGQGTLVTVSRGGSGDAHKSEVAHRFKDLGEENFKALVLIA
FAQYLQQCPFEDHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLR
ETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLK
KYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKA
SSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECC
HGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPAD
LPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARAGGSGGSGGSGGSGGDIQMT
QSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPS
RFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKGGSGGGSG
GGSGGGSGGGSGEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGK
GLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSR
WGGDGFYAMDYWGQGTLVTVSS

Base construct # 14:

GACATTCAGATGACCCAGTCCCCAAGCTCCCTGTCTGCTAGTGTCGGCGATCGGG
TGACTATCACCTGCAGAGCCTCTCAGGACGTCAACACAGCCGTGGCTTGGTACCA
GCAGAAGCCTGGCAAAGCACCAAAGCTGCTGATCTACTCAGCCAGCTTTCTGTAT
AGCGGGGTGCCTTCCAGATTCTCCGGCTCTAGGAGTGGGACTGATTTTACACTGA
CTATCTCTAGTCTGCAGCCAGAGGACTTCGCCACCTACTATTGCCAGCAGCACTA
TACCACACCCCCTACATTTGGGCAGGGAACTAAAGTGGAAATTAAGGGAGGGTCT
GGAGGAGGGAGTGGAGGAGGGTCAGGCGGAGGGAGCGGAGGAGGGTCCGGCGAGG
TGCAGCTGGTCGAAAGCGGAGGAGGACTGGTGCAGCCTGGAGGCTCTCTGAGGCT
GAGTTGTGCCGCTTCAGGCTTCAACATCAAGGATACCTACATTCATTGGGTCCGA
CAGGCTCCAGGCAAAGGGCTGGAGTGGGTGGCAAGAATCTATCCCACAAATGGCT
ACACTAGATATGCCGATAGCGTGAAGGGGAGGTTCACAATTAGCGCTGACACCTC
CAAAAACACAGCATACCTGCAGATGAATAGTCTGCGGGCTGAGGACACTGCAGTG
TACTATTGTAGCAGATGGGGGGGAGACGGCTTTTACGCCATGGATTATTGGGGAC
AGGGCACTCTGGTGACCGTCTCAAGCGGAGGGAGCGGGGATGCACACAAATCCGA
GGTCGCCCATCGCTTCAAGGACCTGGGAGAGGAAAATTTTAAAGCCCTGGTGCTG
ATTGCATTCGCCCAGTACCTGCAGCAGTGCCCCTTCGAAGACCACGTGAAGCTGG
TCAACGAGGTGACCGAATTTGCCAAAACATGCGTCGCCGACGAGTCAGCTGAAAA
TTGTGATAAGAGCCTGCATACCCTGTTCGGAGATAAACTGTGTACAGTGGCCACT
CTGAGGGAGACATATGGCGAAATGGCAGACTGCTGTGCCAAGCAGGAGCCCGAAC

GCAACGAGTGCTTTCTGCAGCACAAAGACGATAACCCAAATCTGCCCAGGCTGGT
GCGCCCTGAAGTGGACGTCATGTGTACTGCCTTCCACGATAATGAGGAAACCTTT
CTGAAGAAATACCTGTATGAGATCGCCCGGAGACATCCCTACTTCTATGCCCCTG
AACTGCTGTTCTTTGCTAAACGGTACAAGGCAGCCTTTACCGAGTGCTGTCAGGC
TGCAGATAAAGCCGCTTGCCTGCTGCCTAAGCTGGACGAGCTGAGGGATGAAGGA
AAGGCTTCCTCTGCAAAACAGCGCCTGAAGTGTGCCTCCCTGCAGAAATTCGGCG
AGCGGGCTTTTAAGGCTTGGGCAGTGGCACGACTGTCCCAGCGATTCCCAAAGGC
CGAGTTTGCTGAAGTCTCTAAACTGGTGACCGACCTGACAAAGGTGCACACCGAG
TGCTGTCATGGCGACCTGCTGGAATGCGCCGACGATAGAGCAGATCTGGCCAAGT
ACATCTGTGAGAACCAGGACTCCATTAGTTCAAAGCTGAAAGAGTGCTGTGAAAA
ACCCCTGCTGGAGAAGTCTCACTGCATCGCAGAGGTGGAAAACGACGAAATGCCA
GCAGATCTGCCTTCCCTGGCAGCAGACTTCGTCGAGTCTAAAGATGTGTGTAAGA
ATTATGCTGAAGCAAAGGATGTGTTCCTGGGCATGTTTCTGTACGAGTATGCACG
AGCTGGAGGCTCAGGAGGAAGCGGAGGGTCCGGAGGCTCTGGGGGAAGCTCCGAA
CTGACCCAGGACCCCGCTGTGAGCGTCGCACTGGGACAGACTGTGCGCATTACCT
GCCAGGGAGACAGTCTGCGATCATACTATGCTTCCTGGTACCAGCAGAAGCCAGG
CCAGGCACCCGTGCTGGTCATCTATGGGAAAAACAATCGACCTTCCGGCATCCCC
GATCGGTTCTCTGGATCTAGTTCAGGCAACACAGCTAGCCTGACCATCACAGGGG
CACAGGCCGAGGACGAAGCCGATTACTATTGCAACAGCAGAGACAGCTCCGGCAA
TCATGTGGTCTTTGGAGGAGGAACTAAGCTGACCGTGGGAGGAGGATCTGGAGGA
GGAAGTGGCGGGGGATCAGGAGGAGGAAGCGGAGGAGGCAGCGGAGAGGTCCAGC
TGGTGGAAAGCGGAGGAGGAGTGGTCAGGCCAGGAGGGTCTCTGCGACTGAGTTG
TGCTGCATCAGGCTTCACTTTTGACGATTACGGAATGAGCTGGGTCAGGCAGGCA
CCAGGGAAGGGACTGGAG**TGG**GTGAGCGGCATCAACTGGAATGGAGGCTCTACAG
GATACGCTGATAGTGTGAAGGGCCGCTTCACTATTAGTCGAGACAACGCCAAAAA
TTCACTGTATCTGCAGATGAATAGCCTGCGCGCCGAGGACACAGCTGTGTACTAT
TGCGCCAGAGGAAGGTCACTGCTGTTTGATTATTGGGGGCAGGGCACACTGGTCA
CCGTCTCCCGCTGAGGATCC

