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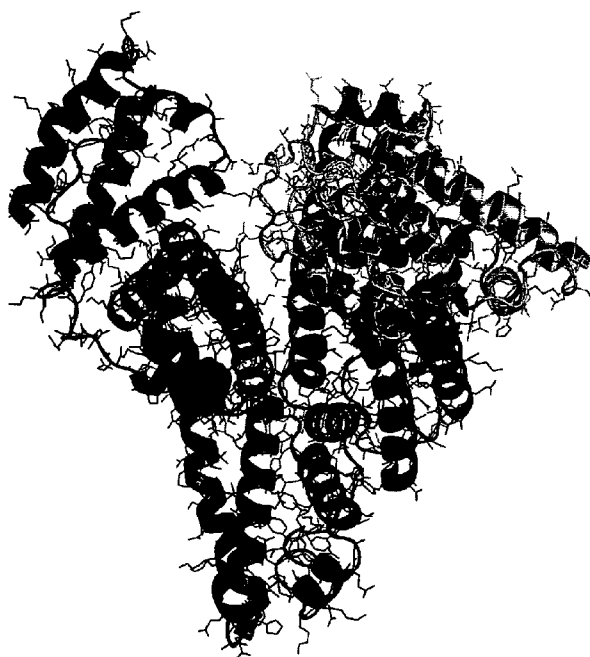
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[Continued on next page]

(54) **Title:** MULTIVALENT HETEROMULTIMER SCAFFOLD DESIGN AND CONSTRUCTS

Figure 1



(57) **Abstract:** Provided herein are multifunctional heteromultimer proteins. In specific embodiments is a heteromultimer comprising: at least two polypeptide constructs, each polypeptide construct comprising at least one cargo polypeptide attached to a transporter polypeptide, said transporter polypeptides derived from a monomeric native protein such that said monomeric constructs associate to form the heteromultimer and said transporter polypeptides associate to form a quasi-native structure of the monomeric native protein or analog thereof. These therapeutically novel molecules encompass heteromultimers comprising constructs that function as scaffolds for the conjugation or fusion of therapeutic molecular entities (cargo polypeptides) resulting in the creation of bispecific or multivalent molecular species. Provided herein is a method for creation of bispecific or multivalent molecular species.



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MULTIVALENT HETEROMULTIMER SCAFFOLD DESIGN AND CONSTRUCTS**Cross-Reference to Related Applications**

[0001] This application claims the benefit of U.S. Application Serial No. 61/671,640, filed July 13, 2012; U.S. Application Serial No. 61/697,245, filed September 05, 2012; U.S. Application Serial No. 61/758,701, filed January 30, 2013; and U.S. Application Serial No. 61/845,945, filed July 12, 2013; which are hereby incorporated by reference in their entirety.

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. HSA is also known to penetrate and be retained in the interstitium of tumors (see Elsadek and Kratz, J. Control. Release (2012) 157:4-28). 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] An object of the present invention is to provide multivalent heteromultimer scaffolds and methods of designing same. In one aspect of the invention there is provided a heteromultimer comprising: a first polypeptide construct that comprises (i) a first transporter polypeptide; and a second polypeptide construct that comprises (ii) a second transporter polypeptide; wherein each of said first and second transporter polypeptide comprises an amino acid sequence with at least 90% identity to a segment of an albumin polypeptide; and wherein said first and second transporter polypeptides are obtained by segmentation of said albumin polypeptide at a segmentation site, such that the segmentation results in a deletion of zero to 3 amino acid residues at the segmentation site, wherein said transporter polypeptides self-assemble to form a quasi-native structure of the monomeric form of said albumin polypeptide.
- [0008] In another aspect of the invention there is provided a heteromultimer comprising: a first polypeptide construct that comprises (i) a first transporter polypeptide; and a second polypeptide construct that comprises (ii) a second transporter polypeptide; wherein each of said first and second transporter polypeptide comprises an amino acid sequence with at least 90% identity to a segment of an albumin polypeptide; and wherein said first and second transporter polypeptides are obtained by segmentation of said albumin polypeptide at a segmentation site, and wherein at least one of said first and second transporter polypeptides comprises at least one mutation of an amino acid residue to cysteine or an analog thereof such that said cysteine forms a disulfide bond with the other transporter polypeptide, such that said transporter polypeptides self-assemble to form a quasi-native structure of the monomeric form of said albumin polypeptide.
- [0009] In another aspect of the invention there is provided a heteromultimer comprising: a first polypeptide construct that comprises (i) a first transporter polypeptide; and (ii) at least one first cargo polypeptide that is an antigen-binding polypeptide construct that binds to CD3, CD19, CD20, HER2 or HER3, and a second polypeptide construct that comprises (iii) a second transporter polypeptide; and (iv) at least one second cargo polypeptide wherein each of said first and second transporter polypeptide comprises an amino acid sequence with at

least 90% identity to a segment of an albumin polypeptide; and wherein said first and second transporter polypeptides are obtained by segmentation of said albumin polypeptide at a segmentation site, wherein said transporter polypeptides self-assemble to form a quasi-native structure of the monomeric form of said albumin polypeptide.

- [0010] In another aspect of the invention there is provided a host cell comprising nucleic acid encoding a heteromultimer of the invention.
- [0011] In another aspect of the invention there is provided a pharmaceutical composition comprising a heteromultimer of the invention and an adjuvant.
- [0012] In another aspect of the invention there is provided a method of treating cancer comprising providing to a patient in need thereof an effective amount of the pharmaceutical composition of the invention.
- [0013] In another aspect of the invention there is provided a method of inhibiting growth of a tumor, comprising contacting the tumor with an effective amount of a heteromultimer the invention.
- [0014] In another aspect of the invention there is provided a method of shrinking a tumor, comprising contacting the tumor with an effective amount of a heteromultimer of the invention.
- [0015] In another aspect of the invention there is provided a method of designing self-associating polypeptides from a protein of interest comprising: segmenting said protein at at-least one segmentation site to obtain at least two polypeptide segments such that said polypeptide segments self-assemble to form a heteromultimer, wherein said heteromultimer forms a quasi-native monomeric structure of said protein, the method comprising the steps of selecting at least one loop of the protein of interest that has a high solvent accessible surface area and limited contact with the rest of the structure of said protein, and introducing one segmentation site per selected loop, resulting in a complementary interface between the at least two polypeptide segments, wherein the interface is apolar, extensive and interdigitate.
- [0016] In another aspect of the invention there is provided a heteromultimer designed by a method of the invention.
- [0017] In another aspect of the invention there is provided a therapeutic scaffold comprising a heteromultimer designed by a method of the invention.
- [0018] Provided herein are multifunctional heteromultimers and methods to design them. In certain embodiments are heteromultimers, each heteromultimer comprising: at least a first polypeptide construct that comprises at least one cargo molecule, and a first transporter polypeptide; and at least a second polypeptide construct that comprises at least one cargo

molecule and a second transporter polypeptide; wherein the transporter polypeptides are derived by segmentation of a protein such that said transporter polypeptides self-assemble to form a quasi-native structure of said protein or analog thereof. In certain embodiments, at least one cargo molecule is a drug, or a therapeutic agent. In certain embodiment more the one cargo molecule of the same nature is present on the transporter polypeptide. 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 transporter polypeptide is unstable and preferentially forms a heteromultimer with at least one other transporter polypeptide. In certain embodiments, each 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.

[0019] In certain embodiments is a heteromultimer that comprises: at least two polypeptide constructs, each polypeptide construct comprising at least one cargo polypeptide attached to a transporter polypeptide, wherein said transporter polypeptides are derived from a protein by segmentation of said protein, each transporter polypeptide comprising an amino acid sequence with at least 90% identity to a segment of said protein, and wherein said transporter polypeptides self-assemble to form a quasi-native monomeric structure of said 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 heteromultimer 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. In certain embodiments, the transporter

polypeptides are derived from a fragment of human serum albumin (HSA or HA), wherein said HAS comprises a sequence as shown in SEQ ID No. 1.

[0020] 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. SEQ ID No.-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.

[0021] 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 sequence 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.

[0022] 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.

[0023] Provided herein are heteromultimers, each heteromultimer comprising: at least a first polypeptide construct that comprises at least one cargo polypeptide and a first transporter polypeptide; and at least a second polypeptide construct 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 heteromultimer 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.

[0024] In certain embodiments, are heteromultimers, each heteromultimer comprising: at least a first polypeptide construct 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 polypeptide construct that comprises at least one cargo polypeptide 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.

[0025] In certain embodiments, are heteromultimers, each heteromultimer comprising: at least a first polypeptide construct 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 polypeptide construct 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 polypeptide construct 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 polypeptide construct 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 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 polypeptide construct comprises two cargo polypeptides. In some embodiments, the second polypeptide construct comprises two cargo polypeptides. In some embodiment, at least one of the polypeptide constructs is engineered by introducing mutations. In certain embodiments, the introduced mutations improve the functionality of the polypeptide construct as compared to the non-mutated form of the construct. In certain embodiments the introduced mutations improve one or more of the stability, half-life and heteromultimer formation of the transporter polypeptide.

[0026] Provided herein is a heteromultimer comprising: at least a first polypeptide construct that comprises (i) a first transporter polypeptide; and (ii) at least one cargo polypeptide and at least a second polypeptide construct that comprises (iii) a second transporter polypeptide and (iv) at least one cargo polypeptide; wherein said transporter polypeptides are derived

from a protein by segmentation of said protein, each transporter polypeptide comprising an amino acid sequence with at least 90% identity to a segment of said protein, and wherein said transporter polypeptides self-assemble to form a quasi-native structure of said protein.

- [0027] In certain embodiments is a heteromultimer described herein, wherein the transporter polypeptides are derived from a protein by segmentation of said protein, each transporter polypeptide comprising an amino acid sequence with at least 95% identity to a segment of said protein.
- [0028] In certain embodiments is a heteromultimer described herein, wherein the transporter polypeptides are derived from a protein by segmentation of said protein, each transporter polypeptide comprising an amino acid sequence with at least 99% identity to a segment of said protein.
- [0029] In certain embodiments, the heteromultimer is a heterodimer. In some embodiments, at least one transporter polypeptide is not derived from an antibody. In exemplary embodiments, each transporter polypeptide is an albumin derivative. In some embodiments, at least one of said first and second transporter polypeptides comprise at least one mutation of an amino acid residue to cystine such that said cystine forms a disulfide bond with a cysteine residue on another transporter polypeptide. In certain embodiments, said first and second transporter polypeptide comprise at least one mutation of an amino acid residue to cystine such that said cystine forms a disulfide bond with a cysteine residue on another transporter polypeptide. In some embodiments are provided heteromultimers wherein each transporter polypeptide is an albumin derivative, the first transporter polypeptide comprising at least one mutation selected from A194C, L198C, W214C, A217C, L331C and A335C. In some embodiments, the second transporter polypeptide is an albumin derivative comprising at least one mutation selected from L331C, A335C, V343C, L346C, A350C, V455C, and N458C.
- [0030] In some embodiments provided are heteromultimers described herein, wherein said first transporter polypeptide has a sequence comprising SEQ ID NO:35 or analog or variant thereof, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:36 or analog or variant thereof. In some embodiments, said first transporter polypeptide has a sequence comprising SEQ ID NO:37 or analog or variant thereof, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:38 or analog or variant thereof. In certain embodiments, said first transporter polypeptide has a sequence comprising SEQ ID NO:39 or analog or variant thereof, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:40 or analog or variant

thereof. In exemplary embodiments, said first transporter polypeptide has a sequence comprising SEQ ID NO:41 or analog or variant thereof, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:42 or analog or variant thereof. In certain embodiments, said first transporter polypeptide has a sequence comprising SEQ ID NO:43 or analog or variant thereof, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:44 or analog or variant thereof. In an embodiment, said first transporter polypeptide has a sequence comprising SEQ ID NO:45 or analog or variant thereof, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:46 or analog or variant thereof. In one embodiment, said first transporter polypeptide has a sequence comprising SEQ ID NO:47 or analog or variant thereof, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:48 or analog or variant thereof. In certain embodiments, said first transporter polypeptide has a sequence comprising SEQ ID NO:49 or analog or variant thereof, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:50 or analog or variant thereof.

- [0031]** In certain embodiments of the heteromultimer described herein, at least one cargo polypeptide binds a target antigen, and wherein said target antigen is at least one of a-chain (CD25) of IL-2R, Amyloid beta, anti-EpCAM, anti-CD3, CD16, 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, TNFR, VEGF-A, glucagon receptor, GLP receptor, and LDL receptor.
- [0032]** Provided herein are heteromultimers, each heteromultimer comprising: at least a first polypeptide construct that comprises at least one cargo polypeptide and a first transporter polypeptide; and at least a second polypeptide construct 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

polypeptide construct that comprises at least one cargo polypeptide and a first transporter polypeptide; and at least a second polypeptide construct 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 relevant 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.

[0033] Provided herein are heteromultimers, each heteromultimer comprising: at least a first polypeptide construct that comprises at least one cargo polypeptide and a first transporter polypeptide; and at least a second polypeptide construct 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.

- [0034] Provided herein are heteromultimers, each heteromultimer comprising: at least a first polypeptide construct that comprises at least one cargo molecule, fragment; and at least a second polypeptide construct 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 difitox 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 botulinum toxin type A. In some embodiments, the toxin is ricin or a fragment thereof. In some embodiments, the toxin is a toxin from RTX family of toxins.
- [0035] Provided herein are heteromultimers, each heteromultimer comprising: at least a first polypeptide construct that comprises at least one cargo polypeptide and a first transporter polypeptide; and at least a second polypeptide construct 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.
- [0036] Provided herein are host cells comprising nucleic acid encoding a heteromultimer described herein. In certain embodiments, the nucleic acid encoding the first polypeptide construct and the nucleic acid encoding the second polypeptide construct are present in a single vector. In certain embodiments, the nucleic acid encoding the first polypeptide construct and the nucleic acid encoding the second polypeptide construct are present in separate vectors.

- [0037] 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 described 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.
- [0038] 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. Provided are methods of treating cancer comprising providing to a patient in need thereof an effective amount of the pharmaceutical composition described herein. In certain embodiments is a method of inhibiting growth of a tumor, comprising contacting the tumor with an effective amount of the heteromultimer described herein. In some embodiments is a method of shrinking a tumor, comprising contacting the tumor with an effective amount of the heteromultimer provided herein.

- [0039] 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.
- [0040] Provided herein are heteromultimer proteins that comprise at least two polypeptide constructs, wherein each polypeptide construct comprises at least one cargo polypeptide, and an albumin based polypeptide, such that said polypeptide constructs self-assemble to form the heteromultimer.
- [0041] 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.
- [0042] Provided herein are heteromultimer proteins that comprise at least two polypeptide constructs, wherein each polypeptide construct 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.

- [0043] 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 polypeptide construct functions in synergy with the cargo polypeptide of another polypeptide construct.
- [0044] Provided herein are heteromultimer proteins that comprise at least two polypeptide constructs, wherein each polypeptide construct 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 monomeric 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 polypeptide construct functions in synergy with the cargo polypeptide of another polypeptide construct. In some embodiments the cargo polypeptide can be an agonist or antagonist to the cargo polypeptide of another polypeptide construct.
- [0045] 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.
- [0046] 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.

[0047] In certain embodiments are heteromultimer proteins comprising a first polypeptide construct which comprises at least one cargo polypeptide fused to an alloalbumin derived polypeptide; and a second polypeptide construct 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 polypeptide construct is different from the at least one cargo polypeptide of the second polypeptide construct. In certain embodiments, the at least one cargo polypeptide of the first polypeptide construct is the same as the at least one cargo polypeptide of the second polypeptide construct.

[0048] 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 therapeutic 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.

[0049] 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.

[0050] Provided herein is a method of obtaining fusion protein scaffolds with a known number of conjugation sites based on any transport protein of interest.

[0051] Also provided are transgenic organisms modified to contain nucleic acid molecules described herein to encode and express monomeric fusion proteins described herein.

[0052] 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

[0053] In drawings which illustrate embodiments of the invention,

BRIEF DESCRIPTION OF THE DRAWINGS

[0054] In drawings which illustrate embodiments of the invention,

[0055] **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.

[0056] **Figure 2** is a plot of buried solvent accessible surface area at the interface of two albumin-based polypeptides. ABH1 is represented by the structure to the left, while ABH2 is represented by the right structure.

[0057] **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.

[0058] **Figure 4** is a schematic representation of bispecific and other multifunctional therapeutics based on the multispecific heteromultimer described herein. The albumin-based, or

albumin-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.

- [0059] **Figure 5** is a schematic of a bispecific antibody derived from a heterodimeric Fc domain. Albumin or albumin based polypeptides are connected to the C-terminal of the Fc to selectively drive the formation of heterodimers.
- [0060] **Figure 6B** shows 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. **Figure 6A** shows a non-reducing SDS-PAGE gel profile of ABH2, ABH1 and WT HSA. The two fragment polypeptides of ABH1 and ABH2 were co-expressed in different DNA ratios, as indicated by the ratios at the top of the figure. As controls and to observe homodimerization, fragments A and B were expressed independently. **Figure 6C** is a native gel of initial, flowthrough, wash and final elute after the Blue Sepharose purification of WT-HSA (v225) and ABH2 (v221).
- [0061] **Figure 7** shows stability of wild type HSA and heterodimer scaffolds ABH1 and ABH2 studied using Differential Scanning Calorimetry
- [0062] **Figures 8A-8B** show equilibrium binding isotherms 3000 nM FcRN 3x dilution series over 3000 RUs. Figure 8B shows Albumin and Figure 8A shows heteromultimer scaffold ABH1
- [0063] **Figure 9** shows scheme for multivalent Albumin based heteromultimers comprising anti-Her2/neu and anti-CD16 scFv bioactive fusions
- [0064] **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.
- [0065] **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.
- [0066] **Figure 12** shows a plot of the buried solvent accessible surface area at the interface of Annexin based transporter polypeptide-1, and Annexin based transporter polypeptide-2.
- [0067] **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.

- [0068] **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 Å² of buried surface area.
- [0069] **Figure 15A-15P** depict heteromultimers comprising albumin based transporter polypeptides that self-associate to form quasi-native monomeric albumin. Each pair of transporter polypeptides is obtained by segmentation of albumin at a unique site, different from the other
- [0070] **Figures 16A and 16B** provides the structures of ABH2 and ABH1 respectively. Both diagrams include a) the location of the cut site in WT-HSA to create the albumin-based protein, b) location of the new C' and N' cut sites and c) a simplified circular rendering of both ABH1 and ABH2, utilized in numerous later examples.
- [0071] **Figure 17** depicts various mono, bi, tri and tetra-valent configurations of anti-Her2 scFv that can be attached to ABH2. Controls included the ABH2 scaffold, the anti-HER2 scFV, the one-armed Fc and the bivalent Fc. Additionally, the ability of the various ABH2 constructs to bind to SKOV cells expressing Her2 is indicated with the K_D values within the tables.
- [0072] **Figure 18A** shows the binding curves of four anti-Her3 x Her2 loaded constructs (v1087, v1088, v1089 and v1090) to MALME-3M cells. Affinity for the cells was evaluated using FACS with FITC-labeled anti-HSA antibodies. The lower left graph displays the concentration on a linear scale while the lower right is on a logarithmic scale. **Figure 18B** shows the binding curves of the aforementioned four constructs to SKOV-3 cells. The lower left graph displays a linear scale while the lower right displays a logarithmic one.
- [0073] **Figure 19A –D** demonstrate the stability of four anti-Her2 (v519, v520, v518 and v517) loaded ABH2 in human sera. Binding affinity to SKOV-3 cells was assessed using FACS with FITC-labeled anti-HSA antibody.
- [0074] **Figure 20A and 20B** shows the binding affinity of bispecific anti-Her2 x Her3 ABH2 (v1378) to FcRn as compared to the v1087 (MM-111) control. The graphs represent the surface plasmon resonance (SPR) curves of the two compounds represented as Resp. Diff. over Time.
- [0075] **Figure 21** depicts the native gel profiles of both the ABH2 scaffold (v221) and a monovalent anti-Her2 ABH2 (v517) incubated for five days at three temperatures (37°C, room temperature and 4°C).