Base construct # 14 protein:

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLY
SGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKGGS
GGGSGGGSGGGSGGGSGEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVR
QAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAV

YYCSRWGGDGFYAMDYWGQGTLVTVSSGGSGDAHKSEVAHRFKDLGEENFKALVL
IAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVAT
LRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETF
LKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEG
KASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTE
CCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMP
ADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARAGGSGGSGGSGGSGGSSE
LTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPSGIP
DRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGGGSGG
GSGGGSGGGSGGGSGEVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWVRQA
PGKGLEWVSGINWNGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYY
CARGRSLLFDYWGQGTLVTVSR

Base construct # 15:

TCTTCAGAACTGACCCAGGACCCCGCAGTGAGCGTCGCACTGGGCCAGACCGTGA
GAATCACATGCCAGGGGGATTCCCTGAGGTCTTACTATGCTAGCTGGTACCAGCA
GAAGCCAGGCCAGGCACCCGTGCTGGTCATCTATGGCAAAAACAATAGGCCTTCA
GGGATTCCAGACCGCTTTAGCGGAAGCTCCTCTGGCAACACAGCAAGCCTGACAA
TTACTGGCGCTCAGGCAGAGGACGAAGCCGATTACTATTGCAACAGCAGGGATAG
TTCAGGCAATCACGTGGTCTTCGGAGGAGGAACTAAGCTGACCGTGGGAGGAGGA
TCTGGAGGAGGAAGTGGCGGGGGATCAGGAGGAGGAAGCGGAGGAGGCAGCGGAG
AGGTGCAGCTGGTCGAAAGCGGAGGAGGAGTGGTCCGCCCAGGAGGGTCTCTGCG
ACTGAGTTGTGCCGCTTCAGGATTCACCTTTGACGATTACGGAATGTCCTGGGTG
AGGCAGGCACCAGGGAAGGGACTGGAGTGGGTCTCTGGCATCAACTGGAATGGAG
GCTCTACAGGGTACGCTGACAGTGTGAAGGGACGGTTCACCATTTCCCGGGATAA
CGCCAAAAATTCTCTGTATCTGCAGATGAATAGTCTGCGCGCTGAGGACACCGCA
GTGTACTATTGTGCCAGGGGCCGCAGTCTGCTGTTCGATTACTGGGGCCAGGGAA
CACTGGTGACTGTCAGCCGAGGAGGAAGTGGAGGGTCAGGAGGCAGCGGAGGCAG
CGGAGGGTCTGTGGTCCTGCTGCTGAGACTGGCTAAGACATACGAGACCACACTG
GAAAAATGCTGTGCAGCCGCTGACCCCCATGAGTGCTATGCCAAGGTGTTCGATG
AGTTCAAGCCACTGGTCGAGGAACCCCAGAACCTGATCAAGCAGAATTGTGAGCT
GTTCGAACAGCTGGGCGAGTACAAATTTCAGAACGCCCTGCTGGTGCGCTATACC
AAGAAAGTGCCTCAGGTCTCAACCCCAACACTGGTGGAGGTCAGCAGGAATCTGG
GCAAGGTCGGGTCCAAATGCTGTAAGCACCCCGAGGCAAAACGCATGCCTTGCGC

CGAAGACTACCTGTCCGTGGTCCTGAACCAGCTGTGTGTGCTGCATGAGAAGACA
CCTGTGTCTGATCGGGTCACTAAATGCTGTACCGAATCTCTGGTGAACCGGAGAC
CTTGCTTTAGTGCCCTGGAGGTGGACGAAACTTATGTCCCAAAGGAGTTCAATGC
TGAAACTTTCACCTTTCACGCAGATATCTGTACCCTGAGCGAGAAGGAAAGACAG
ATTAAGAAACAGACAGCCCTGGTGGAGCTGGTCAAGCATAAACCAAAGGCCACCA
AGGAACAGCTGAAAGCTGTGATGGACGATTTCGCAGCCTTTGTCGAGAAATGCTG
TAAGGCTGACGATAAGGAAACATGCTTCGCAGAGGAAGGGAAGAAACTGGTGGCT
GCATCCCAGGCAGCTCTGGGACTGGGAGGCAGTGGAGGATCAGGAGGGAGCGGAG
GCTCCGGAGGAGACATCCAGATGACTCAGTCCCCAAGCTCCCTGTCAGCAAGCGT
GGGCGACCGGGTCACAATTACTTGTAGAGCTTCTCAGGATGTGAATACCGCCGTC
GCTTGGTACCAGCAGAAACCCGGCAAGGCCCCTAAACTGCTGATCTACTCCGCTT
CTTTCCTGTATAGCGGAGTGCCATCCCGGTTCAGCGGGTCAAGGAGCGGAACTGA
CTTCACCCTGACAATTTCTAGTCTGCAGCCTGAGGATTTTGCCACCTACTATTGC
CAGCAGCACTATACTACCCCCCCTACTTTCGGACAGGGCACCAAGGTGGAAATCA
AAGGAGGGTCTGGAGGAGGGAGTGGAGGAGGGTCAGGCGGAGGGAGCGGAGGAGG
GTCCGGCGAAGTCCAGCTGGTCGAATCCGGAGGAGGACTGGTGCAGCCTGGAGGC
TCTCTGAGGCTGAGTTGTGCAGCCTCAGGCTTTAACATCAAGGACACCTACATTC
ATTGGGTGCGGCAGGCACCAGGGAAAGGACTGGAGTGGGTGGCCAGAATCTATCC
CACAAATGGATACACTCGATATGCCGACTCTGTGAAGGGCCGGTTCACAATTAGC
GCAGATACC**TCC**AAAAACACAGCCTACCTGCAGATGAACAGCCTGCGCGCCGAGG
ATACTGCTGTGTACTATTGCAGCCGATGGGGCGGGGACGGCTTCTACGCTATGGA
CTATTGGGGGCAGGGGACTCTGGTGACAGTGAGCAGCTGAGGATCC

Base construct # 15 protein:

SSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPS
GIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGGG
SGGGSGGGSGGGSGGGSGEVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWV
RQAPGKGLEWVSGINWNGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTA
VYYCARGRSLLFDYWGQGTLVTVSRGGSGGSGGSGGSGGSVVLLLRLAKTYETTL
EKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYT
KKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKT
PVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQ
IKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVA
ASQAALGLGGSGGSGGSGGSGGDIQMTQSPSSLSASVGDRVTITCRASQDVNTAV

AWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYC
QQHYTTPPTFGQGTKVEIKGGSGGGSGGGSGGGSGGGSGEVQLVESGGGLVQPGG
SLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTIS
ADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS

Base construct # 16:

GATATTCAGATGACCCAGAGCCCAAGCTCCCTGAGTGCATCAGTGGGCGACAGAG
TCACAATCACTTGCAGGGCTAGCCAGGATGTGAACACAGCTGTCGCATGGTACCA
GCAGAAACCAGGCAAGGCTCCCAAACTGCTGATCTACAGCGCATCCTTCCTGTAT
TCCGGCGTGCCCTCTAGGTTTTCTGGGAGTCGCTCAGGAACTGACTTCACCCTGA
CAATCTCTAGTCTGCAGCCTGAGGATTTTGCCACCTACTATTGCCAGCAGCACTA
CACCACACCCCCTACTTTCGGCCAGGGGACCAAGGTGGAGATCAAGGGCGGGAGT
GGAGGCGGGTCAGGCGGAGGGAGCGGAGGAGGGTCCGGAGGAGGGTCTGGCGAGG
TGCAGCTGGTCGAAAGCGGAGGAGGACTGGTGCAGCCTGGAGGCAGTCTGCGGCT
GTCATGTGCCGCTAGCGGCTTCAACATCAAGGACACCTACATTCATTGGGTGCGC
CAGGCACCAGGAAAAGGCCTGGAGTGGGTCGCCCGAATCTATCCCACCAATGGGT
ACACAAGATATGCCGACTCCGTGAAGGGACGCTTTACAATTTCCGCTGATACTTC
TAAAAACACCGCATACCTGCAGATGAATAGTCTGAGAGCAGAGGATACTGCCGTG
TACTATTGTAGCAGATGGGGGGGAGACGGCTTCTACGCCATGGACTACTGGGGCC
AGGGCACTCTGGTGACCGTCTCAAGCGGAGGGAGCGGAGGCTCCGGAGGATCTGG
AGGGAGTGGAGGCTCAGTGGTCCTGCTGCTGAGGCTGGCTAAGACCTACGAGACT
ACCCTGGAAAAATGCTGTGCAGCCGCTGACCCCCACGAGTGCTATGCCAAGGTGT
TCGATGAGTTCAAGCCACTGGTCGAGGAACCCCAGAACCTGATCAAGCAGAATTG
TGAGCTGTTCGAACAGCTGGGCGAGTACAAATTTCAGAACGCCCTGCTGGTGCGC
TATACAAAGAAAGTGCCTCAGGTCAGTACTCCAACCCTGGTGGAAGTCTCACGGA
ATCTGGGAAAGGTCGGCAGCAAGTGCTGTAAACACCCCGAGGCAAAAAGAATGCC
TTGCGCCGAAGACTACCTGAGCGTGGTCCTGAATCAGCTGTGTGTGCTGCATGAG
AAGACACCTGTGAGCGATAGGGTCACAAAATGCTGTACTGAATCCCTGGTGAACC
GGAGACCTTGCTTTTCTGCTCTGGAGGTGGACGAAACTTATGTCCCAAAGGAGTT
CAATGCCGAAACATTCACTTTTCACGCTGATATCTGTACCCTGAGCGAGAAGGAA
CGCCAGATTAAGAAACAGACAGCCCTGGTGGAGCTGGTCAAGCATAAACCAAAGG
CAACTAAGGAACAGCTGAAAGCCGTGATGGACGATTTCGCAGCCTTTGTCGAGAA
GTGCTGTAAAGCCGACGATAAGGAAACCTGCTTTGCTGAGGAAGGCAAGAAACTG
GTGGCTGCAAGCCAGGCAGCTCTGGGACTGGGAGGAAGCGGAGGGTCCGGAGGCT