- [0076] **Figure 22A** depicts the native gel profile of the total, flowthrough, wash and elution fractions of WT HSA after Blue Sepharose purification. **Figure 22B** is a chromatogram of WT-HSA after Blue Sepharose purification applied to a gel filtration column. Different peaks represent different protein products. **Figure 22C** is a chromatogram of WT-HSA after AlbuPure purification, fractionated via application to gel filtration column.
- [0077] **Figure 23A** is a chromatogram of the liquid chromatography analysis of WT-HSA (v225) after AlbuPure purification and gel filtration. **Figures 23B-23E** are chromatograms of AlbuCORE scaffolds 1 (v225), 3 (v1636), 6 (v1638) and 9 (v1640) respectively after AlbuPure purification, separated into fractions and analyzed via gel filtration LC.
- [0078] **Figure 24A** represents the gel filtration LC analysis of an albumin scaffold (v221) after AlbuPure purification. The express product was scaled-up via via transient co-expression in CHO cells. **Figure 24B** shows non-reducing (indicated by the -DTT) and reducing (indicated by the +DTT) SDS-PAGE gel profiles of the same variant.
- [0079] **Figure 25A** represents the native gel profiles of the total, flowthrough, wash and final elute of WT-HSA and AlbuCORE-A proteins after the AlbuPure purification. **Figure 25B** represents an LC/MS analysis of the same two proteins.
- [0080] **Figure 26A** is a diagram indicating the degree of spatial separation between the four termini in ABH2. **Figure 26B** is a comparative diagram demonstrating the degree of spatial separation between the variable regions of a normal antibody molecule. **Figure 26C** shows four AlbuCore scaffolds which have different and variable inter-termini distances for alternative antigen binding.
- [0081] **Figure 27** shows the DSC melting curves of AlbuCORE scaffolds 1, 3, 6 and 9. In each graph, the curve and melting point of each albumin-based scaffold is compared to the curve and 75°C melting point of the WT HSA.
- [0082] **Figure 28A** is the schematic rendering of anti-CD3xCD19 bispecific bivalent heteromultimers on either a WT-HSA or a spliced albumin scaffold. **Figure 28B** represents the schematic renderings of additional monovalent anti-CD3 heteromultimer constructs as well as both monovalent and multivalent anti-CD19 heteromultimer constructs. **Figure 28C** represents a non-reducing PAGE gel of the previous described constructs after each was expressed in a suspension growing human CHO system.
- [0083] **Figure 29** provides the K_a and Hill Slope measurements of four anti-CD3xCD19 bispecific bivalent heteromultimers on either a WT-HSA or spliced albumin scaffold. The binding tests assessed the variant's ability to bind Jurkat or Raji cells as analyzed using FACS.

- [0084] **Figure 30A** displays FACS cell population graphs of the BiTE-like control (v891), an anti-CD3xCD19 albumin-based heteromultimer (v1093) and an anti-CD3xCD19 Het-Fc-based heteromultimer (v873). Binding to Raji and Jurkat cells was assessed. **Figure 30B** shows additional FACS graphs of the three aforementioned constructs and as compared against an albumin control (v221).
- [0085] **Figure 31A** shows a SDS-PAGE of the v218 expression product before, during and after purification using Co²⁺ affinity. **Figure 31B** is a chromatogram of the same construct after additional purification via gel filtration.
- [0086] **Figure 32** Plot of residue contacts made by a subset of residues in the human serum albumin derived from its 3-dimensional structure.
- [0087] **Figure 33** Plot of nature of contacts and interactions achieved by select hotspot residues in human serum albumin.
- [0088] **Figure 34** A graphical representation of hot spot residues or clusters (patches) of hotspot residues in human serum albumin.
- [0089] **Figure 35** shows a gel demonstrating the expression of several albumin-based heteromultimers comprising introduced cysteine residues.
- [0090] **Figure 36A** provides a non-reducing gel showing the expression of heteromultimer variants 1635, 1638, 1639, and 1641; **Figure 36B** provides a non-reducing gel showing the expression of heteromultimer variants 1634, 1636, 1637, 1640, and 221. CHO cells were transfected with the following DNA ratios: Ratio A = plasmid A:plasmid B 100%:0%; Ratio D = plasmid A: plasmid B 66%:34%; Ratio B = plasmid A: plasmid B 50%:50%; Ratio H = plasmid A: plasmid B 34%:66%; and Ratio F = plasmid A:plasmid B 0%/100%.

DETAILED DESCRIPTION

- [0091] 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.

- [0092] The human serum albumin (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].
- [0093] 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].

- [0094] 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 resembling the monomeric native protein 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.
- [0095] The present invention provides heteromultimers comprising a first monomer that comprises (i) a first transporter polypeptide; and a second monomer that comprises (ii) a second transporter polypeptide; wherein each of said first and second transporter polypeptide comprises an amino acid sequence with at least 90% identity to a segment of an albumin polypeptide; and wherein said first and second transporter polypeptides are obtained by segmentation of said albumin polypeptide at a segmentation site, wherein said transporter polypeptides self-assemble to form a quasi-native structure of the monomeric form of said albumin polypeptide. In one embodiment, the segmentation site is selected such that it resides on a loop of the albumin polypeptide that has a high solvent accessible surface area (SASA) and limited contact with the rest of the albumin structure, b) results in a complementary interface between the transporter polypeptides, wherein the interface is apolar, extensive and interdigitate. Such heteromultimers exhibit stability comparable to that of wild-type albumin. The first and second monomers may further comprise at least one cargo polypeptide. Heteromultimers comprising said at least one cargo polypeptide may be used as therapeutics for the treatment of disease.

[0096] **Definitions**

- [0097] 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.
- [0098] 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.
- [0099] 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.
- [00100] 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.
- [00101] 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 interact 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 interact 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 interact 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 interact with each other by means of cation- π 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.

[00102] 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.

[00103] 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.

[00104] "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.

[00105] 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.

[00106] 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.

[00107] 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.

[00108] "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.

[00109] "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.

[00110] 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.

[00111] 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.

[00112] 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.

[00113] 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, phosphoramidates, 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 (Batzner 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)).

[00114] 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.

[00115] 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, proline, 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 α 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.

[00116] 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.

[00117] "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.

[00118] 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.

[00119] 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:

[00120] 1) Alanine (A), Glycine (G);

[00121] 2) Aspartic acid (D), Glutamic acid (E);

[00122] 3) Asparagine (N), Glutamine (Q);

[00123] 4) Arginine (R), Lysine (K);

[00124] 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);

[00125] 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W);

[00126] 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))

[00127] 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.

[00128] 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.

[00129] 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)).

[00130] 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) of 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.

[00131] 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.

[00132] 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).

[00133] 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).

- [00134] 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.
- [00135] 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*, *Aeurypyrum pernix*, etc.) phylogenetic domain.
- [00136] 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).
- [00137] 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.
- [00138] 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.
- [00139] 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.

[00140] 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.

[00141] 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-native protein structure. Alternatively, segmentation can include deletion of more than one amino acid residue. In one embodiment, the deletion is one amino acid residue. In another embodiment, the deletion is two amino acid residues. In another embodiment, the deletion is three amino acid residues.

[00142] Quasi-native Monomer Structure:

[00143] With reference to a monomeric native protein or its structure, quasi-native proteins and/or 'quasi-native structures' or quasi-native monomeric structures are heteromultimer assemblies of polypeptides derived from segments of the monomeric native protein such that said heteromultimer assemblies present functional and structural characteristics comparable to the monomeric native protein. In some embodiments, the segments are components of a polypeptide construct that also comprise other molecular entities. Proteins are naturally dynamic 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 quasi-native structures can be considered to resemble one of the alternate structural configurations in the ensemble. 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 polypeptide constructs each of which have a transporter polypeptide component, the transporter polypeptides assemble to form a quasi-native