CTGGGGGAAGTGGAGGGTCCTCTGAGCTGACCCAGGACCCCGCTGTGTCCGTCGC
ACTGGGACAGACCGTGCGAATTACATGTCAGGGCGATTCACTGCGGAGCTACTAT
GCTTCTTGGTACCAGCAGAAGCCTGGCCAGGCACCAGTGCTGGTCATCTATGGAA
AAAACAATCGGCCCAGTGGCATTCCTGACAGATTTTCAGGCAGTTCAAGCGGGAA
CACCGCATCCCTGACCATCACAGGCGCCCAGGCTGAGGACGAAGCCGATTACTAT
TGCAACTCTAGGGATTCCTCTGGCAATCATGTGGTCTTCGGAGGCGGGACAAAGC
TGACTGTGGGAGGAGGGAGTGGCGGAGGGTCAGGCGGCGGGAGCGGCGGCGGGTC
CGGCGGCGGGTCTGGAGAAGTGCAGCTGGTCGAATCCGGAGGAGGAGTGGTCCGC
CCAGGAGGCAGTCTGCGACTGTCATGTGCAGCCAGCGGGTTCACCTTTGACGATT
ACGGAATGTCCTGGGTGCGGCAGGCACCAGGCAAGGGACTGGAGTGGGTGTCTGG
CATCAACTGGAATGGGGGCAGCACAGGCTACGCTGACTCTGTGAAGGGGCGATTC
ACTATTAGCCGGGATAACGCCAAAAATTCCCTGTATCTGCAGATGAACAGCCTGA
GAGCCGAGGACACAGCTGTGTACTATTGCGCCAGGGGGCGGTCACTGCTGTTTGA
TTATTGGGGGCAGGGAACTCTGGTCACTGTCTCTAGGTGAGGA**TCC**

Base construct # 16 protein:

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLY
SGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKGGS
GGGSGGGSGGGSGGGSGEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVR
QAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAV
YYCSRWGGDGFYAMDYWGQGTLVTVSSGGSGGSGGSGGSGGSVVLLLRLAKTYET
TLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVR
YTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHE
KTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKE
RQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKL
VAASQAALGLGGSGGSGGSGGSGGSSELTQDPAVSVALGQTVRITCQGDSLRSYY
ASWYQQKPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYY
CNSRDSSGNHVVFGGGTKLTVGGGSGGGSGGGSGGGSGGGSGEVQLVESGGGVVR
PGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWVSGINWNGGSTGYADSVKGRF
TISRDNAKNSLYLQMNSLRAEDTAVYYCARGRSLLFDYWGQGTLVTVSR

Sequence for v593:

GACATTCAGATGACACAGAGCCCAAGCTCCCTGTCTGCAAGTGTCGGCGATCGAG
TGACAATCACTTGCCGGGCTTCCCAGGACGTCAACACTGCCGTGGCTTGGTACCA
GCAGAAACCTGGGAAGGCCCCAAAACTGCTGATCTACTCAGCTAGCTTTCTGTAT
AGCGGAGTGCCCTCCCGGTTCTCCGGATCTAGAAGTGGCACCGATTTTACCCTGA
CAATCTCTAGTCTGCAGCCTGAGGACTTCGCCACATACTATTGCCAGCAGCACTA
TACCACACCCCCTACCTTTGGGCAGGGAACAAAGGTGGAAATCAAAGGAGGGTCT
GGAGGAGGGAGTGGAGGAGGGTCAGGCGGAGGGAGCGGAGGAGGGTCCGGCGAGG
TGCAGCTGGTCGAAAGCGGAGGAGGACTGGTGCAGCCTGGAGGCTCTCTGAGGCT
GAGTTGTGCCGCTTCAGGCTTCAACATCAAAGATACCTACATTCATTGGGTCCGC
CAGGCTCCAGGCAAGGGACTGGAGTGGGTGGCACGAATCTATCCCACAAATGGAT
ACACTCGGTATGCCGATTCCGTGAAAGGCAGATTCACTATTAGCGCTGACACCTC
CAAGAACACAGCATACCTGCAGATGAATAGTCTGCGAGCAGAGGACACCGCCGTG
TACTATTGCTCACGGTGGGGGGGAGACGGCTTTTACGCCATGGATTATTGGGGAC
AGGGCACTCTGGTGACCGTCTCAAGCGGAGGGAGCGGAGATGCACACAAGTCCGA
GGTCGCTCATCGCTTCAAAGACCTGGGCGAGGAAAACTTTAAGGCCCTGGTGCTG
ATTGCATTCGCCCAGTACCTGCAGCAGTGCCCATTCGAGGACCACGTGAAACTGG
TCAACGAAGTGACTGAATTTGCCAAGACCTGCGTGGCTGACGAGTCAGCAGAAAA
TTGTGATAAAAGCCTGCATACACTGTTCGGCGATAAGCTGTGTACAGTGGCCACT
CTGAGGGAGACTTATGGGGAAATGGCCGACTGCTGTGCTAAACAGGAGCCAGAAC
GCAACGAGTGCTTTCTGCAGCACAAGGACGATAACCCAAATCTGCCCAGACTGGT
GAGGCCCGAAGTGGACGTCATGTGTACAGCCTTCCACGATAATGAGGAAACTTTT
CTGAAGAAATACCTGTATGAGATCGCTCGGAGACATCCCTACTTCTATGCCCCTG
AACTGCTGTTCTTTGCTAAGAGGTACAAAGCAGCCTTTACCGAGTGCTGTCAGGC
TGCAGATAAGGCCGCTTGCCTGCTGCCAAAACTGGACGAGCTGAGAGATGAAGGC
AAGGCATCCTCTGCCAAGCAGAGGCTGAAATGTGCCTCCCTGCAGAAGTTCGGGG
AGAGGGCTTTTAAAGCTTGGGCAGTGGCACGACTGAGCCAGCGATTCCCAAAGGC
TGAGTTTGCAGAAGTCTCCAAGCTGGTGACCGACCTGACAAAAGTGCACACCGAG
TGCTGTCATGGCGACCTGCTGGAATGCGCCGACGATCGCGCCGATCTGGCTAAGT
ACATCTGTGAGAACCAGGACAGCATTAGTTCAAAGCTGAAAGAGTGCTGTGAAAA
GCCTCTGCTGGAGAAATCCCACTGCATTGCAGAGGTGGAAAACGACGAAATGCCA
GCAGATCTGCCTTCCCTGGCAGCAGACTTCGTCGAGTCTAAGGATGTGTGTAAAA
ATTACGCTGAAGCAAAGGATGTGTTCCTGGGCATGTTTCTGTACGAGTATGCCAG
GCGCCACCCTGACTACAGCGTGGTCCTGCTGCTGCGGCTGGCTAAAACCTATGAG

ACTACCCTGGAAAAGTGCTGTGCTGCAGCCGATCCACATGAGTGCTATGCCAAGG
TCTTCGACGAGTTCAAGCCACTGGTGGAGGAACCCCAGAACCTGATCAAACAGAA
TTGTGAGCTGTTTGAACAGCTGGGCGAGTACAAGTTCCAGAACGCCCTGCTGGTG
AGATATACAAAGAAAGTCCCTCAGGTGAGTACTCCAACCCTGGTGGAAGTCTCAC
GGAATCTGGGCAAAGTGGGGAGCAAGTGCTGTAAACACCCCGAGGCAAAGAGAAT
GCCTTGCGCCGAAGATTACCTGTCTGTGGTCCTGAATCAGCTGTGTGTGCTGCAT
GAGAAAACTCCTGTCAGCGACCGGGTGACTAAGTGCTGTACCGAATCCCTGGTGA
ACCGACGGCCTTGCTTCTCTGCCCTGGAGGTCGATGAAACATATGTGCCAAAGGA
GTTTAATGCAGAAACATTCACTTTTCACGCCGACATCTGTACTCTGAGCGAGAAG
GAAAGACAGATTAAGAAACAGACCGCCCTGGTCGAGCTGGTGAAGCATAAACCAA
AGGCTACCAAGGAACAGCTGAAAGCAGTCATGGACGATTTCGCTGCATTTGTGGA
GAAGTGCTGTAAAGCAGACGATAAGGAAACATGCTTCGCCGAGGAAGGGAAGAAA
CTGGTGGCAGCTAGCCAGGCAGCACTGGGACTGGGAGGCTCAGGAGGAAGCGGAG
GGTCCGGAGGCTCTGGAGGAAGCTCCGAGCTGACCCAGGACCCCGCAGTGTCTGT
CGCACTGGGACAGACAGTGAGGATTACTTGTCAGGGGGACAGTCTGCGCTCATAC
TATGCTAGCTGGTACCAGCAGAAACCAGGCCAGGCACCCGTGCTGGTCATCTATG
GCAAGAACAATCGCCCTTCCGGGATTCCAGATCGATTCTCTGGGTCTAGTTCAGG
AAACACCGCATCTCTGACCATCACAGGCGCCCAGGCTGAGGACGAAGCTGATTAC
TATTGCAACAGCAGAGACAGCTCCGGCAATCACGTGGTCTTTGGAGGAGGAACTA
AGCTGACCGTGGGAGGAGGATCTGGAGGAGGAAGTGGCGGGGGATCAGGAGGAGG
AAGCGGAGGAGGCAGCGGAGAGGTCCAGCTGGTGGAAAGCGGAGGAGGCGTGGTC
AGACCAGGAGGGTCTCTGAGACTGTCCTGTGCTGCATCAGGATTCACCTTTGACG
ATTACGGCATGTCTTGGGTCAGGCAGGCACCTGGGAAGGGCCTGGAATGGGTGAG
TGGCATCAACTGGAATGGAGGCTCTACCGGGTACGCCGATAGTGTGAAAGGAAGG
TTCACAATTAGTCGCGACAACGCTAAGAACAGCCTGTATCTGCAGATGAATAGCC
TGCGCGCTGAGGACACAGCAGTGTACTATTGCGCCAGGGGGAGGTCACTGCTGTT
TGATTATTGGGGGCAGGGAACTCTGGTCACTGTGTCACGGTGAGGATCC