structure similar to the native monomeric form of the protein that the transporter polypeptides are derived from. The reference native protein in this case is the monomer protein from which each 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 heteromultimer with structural and functional characteristics comparable to the native protein which itself is a monomeric entity. In some embodiments, the assembled heteromultimer has at least 50% of the activity of the monomeric native protein. In some embodiments, the assembled heteromultimer has at least 60% of the activity of the monomeric native protein. In specific embodiments, the assembled heteromultimer has at least 75% of the activity of the monomeric native protein. In some embodiments, the assembled heteromultimer has at least 80% of the activity of the monomeric native protein. In particular embodiments, the assembled heteromultimer has at least 90% of the activity of the monomeric native protein. In some embodiments, the assembled heteromultimer has at least 95% of the activity of the monomeric native protein. In some embodiments, the assembled heteromultimer has at least 99% of the activity of the monomeric native protein. In certain embodiments provided are, heteromultimers comprising transporter polypeptides derived from albumin or allo-albumin such that the transporter polypeptides self-assemble to form a heteromultimer that exhibits native albumin like structural and/or functional characteristics such as FcRn, SPARC and/or gp60 binding. In certain embodiments are heteromultimers comprising transporter polypeptides, each transporter polypeptide comprising an amino acid sequence that has at least 75% sequence identity with an amino acid segment obtained from the native monomeric protein. In some embodiments are heteromultimers comprising transporter polypeptides, each transporter polypeptide comprising an amino acid sequence that has at least 80% sequence identity with an amino acid segment obtained from the native monomeric protein. In exemplary embodiments are heteromultimers comprising transporter polypeptides, each transporter polypeptide comprising an amino acid sequence that has at least 85% sequence identity with an amino acid segment obtained from the native monomeric protein. In specific embodiments are heteromultimers comprising transporter polypeptides, each transporter polypeptide comprising an amino acid sequence that has at least 90% sequence identity with an amino acid segment obtained from the native monomeric protein. In certain embodiments are heteromultimers comprising transporter polypeptides, each transporter polypeptide comprising an amino acid sequence that has at least 95% sequence identity with an amino

acid segment obtained from the native monomeric protein. In some embodiments are heteromultimers comprising transporter polypeptides, each transporter polypeptide comprising an amino acid sequence that has at least 99% sequence identity with an amino acid segment obtained from the native monomeric protein. In some embodiments, the native monomeric protein is a mammalian albumin or derivative or analog thereof. In certain embodiments, the native monomeric protein is human serum albumin. 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.

[00144] Transporter polypeptide

[00145] 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.

[00146] In certain embodiments, transporter polypeptides are segments of a monomeric whole protein, wherein said segments are capable of assembling to form a heteromultimer such that said heteromultimer forms a quasi-native structure similar to the native monomeric whole protein. In an embodiment, the transporter polypeptides are segments from a beta-barrel protein. In certain embodiments provided are, heteromultimers

comprising transporter polypeptides that assemble to form a quasi-native structure similar to a native monomeric protein, each transporter polypeptide comprising an amino acid sequence with at least 50% identity to a segment of the native monomeric protein. In certain embodiments provided are, heteromultimers comprising transporter polypeptides that assemble to form a quasi-native structure similar to a native monomeric protein, each transporter polypeptide comprising an amino acid sequence with at least 60% identity to a segment of the native monomeric protein. In certain embodiments provided are, heteromultimers comprising transporter polypeptides that assemble to form a quasi-native structure similar to a native monomeric protein, each transporter polypeptide comprising an amino acid sequence with at least 75% identity to a segment of the native monomeric protein. In certain embodiments provided are, heteromultimers comprising transporter polypeptides that assemble to form a quasi-native structure similar to a native monomeric protein, each transporter polypeptide comprising an amino acid sequence with at least 80% identity to a segment of the native monomeric protein. In certain embodiments provided are, heteromultimers comprising transporter polypeptides that assemble to form a quasi-native structure similar to a native monomeric protein, each transporter polypeptide comprising an amino acid sequence with at least 90% identity to a segment of the native monomeric protein. In certain embodiments provided are, heteromultimers comprising transporter polypeptides that assemble to form a quasi-native structure similar to a native monomeric protein, each transporter polypeptide comprising an amino acid sequence with at least 95% identity to a segment of the native monomeric protein. In certain embodiments provided are, heteromultimers comprising transporter polypeptides that assemble to form a quasi-native structure similar to a native monomeric protein, each transporter polypeptide comprising an amino acid sequence with at least 99% identity to a segment of the native monomeric protein. In an embodiment, the native monomeric protein is a beta-propeller protein. In some embodiments, the native monomeric protein is a helical bundle protein. In embodiments, the transporter 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 comprise segments obtained from non-immunogenic proteins that are structurally stable, and have favorable biological properties.

[00147] Transporter polypeptides are derived from a protein by segmentation of the protein at a segmentation site. The segmentation site is designed on the basis of the following two decision criteria.

- [00148] First, the segmentation site is selected such that it resides on a loop within the protein that has a high relative SASA and limited contact with the rest of the protein structure. In one embodiment, the segmentation site resides in a loop that is flexible and accessible. In another embodiment, the segmentation site resides in a loop that is less likely to contribute to the protein core stability and thus impact stability of the assembled quasi-native protein structure relative to the intact protein structure.
- [00149] Second, the segmentation site is selected such that it results in a complementary interface between the transporter polypeptides, wherein the interface is apolar, extensive and interdigitated. The apolarity of the interface relates to the number of apolar amino acids in the interface. As is known in the art, amino acids can be broadly classified as polar and apolar residues. While polar residues interact favourably with water in the solvent environment, apolar residues do not make energetically favourable contact with water. For this reason, the apolar residues in a protein tend to cluster with other apolar residues and exclude water molecules from their local environment and this is energetically favourable. Such interactions involving apolar residues is also referred to as hydrophobic contact.
- [00150] The buried surface area at the interface is derived from the loss in the molecular or solvent accessible surface between the unassociated and associated states of the two interacting polypeptides. In one embodiment, the interface buried area is between about 6000 Å² and about 2000 Å². In another embodiment, the interface buried area is between about 4000 Å² and 2000 Å². In another embodiment, the interface buried area is between about 3000 Å² and 2000 Å². In one embodiment, the apolar contribution to the interface buried area is greater than 50% of the net buried surface area at the interface.
- [00151] With respect to the interface and its interdigitation, an important factor driving any protein-protein interaction and complex formation is the contact of surface residues at the interface formed between the two contacting proteins. Physico-chemical and structural complementarity of the two contacting protein surfaces in the associated form is a hallmark of favourable and stable complex formation. The physico-chemical complementarity is defined by the preferable interactions and could comprise hydrogen bonds, charge based salt-bridges, various types of electrostatic interactions, hydrophobic and van der Waals contacts across interacting protein surface. The protein surface at the 3-dimensional level is defined by protuberances and cavities formed due to the differences in size and orientation of various amino acids that constitute the surface region. In an favourable protein-protein interface, the protuberance and cavities from the two interacting protein surfaces interdigitate to result in structural complementarity. The larger and more extensive the

interface between the interacting proteins, the protein-protein contact is potentially more stable.

[00152] In one embodiment, the interface between the two transported polypeptides is rich in hotspot residues, i.e. individual residues or group of residues involved in a well-connected network of interactions. The interactions could be based on typical interactions observed in proteins such as hydrogen bonds, charged residue interactions such as salt-bridges, van der Waals contacts which show strong structural complementarity and hydrophobic residue clusters. This maximizes the interface stability and prevents the complex from dissociating.

[00153] In one embodiment, the segmentation site is selected to retain a natural disulfide bond present in the protein from which the transporter polypeptides are derived, across the segmented sections so as to covalently connect the two segments. That was meant to further increase the stability of the heterodimerizing quasi-native protein-like structure that self assembles from the two derived segments following segmentation. In this embodiment, loop regions in the protein sequence are chosen that satisfy the disulfide criteria described here i.e. have a disulfide very close to the segmentation site in the loop region. Besides the function of covalently crosslinking the two chains, this disulfide could also serve as a surrogate for the loop being open.

[00154] In another embodiment, a disulphide link across the two segments is engineered by introducing individual amino acid to cysteine mutations at selected positions on each of the two derived segments.

[00155] In another embodiment, we engineer amino acids at the interface of the two segments to further increase or improve the hotspots at the interface or enhance the structural and physico-chemical complementarity between the two segments in the assembled quasi-native albumin structure.

[00156] Albumin

[00157] 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. In certain embodiments, albumin refers to a truncated version of albumin.

[00158] The term "quasi-native 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 polypeptides of interest here comprise "segments" that preferentially associate as heteromultimeric pairs to form a quasi-native protein. In some embodiment this quasi-native protein is quasi-native albumin. In some embodiments, the quasi-native albumin has 90% of the activity of the whole albumin. In some embodiments, the quasi-native albumin has 75% of the activity of whole-albumin. In an embodiment, the quasi-native albumin has 50% of the activity of whole albumin. In some embodiments, the quasi-native albumin has 50-75% of the activity of whole albumin. In an embodiment, quasi-native albumin has 80% of the activity of whole albumin. In some embodiments, the quasi-native albumin retains about 90% of the structural characteristics of the whole albumin as determined by structural modeling. In some embodiments, the quasi-native albumin retains about 80% of the structural characteristics of the whole albumin as determined by structural modeling. In some embodiments, the quasi-native albumin retains about 70% of the structural characteristics of the whole albumin as determined by structural modeling. In some embodiments, the quasi-native albumin retains about 50% of the structural characteristics of the whole albumin as determined by structural modeling. In some embodiments, the quasi-native albumin retains about 50-75% of the structural characteristics of the whole albumin as determined by structural modeling.

[00159] 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).

[00160] 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. In certain embodiments variants also could mean alternate heteromultimer species derived by segmenting the albumin at alternate locations in its primary sequence.

[00161] 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 (P_n), where n is 369 to 419).

[00162] 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.