Protein Sequence for v593:

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLY
SGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKGGS
GGGSGGGSGGGSGGGSGEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVR
QAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAV
YYCSRWGGDGFYAMDYWGQGTLVTVSSGGSGDAHKSEVAHRFKDLGEENFKALVL

IAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVAT
LRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETF
LKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEG
KASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTE
CCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMP
ADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYE
TTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLV
RYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLH
EKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEK
ERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKK
LVAASQAALGLGGSGGSGGSGGSGGSSELTQDPAVSVALGQTVRITCQGDSLRSY
YASWYQQKPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADY
YCNSRDSSGNHVVFGGGTKLTVGGGSGGGSGGGSGGGSGGGSGEVQLVESGGGVV
RPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWVSGINWNGGSTGYADSVKGR
FTISRDNAKNSLYLQMNSLRAEDTAVYYCARGRSLLFDYWGQGTLVTVSR

Sequence for v594:

AGTAGCGAACTGACCCAGGACCCCGCAGTGAGCGTCGCACTGGGGCAGACAGTGC
GAATCACTTGCCAGGGAGACAGCCTGCGGTCCTACTATGCTTCCTGGTACCAGCA
GAAACCTGGCCAGGCACCAGTGCTGGTCATCTATGGGAAGAACAATCGGCCCAGC
GGCATCCCCGATAGATTCTCCGGCAGCTCCTCTGGGAACACCGCCTCTCTGACAA
TTACTGGGGCCCAGGCTGAGGACGAAGCTGATTACTATTGCAACAGCAGGGACAG
TTCAGGAAATCACGTGGTCTTTGGAGGAGGAACTAAGCTGACCGTGGGAGGAGGC
AGCGGAGGAGGATCTGGAGGAGGAAGTGGAGGAGGATCAGGAGGAGGAAGCGGAG
AGGTGCAGCTGGTCGAAAGCGGAGGAGGAGTGGTCAGACCTGGAGGGTCCCTGAG
GCTGTCTTGTGCCGCTAGTGGCTTCACCTTTGACGATTACGGAATGAGTTGGGTC
CGGCAGGCACCAGGAAAGGGACTGGAGTGGGTGTCAGGCATCAACTGGAATGGAG
GCAGTACCGGATACGCCGATTCAGTGAAAGGCAGGTTCACAATTTCTCGCGACAA
CGCTAAGAATAGTCTGTATCTGCAGATGAACTCACTGAGAGCTGAGGATACAGCA
GTGTACTATTGCGCCAGAGGCAGGTCTCTGCTGTTTGACTACTGGGGGCAGGGAA
CACTGGTGACTGTCTCACGAGGAGGAAGCGGCGATGCACACAAGTCCGAGGTCGC
TCATAGATTCAAAGACCTGGGGGAGGAAAATTTTAAGGCCCTGGTGCTGATCGCA
TTCGCCCAGTATCTGCAGCAGTGCCCATTCGAGGACCACGTGAAACTGGTCAACG
AGGTGACCGAATTTGCCAAGACATGCGTGGCCGACGAGAGCGCTGAAAATTGTGA