[00163] The sequence of human albumin is as shown, in the preprotein form with the N-terminal signaling residues MKWVTFISLLFLFSSAYSRGVFRR:

[00164] SEQ ID NO: 1

[00165] MKWVTFISLLFLFSSAYSRGVFRRDAHKSEVAHRFKDLGEENFKALVLIAFA
QYLQQCPFEDHVKL VNEVTEFAKTCVADESAENC DKSLHTLFGDKLCTVATLRET
YGEMADCCAKQEPERNECFLQHKDDNP NLPRLVRPEVDVMCTAFHDNEETFLKK
YLYEIARRHPYFYAPELLFFAKRYKAAFT ECCQAADKAACLLPKLDEL RDEGKASS
AKQRLK CASLQKFGERAFAKAWAVARLSQRFPKAEFAEVSKLVTDLT KVHTECCHG
DLLECADD RADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSL
AADFVESKDVCKNYAEAKDVFLGMFLY EYARRHPDYSV VLLLRLAKTYETTLEKC
CAAADPHECYAKVFDEFKPLVEEPQNLIKQ NC ELF EQLGEYKFQNALLVRYTKKVP
QVSTPTLVEVSRNLGKVGSKCKHPEAKRMP CAEDYLSVVLNQLC VLVHEKTPVSD
RVTKCCTESLVNRRPCFSALEVD ETYVPKEFNAETFTFHADICTLSEKERQIKKQTA
LVELVKHKPKATKEQLKAVMDDFAAFVEKCKADDKETCFAEEGKKLV AASQAA
LGL

[00166] Alloalbumin

[00167] 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 (Minchiotti et al. Human Mutations 29(8), 1007-1016(2008)).

[00168] **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 %

[00169] Annexin:

[00170] 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.

[00171] The term “quasi-native 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-native 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.

[00172] The sequence of Human wild-type Annexin A2 is as shown:

[00173] SEQ ID NO: 14

[00174] GSAVSPYPTFNPSSDVAALHKAIMVKGVD EATIIDILTKRNNAQRQQIKAAYLQET
 GKPLDETLKKALTGHLEEVVLALLKTPAQFDADELRAAMKGLGTDEDTLIEILASR
 TNKEIRDINRVYREELKRDLAKDITS DTS GDFRNALLSLAKGDRSEDFGVNEDLADS
 DARALYEAGERRKGT DVNVFNTILTRSY PQLRRVFQKYTKYSKHD MNKVL DLEL
 KGDIEKCLTAIVKCATSKPAFFAEKLHQAMKGVGTRHKALIRIMVSRSEIDMNDIK
 AFYQKMYGISLCQAILDETKGDYEKILVALCGGN

[00175] Transferrin:

[00176] 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.

[00177] 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-native 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.

[00178] The sequence of wildtype Human Transferrin is as shown:

[00179] SEQ ID NO: 19

[00180] 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 DLLFKDSAAG FLKVPPRMDA KMYLGYEYVT AIRNLREGTC
PEAPTDECKP VKWCALSHHE RLKCDWSVN SVGKIECVSA ETTEDCIAKI
MNGEADAMSL DGGFVYIAGK CGLVPVLAEN YNKSDNCEDT PEAGYFAVAV
VKKSASDLTW DNKLGKKSCH TAVGRTAGWN IPMGLLYNKI NHCRFDEFFS
EGCAPGSKKD SSLCKLCMGS GLNLCEPNNK EGYGYTGAF RCLVEKGDVA
FVKHQTV PQN TGGKNPDPWA KNLNEKDYEL LCLDGTRKPV EEYANCHLAR
APNHAVVTRK DKEACVHKIL RQQQHLFGSN VTDCSGNFCL FRSETKDLLF
RDDTVCLAKL HDRNTYEKYL GEEYVKAVGN LRKCSTSSLL EACTFRRP

[00181] Cargo molecule:

[00182] A heteromultimer described herein comprises polypeptides 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 some embodiments, the cargo polypeptide is fused to either the N or C terminus of the transporter polypeptide. In some embodiments, the cargo polypeptides are fused to both the N and C terminus of the transporter polypeptide. In some embodiment the cargo polypeptide is conjugated to Cysteine 37 position of the transporter polypeptide derived by segmenting human albumin. 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 cytotoxic 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, teniposide, 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), cyclophosphamide, 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).

[00183] 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

[00184] 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.

[00185] 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.

- [00186]** 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.
- [00187]** 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.
- [00188]** 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.
- [00189]** "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; Eprex; Erypo; Espo; Epoimmun; EPOGIN; NEORECORMON; HEMOLINK; Dynepo; ARANESP)	Stimulates cellular differentiation of bone-marrow stem cells at an early stage of erythropoiesis; accelerates the proliferation and maturation of terminally differentiating cells into erythrocytes; and modulates the level of circulating erythrocytes.	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-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

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			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.
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 myelogenous 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;	Regulates hematopoietic cell differentiation, gene expression, growth, and	Colony Stimulating Assay: Testa, N. G., et al., "Assays for hematopoietic growth	Bone Marrow Disorders; Bone marrow transplant; Chemoprotection; Hepatitis C; HIV Infections; Cancer; Lung Cancer;

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
Prokine; Molgramostim; Sargramostim; GM-CSF/IL 3 fusion; Milodistim; Leucotropin; PROKINE; LEUKOMAX; Interberin; Leukine; Leukine Liquid; Pixykinine)	function.	factors.” Balkwill FR (edt) Cytokines, A practical Approach, pp 229-44; IRL Press Oxford 1991.	Melanoma; Malignant melanoma; Mycobacterium avium complex; Mycoses; Leukemia; Myeloid Leukemia; Infections; Neonatal infections; Neutropenia; Mucositis; Oral Mucositis; Prostate Cancer; Stem Cell Mobilization; Vaccine Adjuvant; 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	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

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
		Res 1999; 51 Suppl 1: 7-12	treatment; Frailty, AIDS wasting; Muscle Wasting; Short Stature; Diagnostic Agents; Female Infertility; lipodystrophy.
Insulin (Human insulin; Insulin aspart; Insulin Glargine; Insulin lispro; 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)	Stimulates glucose uptake and promotes glycogenesis and lipogenesis.	Insulin 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.
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	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	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)

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
consensus interferon; CGP 35269; RO 253036; RO 258310; INTRON A; PEG- INTRON; OIF; OMNIFERON; PEG- OMNIFERON; VELDONA; PEG- REBETRON; ROFERON A; WELLFERON; ALFERON N/LDO; REBETRON; ALTEMOL; VIRAFERON PEG; PEGASYS; VIRAFERON; VIRAFON; AMPLIGEN; INFERGEN; INFAREX; ORAGEN)		Biol. 19(11): 7305-13.	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 Myelogenous 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; Hemorrhagic Fever; Viral Hemorrhagic 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

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			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.
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; BETA FERON; SIGOSIX)	Modulates MHC antigen expression, NK cell activity and IFN γ 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	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

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
		viral transcript. Mol Cell Biol. 19(11): 7305-13.	Cancer; Glioma; Glioblastoma Multiforme; Cervical Dysplasia; Leukemia; Preleukemia; Bone Marrow Disorders; Bone Disorders; Hairy Cell Leukemia; Chronic Myelogenous Leukemia; 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; Hemorrhagic Fever; Viral Hemorrhagic 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

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.
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;	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."	Cancer; Solid Tumors; Metastatic Renal Cell Carcinoma; Metastatic Melanoma; Malignant Melanoma; Melanoma; Renal Cell Carcinoma; Renal Cancer; Lung Cancer (e.g.,

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
CELEUK; ONCOLIPIN 2; MACROLIN)		Science 223: 1412-1415, 1984. natural killer (NK) cell and CTL cytotoxicity assay "Control of homeostasis of CD8+ memory T cells by opposing cytokines. Science 288: 675-678, 2000; CTLL-2 Proliferation: Gillis et al (1978) J. Immunol. 120, 2027	Non-Small Cell Lung Cancer or Small Cell Lung Cancer); Colon Cancer; Breast Cancer; Liver Cancer; Leukemia; Preleukemia; Hematological 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 conjunction 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;	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
		101(10): 2215-22.	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.
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; Neutrokin alpha; TL7; BAFF; TALL-1; THANK;	Promotes proliferation, differentiation and survival of B cells; Promotes	BLyS activity can be determined using assays known in the art, such as, for example, the	B cell and/or T cell mediated immune disorders, particularly immune system disorders associated with low B cell numbers or low

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
radiolabeled BLyS)	immunoglobulin production by B cells.	costimulatory proliferation assay and other assays disclosed by Moore et al., 1999, Science, 285(5425): 260-3.	serum immunoglobulin; Immunodeficiency (e.g., Common 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

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			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.
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 TNF α and TNF β ; 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	Stimulate Epithelial Cell Proliferation; Stimulate Basal Keratinocytes; Wound Healing; Stimulate Hair Follicle Production; Healing Of Dermal Wounds.

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
		described in U.S. Pat. No. 6,077,692.	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 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

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			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; Inflammamatory 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-2-activated natural killer	04658 Chemokine activities can be determined using assays known in the art:	Inflammatory diseases; wound healing; angiogenesis; AIDS infection.

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
	cells.	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.	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 regeneration; organ

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
		12: 845-854 (1998).	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; Immunoregulatory 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 Ulcers; Metabolic Disorders; Immune

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			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, Ca ²⁺ mobilization and tyrosine phosphorylation of extracellular signal-related kinase 1	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
	(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; Hyperlipidemia; Hyperketonemia;

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
precursor))		1110.; Biochem J. 1993 Nov 15; 296 (Pt 1): 15-9.	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; stimulates glucose uptake; slows	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; Non- insulin dependent Diabetes Mellitus (NIDDM); Insulin-

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
	the digestive process; suppresses appetite; blocks the secretion of glucagon.		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, Scienceexpress (www.scienceexpress.org).	HIV; AIDS; SIV (simian immunodeficiency virus) infection.
T1249 (T1249 HIV inhibitory peptide; T1249 anti-HIV peptide)	a second generation HIV fusion inhibitor	Virus inhibition assays as described in Zhang et al., Sep. 26 2002, Scienceexpress (www.scienceexpress.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 antigen expression, NK	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	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)

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
	cell activity and IFN γ production and IL12 production in monocytes.	Biol. 19(11): 7305-13.	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 Myelogenous 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; Hemorrhagic Fever; Viral Hemorrhagic 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

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			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). Natriuresis 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-related defensin; DEFA1; neutrophil-	Suppression of HIV replication; active against bacteria, fungi, and enveloped viruses.	Virus inhibition assays as described in Zhang et al., Sep. 26 2002, Scienceexpress (www.scienceexpress.org).	HIV, AIDS; ARC.