TAAATCCCTGCATACACTGTTCGGGGATAAGCTGTGTACCGTGGCCACACTGAGG
GAGACTTACGGAGAAATGGCAGACTGCTGTGCCAAACAGGAGCCAGAACGCAACG
AGTGCTTTCTGCAGCACAAGGACGATAACCCAAATCTGCCACGACTGGTGCGACC
AGAAGTGGACGTCATGTGTACAGCCTTCCACGATAATGAGGAAACTTTTCTGAAG
AAATACCTGTATGAGATCGCCCGGAGACATCCCTACTTCTATGCTCCTGAACTGC
TGTTCTTTGCAAAACGGTACAAGGCAGCCTTTACCGAGTGCTGTCAGGCTGCAGA
TAAGGCCGCTTGCCTGCTGCCAAAACTGGACGAGCTGAGAGATGAAGGCAAGGCA
AGCTCCGCCAAGCAGAGGCTGAAATGTGCTAGCCTGCAGAAGTTCGGGGAGAGGG
CCTTCAAGGCTTGGGCAGTGGCACGACTGTCACAGAGATTCCCCAAGGCTGAGTT
TGCAGAAGTCAGCAAGCTGGTGACTGACCTGACCAAAGTGCACACCGAGTGCTGT
CATGGCGACCTGCTGGAATGCGCCGACGATCGCGCCGATCTGGCTAAGTACATCT
GTGAGAACCAGGACAGCATTTCTAGTAAGCTGAAAGAGTGCTGTGAAAAGCCTCT
GCTGGAGAAATCCCACTGCATCGCCGAGGTGGAAAACGACGAAATGCCAGCTGAT
CTGCCCTCTCTGGCAGCCGACTTCGTCGAGAGTAAGGATGTGTGTAAAAATTACG
CTGAAGCAAAGGATGTGTTCCTGGGCATGTTTCTGTACGAGTATGCAAGGCGACA
CCCAGACTACTCCGTGGTCCTGCTGCTGCGGCTGGCTAAAACCTATGAGACCACA
CTGGAAAAGTGCTGTGCTGCAGCCGATCCTCATGAGTGCTATGCCAAGGTCTTCG
ACGAGTTCAAGCCACTGGTGGAGGAACCCCAGAACCTGATCAAGCAGAATTGTGA
GCTGTTTGAACAGCTGGGCGAGTACAAGTTCCAGAACGCCCTGCTGGTGAGATAT
ACAAAGAAAGTCCCTCAGGTGTCAACCCCAACACTGGTGGAGGTCAGCCGGAATC
TGGGGAAAGTGGGCAGCAAATGCTGTAAGCACCCCGAGGCAAAGAGAATGCCTTG
CGCCGAAGATTACCTGTCTGTGGTCCTGAACCAGCTGTGTGTGCTGCATGAGAAA
ACTCCTGTCAGTGACAGGGTGACCAAGTGCTGTACAGAATCTCTGGTGAACCGAC
GGCCTTGCTTCAGTGCCCTGGAGGTCGATGAAACATATGTGCCAAAGGAGTTTAA
TGCCGAAACTTTCACCTTTCACGCTGACATCTGTACTCTGAGCGAGAAGGAACGC
CAGATTAAGAAACAGACCGCCCTGGTCGAGCTGGTGAAGCATAAACCAAAGGCAA
CAAAGGAACAGCTGAAAGCCGTCATGGACGATTTCGCTGCATTTGTGGAGAAATG
CTGTAAGGCCGACGATAAGGAAACTTGCTTCGCTGAGGAAGGAAAGAAACTGGTG
GCAGCTTCCCAGGCAGCACTGGGACTGGGAGGGTCTGGAGGCAGTGGAGGATCAG
GAGGGAGCGGAGGCGACATCCAGATGACCCAGTCCCCCTCAAGCCTGAGTGCCTC
AGTCGGCGATCGCGTGACAATTACTTGTCGAGCTTCTCAGGACGTCAATACAGCC
GTGGCTTGGTATCAGCAGAAGCCTGGAAAGGCACCAAAACTGCTGATCTACAGCG
CCTCCTTTCTGTATTCCGGCGTGCCCTCTCGATTCTCTGGAAGTCGGTCAGGCAC
CGATTTTACCCTGACAATTTCCTCTCTGCAGCCTGAGGACTTCGCCACATACTAT

TGCCAGCAGCACTATACTACCCCCCCTACTTTTGGCCAGGGGACCAAGGTGGAAA
TCAAAGGGGGAAGTGGCGGGGGATCAGGCGGCGGAAGCGGCGGCGGCAGCGGCGG
CGGATCTGGAGAGGTCCAGCTGGTGGAAAGCGGAGGAGGACTG**GTG**CAGCCTGGA
GGGAGTCTGCGACTGTCATGTGCTGCAAGCGGCTTCAACATCAAAGATACCTACA
TTCATTGGGTCAGGCAGGCCCCTGGAAAGGGCCTGGAATGGGTGGCACGAATCTA
TCCCACTAATGGCTACACCAGATATGCCGATTCCGTGAAAGGGCGCTTCACTATT
TCCGCTGACACATCTAAGAACACTGCATACCTGCAGATGAACAGCCTGCGCGCTG
AGGACACCGCAGTGTACTATTGCTCTCGATGGGGCGGCGACGGCTTCTACGCAAT
GGACTACTGGGGGCAGGGGACACTGGTGACTGTGAGCAGCTGAGGATCC