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
specific defensin; CAF)			
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 3.12 Proliferation Assays	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

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
		for T-cell Function John Wiley & Soncs, Inc. (1999).	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 uptake 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 (osteomyelitis and/or infectious arthritis); Charcot's joints; Heel spurs; Sever's disease; Sport's injuries; Cancer; Solid Tumors; Melanoma; Malignant Melanoma; Renal Cancer (e.g., Renal Cell Carcinoma); Lung Cancer (e.g., Non-Small Cell Lung Cancer or

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			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 Myelogenous 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 Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X; Kidney disorders;

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			Hyperinsulinemia; Hypoinsulinemia; Immunological disorders (e.g. arthritis, asthma, immunodeficiency diseases, AIDS, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, T-cell mediated cytotoxicity, host-versus-graft disease, autoimmunity disorders, demyelination, systemic lupus erythematosus, 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 Ulcers; Metabolic Disorders; Immune Disorders; Obesity; Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X; Kidney disorders;

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			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 bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, T-cell mediated cytotoxicity, host- versus-graft disease, autoimmunity disorders, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjorgren's disease, scleroderma)
HDPBQ71	Regulates production and secretion of IFN γ ; 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 Biomolecular Screening 4: 193-204 (1999); Rowland et al., "“Lymphocytes: a practical approach”" Chapter 6: 138-160	Blood disorders and infection (e.g., viral infections, tuberculosis, infections associated with chronic granulomatous disease and malignant osteoporosis); Autoimmune disease (e.g., rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis); Immunodeficiency, boosting a T cell-mediated immune response, 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,

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
		(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)	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; 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,

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			Ewing's tumors, and/or malignant lymphomas); Bone and joint infections (osteomyelitis and/or infectious arthritis); Charcot's joints; Heel spurs; Sever's disease; Sport's injuries
Tumstatin (T5, T7 or T8 peptide; $\alpha 3(IV)NC1$)	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 Myelogenous Leukemia; Hematological Malignancies; Hematological Disorders; Multiple Myeloma; Angiogenesis
CNTF (Ciliary neurotrophic factor)	Enhances myelin formation; Reduces photoreceptor degradation; Regulates calcium currents	Regulation of myelin formation can be assayed as described in J. Neurosci. (2002) Nov. 1; 22(21): 9221-7.	Neurological and neural diseases 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.

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
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 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;

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			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 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

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			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 transcript; APM-1; GBP28; ACRP30; ADIPOQ)	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): 30864-30873).	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;

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			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
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	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,

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
		(2000); and Klemm et al., J Biol Chem 273: 917-923 (1998)).	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 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-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	Most preferred: Treatment of

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
		measured using adipogenesis inhibition assays (Ross et al., Science 2000; 289(5481): 950-953	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 proliferation assays known in the art (Doherty et al., PNAS 1999; 96(19): 10869-10874.	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; Hairy Cell Leukemia; Chronic Myelogenous Leukemia; Hematological Malignancies; Hematological Disorders; Multiple Myeloma.

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
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; Progressive Supranuclear Palsy; Creutzfeld-Jacob Disease; Motor Neuron Disease.
CART (Cocaine- and Amphetamine-Regulated Transcript)	Inhibits food intake and fat storage; promotes lipid oxidation.	Appetite and food intake 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).

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
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 measuring serum cortisol levels. (Frank et al. J. Am. Vet. Med. Assoc. 1998 212(10): 1569-71).	Endocrine; Addison's disease; Cushing's syndrome; pituitary dysfunction; acute adrenal crisis
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.	Treatment of Obesity; treatment of Diabetes; suppression of body weight gain; suppression of appetite; treatment of endocrine disorders;

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
		Nature 2002; 418: 650654)	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; appetite stimulant; treatment of cachexia.
Teriparatide (CAS- 52232- 67-4)	Acts in conjunction 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 lysine vasopressin) (CAS-14636- 12-5)	Analog of vasopressin; induces vasoconstriction.	Terlipressin activity can be measured using assays of vasoconstriction, such	Variceal hemorrhage; cirrhosis; portal hypertension; hepatorenal syndrome; Blood-related disorders

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
		as the isolated arterial ring preparation. (Landstrom et al., Hum Reprod 1999 Jan; 14(1): 151-5).	
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-4117-65-1; CAS- 1402-89-7)	Antibacteria	Aspartocin 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.
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; Osteoporosis; 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

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			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 aggregation assays. (Glusa, Haemostasis 1991; 21 Suppl 1: 116-20).	Blood-related disorder; Thrombosis; thrombocytopenia; hemorrhages.
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-	Hypoglycemia; 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
		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; Endocrine disorders.
Nagrestipen (CAS-166089- 33-4)			Inflammation; Immune
Pentigetide (Pentyde) (CAS-62087- 72-3)			Respiratory; Allergy; Immune
Proinsulin (CAS-67422-14-4)	Stimulates glucose uptake and promotes glycogenesis and lipogenesis.	Insulin 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.
Becaplermin (Regranex; recombinant PDGF-BB) (CAS-165101- 51-9)	Promotes wound healing.	Becaplermin activity can be assessed using animal wound healing models	Stimulate Epithelial Cell Proliferation; Stimulate Basal Keratinocytes; Promote Wound

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
		known in the art. (Saba et al., Ann Plast Surg 2002 Jul; 49(1): 62-6).	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 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

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			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; Inflammamatory 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; 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

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
			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 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.

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
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 NM_000164)	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.
POMC (proopiomelanocortin), including fragments or variants (such as, for example, alpha-melanocyte stimulating hormone, α MSH, gamma melanocyte stimulating hormone, γ MSH, beta-melanocyte stimulating hormone, β MSH, 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; Does, 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.	See U.S. Pat. No.	See U.S. Pat. No.	Resistance to stress; anti-

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
6,350,430)	6,350,430	6,350,430	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 differentiation of T cells into Th2 phenotype.	IL-4 activity can be assessed by measuring Th1/Th2 cytokine responses of isolated spleen cells in vitro. (Waltz et al., Horm Metab Res 2002 Oct; 34(10): 561-9).	Treatment of Psoriasis; Autoimmune disorders; 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	Treatment of coronary heart disease.

Cargo Polypeptide	Biological Activity	Exemplary Activity Assay	Indication
		Targets 2008, 8, 238-243)	

[00190] In one embodiment, the cargo polypeptide is an antigen binding polypeptide construct. In another embodiment, the cargo polypeptide is an antigen-binding polypeptide construct that is an antibody, or fragment or variant thereof. In one embodiment, the antigen-binding polypeptide construct is a Fab fragment of an antibody, or an scFv, or a single domain antibody or fragment thereof.

[00191] In one embodiment the cargo polypeptide is selected from an antigen-binding polypeptide construct that binds to CD3, CD19, CD20, HER2, or HER3. Suitable examples of antigen binding polypeptide constructs are known in the art and include those derived from anti-CD3, anti-CD19, anti-HER2, and anti-HER3 antibodies.

[00192] Functional Activity :

[00193] "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.

[00194] "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).

[00195] 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.

[00196] 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.

[00197] 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.

[00198] 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.

[00199] Biological Activities

[00200] 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.

[00201] 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.

[00202] 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.

[00203] 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.

[00204] 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.

[00205] 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.

[00206] 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.

[00207] 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.

[00208] In some embodiments, heteromultimers described herein and/or polynucleotides encoding the albumin/alboalbumin 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.

[00209] Therapeutic Uses:

[00210] 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.

[00211] 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.

[00212] 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.

[00213] 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.

[00214] Gene Therapy:

[00215] 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.

[00216] Demonstration of Therapeutic or Prophylactic Activity:

[00217] 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.

[00218] Therapeutic/Prophylactic Administration and Composition

[00219] 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.

[00220] 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.

[00221] 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.

[00222] 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.*)

[00223] 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)).

[00224] Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

[00225] In a specific embodiment comprising a nucleic acid encoding a heteromultimer described 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.

[00226] 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.

[00227] 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.

[00228] 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.

[00229] 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.

[00230] Methods of Recombinant and Synthetic Production of Heteromultimer Proteins:

[00231] 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.

[00232] 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.

[00233] 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.

[00234] 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.

[00235] 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).

[00236] 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 homopolymeric tails to form recombinant DNA molecules.

[00237] 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.

[00238] 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.

[00239] 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.

[00240] 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*, *Metschnikowia*, *Rhodospiridium*, *Leucosporidium*, *Botryosacculus*, *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*.

[00241] 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.

[00242] 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 *Aspergillus* include *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* (Teunissen 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).

- [00243] 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).
- [00244] 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.
- [00245] 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.
- [00246] 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.
- [00247] 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 (MF α -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 MF α -1, 0 glucanase (BGL2)

and killer toxin; *S. diastaticus* glucoamylase II; *S. carlsbergensis* α -galactosidase (MEL1); *K. lactis* killer toxin; and *Candida* glucoamylase.

[00248] 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.

[00249] 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.

[00250] 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.

[00251] 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.

[00252] 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, Carlsbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.

[00253] 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.

[00254] 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).

[00255] Vectors which use glutamine synthase (GS) or DHFR as the selectable markers can be amplified in the presence of the drugs methionine sulfoximine or methotrexate,

respectively. An advantage of glutamine synthase based vectors are the availability 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 entirety 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.

[00256] 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.

[00257] 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.

[00258] 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.

[00259] 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 entirety).

[00260] 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.

[00261] 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.

[00262] 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.

[00263] 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, $C\alpha$ -methyl amino acids, $N\alpha$ -methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

[00264] 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.

[00265] 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.

[00266] 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.

[00267] In specific embodiments, heteromultimer proteins or fragments or variants thereof are attached to macrocyclic chelators that associate with radiometal ions.

[00268] 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 phosphatidylinositol, 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)).

[00269] 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.

[00270] 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.

[00271] 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.

[00272] 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.

[00273] 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.

[00274] 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.

[00275] Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

EXAMPLES

The examples below are given so as to illustrate the practice of this invention. They are not intended to limit or define the entire scope of this invention.

Example 1: The Protein Splitting Method

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. It is reasonable to assume that there would be aspects of both the processes in action during the quaternary structure formation of a complex protein. In the context of some protein-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)

As described here, 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. In some embodiment, the functional role of the split protein segments is to act as transporter polypeptides that drive heteromultimerization and spatial localization of the multiple cargo molecules fused to the transporter polypeptides.

The segmentation sites within the human serum albumin invented here were designed on the basis of following decision criteria. (1) The segmentation site should reside on a loop with high relative SASA and limited residue contact count to rest of the protein. This was aimed to maximize the chances of finding flexible and accessible loops that are less likely to contribute to the protein core stability and thus impact stability of the assembled quasi-native albumin structure relative to the human serum albumin. (2) The interface should be rich in hotspot residues. By hotspot we mean individual or group of residues involved in a well-connected networks of interactions. The interactions could be based on typical interactions observed in proteins such as hydrogen bonds, charged residue interactions such as salt-bridges, van der Waals contacts which show strong structural complementarity and hydrophobic residue clusters. That is to maximize the interface stability and prevent the complex from dissociating. (3) The interface should be as apolar, extensive and interdigitate as possible. Again, that was to maximize the chances that the two chains exist as a permanent complex and do not dissociate into its individual components. (4) In one embodiment we aimed to retain a natural disulfide bond present in the human serum albumin across the segmented sections so as to covalently connect the two chains. That was meant to further increase the stability of the heterodimerizing quasi-native albumin like structure that self assembles from the two derived segments following segmentation. We locate loop regions in the human serum albumin structure / sequence that satisfy the disulfide criteria described here i.e. have a disulfide very close to the segmentation site in the loop region. Besides the obvious advantage of covalently crosslinking the two chains, this disulfide could also serve as a surrogate for the loop being open on segmentation. (5) In one embodiment, we engineer a disulphide link across the two segments by introducing individual amino acid to cysteine mutations at selected positions on each of the two derived segments. (6) In one embodiment, we engineer amino acids at the interface of the two segments to further increase or improve the hotspots at the interface or enhance the structural and

physico-chemical complementarity between the two segments in the assembled quasi-native albumin structure.

Table 3 below shows the interface parameters and association free energies for AlbuCORE scaffolds. The buried interface area between the quasi-native structure derived from assembly of the first and second segment derived from HSA can be computed using a structure based computation using algorithms known in the art (e.g. H. Edelsbrunner and P. Koehl. The weighted volume derivative of a space filling diagram. Proc. Natl. Acad. Sci. (USA) 100, 2203-2208 (2003)). The computed area can be further classified into polar and apolar surface area. The apolar area is an indicator of buried hydrophobic amino acid side chains at the interface. The $\Delta\Delta G_{\text{assoc}}$ refers to the computed free energy difference between the associated state and the unassociated state of the two segment structures. The theoretically computed free energy accounts for hydrogen bonds and other electrostatic interactions, van der waals contact, desolvation effects and surface complementarity between the two contacting segment surfaces. Methods to compute free energy of association are known in the art (e.g. Simonson T, Archontis G, Karplus M. Free energy simulations come of age: protein-ligand recognition. Acc Chem Res. (2002) 35, 430-7)

Table 3: Interface parameters and association free energies for AlbuCORE scaffolds

Scaffold	Interface Buried Area ($\text{\AA}^2$)	Carbon (apolar) Area ($\text{\AA}^2$)	Carbon (apolar) contribution (%)	$\Delta\Delta G_{\text{assoc}}$ (kcal/mol)
AlbuCORE_1A	2892.0	1963.2	67.88	-39.13
AlbuCORE_2A	2282.4	1508.2	66.08	-41.11
AlbuCORE_3	3395.6	2298.3	67.68	-52.69
AlbuCORE_4	5550.6	3690.8	66.49	-180.99
AlbuCORE_6	3278.3	2149.2	65.56	-55.57
AlbuCORE_7	3925.9	2541.2	64.73	-75.20
AlbuCORE_9	4227.0	2709.1	64.09	-122.49
AlbuCORE_13	2982.1	1978.7	66.35	-71.80

Figure 32 shows a plot of residue contacts made by a subset of residues in the human serum albumin derived from its 3-dimensional structure. Residue contacts can be defined by the inter-residue or certain intra-residue atom-atom interaction that fall within a specified distance range and are of certain physico-chemical nature. Residues or groups of residues with strong residue contacts

can be recognized as hotspots. Such residue contact analysis can also be employed to determine loop (or unstructured secondary structural regions in the 3D protein structure) residues, such as those making limited residue contact. The plot also shows residue-wise solvent accessible surface area (SASA) which can be computed using algorithms known in the art (e.g. H. Edelsbrunner and P. Koehl. The weighted volume derivative of a space filling diagram. Proc. Natl. Acad. Sci. (USA) 100, 2203-2208 (2003)).

Figure 33 shows a plot of nature of contacts and interactions achieved by select hotspot residues in human serum albumin. Figure 34 is a graphical representation of hot spot residues or clusters (patches) of hotspot residues in human serum albumin.

Table 4. New segmentation sites in HSA (AlbuCORE) and nature of loop where the segmentation was introduced. The column "loop length" refer to the length (in amino acid residues) of the loop connecting the two nearest secondary structure elements, and the column "sec str distance" shows the distance between the C α carbons of the N- and C- termini of those same secondary structure elements (all α -helices in this case). Interface S-S indicates the disulfide bond at the interface of the derived segments (transporter polypeptides).

Scaffold	Segmentation/Deletion	Interface S-S	loop length	sec str distance
AlbuCORE_1A	segment@(339-340)	316.CYS - 361.CYS	5 res	12.3 Å
AlbuCORE_2A	segment@(300-301)	none	13 res	28.4 Å
AlbuCORE_3	segment@(364-365)	360.CYS - 369.CYS	3 res	7.2 Å
AlbuCORE_4	segment@(441-442)	437.CYS - 448.CYS	7 res	6.0 Å
AlbuCORE_6	delete(84)	75.CYS - 91.CYS	12 res	12.2 Å
AlbuCORE_7	segment@(171-172)	168.CYS - 177.CYS	4 res	5.7 Å
AlbuCORE_9	segment@(281-282)	278.CYS - 289.CYS	2 res	9.1 Å
AlbuCORE_13	segment@(114-115)	none	14 res	36.4 Å

Example 2: Preparation of HA/Alloalbumin based heteromultimer proteins

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 self-assemble 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.

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 dashed marking at the bottom indicate the helical secondary structural regions in the protein structure. The analysis indicates that a large surface area, of the order of about 2000 Å² or greater is buried when the split segmentation is introduced anywhere between residues 30 and 520 with a few exceptions, retaining hydrophobic interfaces. In some embodiment the computed molecular surface area is employed instead of the solvent accessible surface area. In some embodiment the buried surface area is in the range of 2000 Å² to 6000 Å². A larger buried interface was observed for the non-covalent ABH1. Additionally, the analysis also revealed that some loop regions are relatively surface exposed. Albumin has an exceptionally large number of disulfide bridges that contribute 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 Å². This is considerably larger than the average of 1910 - 3880 Å² observed in a number of protein-protein heterodimeric and homodimeric co-complex structures [Bahadur 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.

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 transporter 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 half-life and stability in serum, tissue penetrability transport characteristics and low

immunogenicity. Traditional linkers such as (Gly₄Ser)_x can be used for the fusion of the cargo protein with the transporter polypeptide.

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.

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

SEQ ID No. 4: Residue 1-24 (EFATMAVMAPRTLVLALLSGALALTQTWAG) 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.

SEQ ID No. 1: gi|4502027|ref|NP_000468.1| serum albumin preprotein [Homo sapiens] In parentheses is the N-terminal export sequence that is cleaved resulting in serum albumin.

(EFATMAVMAPRTLVLALLSGALALTQTWAG) DAHKSEVAHRFKDLGEENFKA
LVLIAFAQYLQQCPFEDHVKLNVNEVTEFAKTCVADESAENC DKSLHTLFGDK
LCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNP NLPRLVRPEVDVMCT
AFHDNEETFLKKYLYE IARRHPYFYAPELLFFAKRYKAAFTECCQAADKAAC
LLPKLDEL RDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAEFA
EVSKLVTDLT KVHTECCHGDLLECADDRADLAKY ICENQDSISSKLKECCEK
PLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYE
YARRHPDYSVVL LRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQ
NLIKQNC ELFELGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKC
CKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFS
ALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATK
EQLKAVMDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGL

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

GAATTCGCCACTATGGCTGTGATGGCCCCTAGGACCCTGGTGCTGCTGCTGTCCGGA
GCTCTGGCTCTGACTCAGACCTGGGCTGGAGATGCACACAAGAGTGAGGTTGCTCAT
CGGTTTAAAGATTTGGGAGAAGAAAATTTCAAAGCCTTGGTGTTGATTGCCTTTGCT
CAGTATCTTCAGCAGTGTCCATTTGAAGATCATGTAAAATTAGTGAATGAAGTAACT