Protein Sequence for v594:

SSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPS
GIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGGG
SGGGSGGGSGGGSGGGSGEVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWV
RQAPGKGLEWVSGINWNGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTA
VYYCARGRSLLFDYWGQGTLVTVSRGGSGDAHKSEVAHRFKDLGEENFKALVLIA
FAQYLQQCPFEDHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLR
ETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLK
KYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKA
SSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECC
HGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPAD
LPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETT
LEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRY
TKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEK
TPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKER
QIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLV
AASQAALGLGGSGGSGGSGGSGGDIQMTQSPSSLSASVGDRVTITCRASQDVNTA
VAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYY
CQQHYTTPPTFGQGTKVEIKGGSGGGSGGGSGGGSGGGSGEVQLVESGGGLVQPG
GSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTI
SADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2012/050131

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***C07K 19/00*** (2006.01) , ***C07K 14/47*** (2006.01) , ***C07K 14/485*** (2006.01) , ***C07K 14/605*** (2006.01) , ***C07K 14/76*** (2006.01) , ***C07K 16/00*** (2006.01), ***C07K 16/18*** (2006.01) , ***C07K 16/30*** (2006.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: ***C07K 19/00*** (2006.01) , ***C07K 14/47*** (2006.01) , ***C07K 14/485*** (2006.01) , ***C07K 14/605*** (2006.01) , ***C07K 14/76*** (2006.01) , ***C07K 16/00*** (2006.01), ***C07K 16/18*** (2006.01) , ***C07K 16/30*** (2006.01)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

Canadian patent database, TotalPatent, Pubmed, Scopus, CaPlus (STN)

Keywords: heteromultimer*, multimer*, cargo, transporter, protein scaffolds, monomer*, bispecific, self-assemble, quasi-native, split-proteins, carrier, albumin, annexin, transferrin, drug, deliver*, native-like

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93/11162 (TSO, J.Y et al.) 10 June 1993 (10-06-1993) (Whole document)	1-3, 17-30, 34-40, 44-47 and 51-54
X	WO 03/012069 (WANG, C. et al.) 13 February 2003 (13-02-2003) (Whole document)	1-3, 18-40, 44-47 and 51-54
A	Tafelmeyer, P. et al., "Transforming a $(\beta/\alpha)_8$-barrel enzyme into a split-protein sensor through directed evolution". Chemistry & Biology. May 2004 (05-2004), Vol. 11, pages 681-689. (Whole document)	1-54
A	Stains, C.I. et al., "A general approach for receptor and antibody-targeted detection of native proteins utilizing split-luciferase reassembly". ACS Chemical Biology. Published online 23 July 2010 (23-07-2010), Vol. 5, No. 10, pages 943-952. (Whole document)	1-54

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
08 May 2012 (08-05-2012)	23 May 2012 (23-05-2012)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Nathalie Chartrand (819) 994-2341

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, A	Shekhawat, S.S. and Ghosh, I., "Split-protein systems: beyond binary protein-protein interactions". Current Opinion in Chemical Biology. 7 November 2011 (07-11-2011), Vol. 15, pages 789-797. (Whole document)	1-54
A	Kratz, F., "Albumin as a drug carrier: design of prodrugs, drug conjugates and nanoparticles". Journal of Controlled Release. Published online 17 May 2008 (17-05-2008), Vol. 132, pages 171-183. (Whole document)	1-54
A	Neumann, E., et al., "Native albumin for targeted drug delivery". Expert Opinion on Drug Delivery. August 2010 (08-2010), Vol. 7 No. 8, pages 915-925. (Whole document)	1-54

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2012/050131

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO9311162A1	10 June 1993 (10-06-1993)	AU682141B2	18 September 1997 (18-09-1997)
		AU3147293A	28 June 1993 (28-06-1993)
		AU7411896A	20 February 1997 (20-02-1997)
		CA2123894A1	10 June 1993 (10-06-1993)
		EP0618929A1	12 October 1994 (12-10-1994)
		EP0618929A4	25 January 1995 (25-01-1995)
		JPH07501698A	23 February 1995 (23-02-1995)
		KR100236156B1	15 December 1999 (15-12-1999)
		US5932448A	03 August 1999 (03-08-1999)
WO03012069A2	13 February 2003 (13-02-2003)	CA2455237A1	13 February 2003 (13-02-2003)
		CN1558916A	29 December 2004 (29-12-2004)
		CN101654483A	24 February 2010 (24-02-2010)
		EP1421117A2	26 May 2004 (26-05-2004)
		EP1421117A4	28 December 2005 (28-12-2005)
		JP2005506064A	03 March 2005 (03-03-2005)
		JP2010053132A	11 March 2010 (11-03-2010)
		MXPA04001053A	17 February 2005 (17-02-2005)
		US2003027247A1	06 February 2003 (06-02-2003)
		US6833441B2	21 December 2004 (21-12-2004)
		US2005009139A1	13 January 2005 (13-01-2005)
		US7429652B2	30 September 2008 (30-09-2008)
		WO03012069A3	06 November 2003 (06-11-2003)