GAATTTGCAAAAACATGTGTTGCTGATGAGTCAGCTGAAAATTGTGACAAATCACTT
CATACCCTTTTTGGAGACAAATTATGCACAGTTGCAACTCTTCGTGAAACCTATGGT
GAAATGGCTGACTGCTGTGCAAAACAAGAACCTGAGAGAAATGAATGCTTCTTGCAA
CACAAAGATGACAACCCAAACCTCCCCGATTGGTGAGACCAGAGGTTGATGTGATG
TGCACTGCTTTTCATGACAATGAAGAGACATTTTTGAAAAATACTTATATGAAATT
GCCAGAAGACATCCTTACTTTTATGCCCCGGAACCTCTTTTCTTTGCTAAAAGGTAT
AAAGCTGCTTTTACAGAATGTTGCCAAGCTGCTGATAAAGCTGCCTGCCTGTTGCCA
AAGCTCGATGAACTTCGGGATGAAGGGAAGGCTTCGTCTGCCAAACAGAGACTCAAG
TGTGCCAGTCTCCAAAAATTTGGAGAAAGAGCTTTCAAAGCATGGGCAGTAGCTCGC
CTGAGCCAGAGATTTCCCAAAGCTGAGTTTGCAGAAGTTTCCAAGTTAGTGACAGAT
CTTACCAAAGTCCACACGGAATGCTGCCATGGAGATCTGCTTGAATGTGCTGATGAC
AGGGCGGACCTTGCCAAGTATATCTGTGAAAATCAAGATTCGATCTCCAGTAAACTG
AAGGAATGCTGTGAAAACCTCTGTTGGAAAAATCCCACTGCATTGCCGAAGTGGAA
AATGATGAGATGCCTGCTGACTTGCTTCATTAGCTGCTGATTTTGTGAAAGTAAG
GATGTTTGCAAAAACCTATGCTGAGGCAAAGGATGTCTTCCTGGGCATGTTTTTGTAT
GAATATGCAAGAAGGCATCCTGATTACTCTGTTCGTGCTGCTGCTGAGACTTGCCAAG
ACATATGAAACCACTCTAGAGAAGTGCTGTGCCGCTGCAGATCCTCATGAATGCTAT
GCCAAAGTGTTTCGATGAATTTAAACCTCTTGTGGAAGAGCCTCAGAATTTAATCAAA
CAAAATTGTGAGCTTTTTTGAGCAGCTTGGAGAGTACAAATTCAGAATGCGCTATTA
GTTTCGTTACACCAAGAAAGTACCCCAAGTGTCAACTCCAACCTTGTAGAGGTCTCA
AGAAACCTAGGAAAAGTGGGCAGCAAATGTTGTAAACATCCTGAAGCAAAAAGAATG
CCCTGTGCAGAAGACTATCTATCCGTGGTCCTGAACCAGTTATGTGTGTTGCATGAG
AAAACGCCAGTAAGTGACAGAGTCACCAAATGCTGCACAGAATCCTTGGTGAACAGG
CGACCATGCTTTTTCAGCTCTGGAAGTCGATGAAACATACGTTCCCAAAGAGTTTAAT
GCTGAAACATTACCTTCCATGCAGATATATGCACACTTTCTGAGAAGGAGAGACAA
ATCAAGAAACAAACTGCACTTGTTGAGCTCGTGAAACACAAGCCCAAGGCAACAAAA
GAGCAACTGAAAGCTGTTATGGATGATTTTCGCAGCTTTTGTAGAGAAGTGCTGCAAG
GCTGACGATAAGGAGACCTGCTTTGCCGAGGAGGGTAAAAAACTTGTTGCTGCAAGT
CAAGCTGCCTTAGGCTTATGA

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

Albumin based heteromultimer 1:

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

DAHKSEVAHREFKDLGEENFKALVLI AFAQYLQQCPFEDHVKLVNEVTEFAKTCVADE
SAENCCKSLHTLFGDKLCTVATLRETYGEMADCCAKQE PERNECFLQHKDDNP NLPR
LVRPEVDVMCTAFHDNEETFLKKYLYE IARRHPYFYAPELLFFAKRYKAAFT ECCCQA
ADKAACLLPKLDEL RDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAEF
AEVSKLVTDLT KVHTECCHGDLLECADD RADLAKY ICENQDSISSKLKECCEKPLLE
KSHCIAEV

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

GATGCACACAAGAGTGAGGTTGCTCATCGGTTTAAAGATTTGGGAGAAGAAAATTTT
AAAGCCTTGGTGTTGATTGCCTTTGCTCAGTATCTTCAGCAGTGTCCATTTGAAGAT
CATGTAAAATTAGTGAATGAAGTAACTGAATTTGCAAAAACATGTGTTGCTGATGAG
TCAGCTGAAAATTGTGACAAATCACTTCATACCCTTTTTGGAGACAAATTATGCACA
GTTGCAACTCTTCGTGAAACCTATGGTGAAATGGCTGACTGCTGTGCAAAAACAAGAA
CCTGAGAGAAATGAATGCTTCTTGCAACACAAAGATGACAACCCAAACCTCCCCCGA
TTGGTGAGACCAGAGGTTGATGTGATGTGCACTGCTTTTCATGACAATGAAGAGACA
TTTTTGAAA**AAA**TACTTATATGAAATTGCCAGAAGACATCCTTACTTTTATGCCCCG
GAACTCCTTTTTCTTTGCTAAAAGGTATAAAGCTGCTTTTACAGAATGTTGCCAAGCT
GCTGATAAAGCTGCCTGCCTGTTGCCAAAGCTCGATGAACTTCGGGATGAAGGGAAG
GCTTCGTCTGCCAAACAGAGACTCAAGTGTGCCAGTCTCCAAAAATTTGGAGAAAGA
GCTTTCAAAGCATGGGCAGTAGCTCGCCTGAGCCAGAGATTTCCCAAAGCTGAGTTT
GCAGAAGTTTCCAAGTTAGTGACAGATCTTACCAAAGTCCACACGGAATGCTGCCAT
GGAGATCTGCTTGAATGTGCTGATGACAGGGCGGACCTTGCCAAGTATATCTGTGAA
AATCAAGATTCGATCTCCAGTAAACTGAAGGAATGCTGTGAAAAACCTCTGTTGGAA
AAATCCCCTGCATTGCCGAAGTGTGA

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

SLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLRLAKTYETTTLEKC
 CAAADPHECYAKVFDEFKPLVEEPQNLIKQNCSELFELGEYKFQNALLVRYTKKVPQ
 VSTPTLVEVSRNLGKVGSKCKKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVT
 KCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVE
 LVKHKPKATKEQLKAVMDDFAAFVEKCKADDKETCFEEGKKLVAASQAALGL

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

TCATTAGCTGCTGATTTTGTGAAAGTAAGGATGTTTGCAAAAACCTATGCTGAGGCA
 AAGGATGTCTTCCTGGGCATGTTTTTGTATGAATATGCAAGAAGGCATCCTGATTAC
 TCTGTCTGCTGCTGCTGAGACTTGCCAAGACATATGAAACCACTCTAGAGAAGTGC
 TGTGCCGCTGCAGATCCTCATGAATGCTATGCCAAAGTGTTTCGATGAATTTAAACCT
 CTTGTGGAAGAGCCTCAGAATTTAATCAAACAAAATTGTGAGCTTTTTGAGCAGCTT
 GGAGAGTACAAATTCCAGAATGCGCTATTAGTTCGTTACACCAAGAAAGTACCCCAA
 GTGTCAACTCCAACTCTTGTAGAGGTCTCAAGAAACCTAGGAAAAGTGGGCAGCAAA
 TGTTGTAAACATCCTGAAGCAAAAAGAATGCCCTGTGCAGAAGACTATCTATCCGTG
 GTCCTGAACCAGTTATGTGTGTTGCATGAGAAAACGCCAGTAAGTGACAGAGTCACC
 AAATGCTGCACAGAATCCTTGGTGAACAGGCGACCATGCTTTTCAGCTCTGGAAGTC
 GATGAAACATACGTT**CCC**AAAGAGTTTAATGCTGAAACATTCACCTTCCATGCAGAT
 ATATGCACACTTTCTGAGAAGGAGAGACAAATCAAGAAACAACTGCACTTGTTGAG
 CTCGTGAAACACAAGCCCAAGGCAACAAAAGAGCAACTGAAAGCTGTTATGGATGAT
 TTCGCAGCTTTTGTAGAGAAGTGCTGCAAGGCTGACGATAAGGAGACCTGCTTTGCC
 GAGGAGGGTAAAAAACTTGTTGCTGCAAGTCAAGCTGCCTTAGGCTTATGA

Albumin based heteromultimer 2:

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

DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQCPFEDHVKLVNEVTEFAKTCVADE
 SAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQE PERNECFLQHKDDNP NLPR
 LVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQA
 ADKAACLLPKLDEL RDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAEF
 AEVSKLVTDLTKVHTECCHGDLLECADDRADLAKY ICENQDSISSKLKECCEKPLLE
 KSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARA

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

GATGCACACAAGAGTGAGGTTGCTCATCGGTTTAAAGATTTGGGAGAAGAAAATTTCA
AAAGCCTTGGTGTGATTGCCTTTGCTCAGTATCTTCAGCAGTGTCCATTTGAAGAT
CATGTAAAATTAGTGAATGAAGTAACTGAATTTGCAAAAACATGTGTTGCTGATGAG
TCAGCTGAAAATTGTGACAAATCACTTCATACCCTTTTTGGAGACAAATTATGCACA
GTTGCAACTCTTCGTGAAACCTATGGTGAAATGGCTGACTGCTGTGCAAAACAAGAA
CCTGAGAGAAATGAATGCTTCTTGCAACACAAAGATGACAACCCAAACCTCCCCCGA
TTGGTGAGACCAGAGGTTGATGTGATGTGCACTGCTTTTCATGACAATGAAGAGACA
TTTTTGAAAAAATACTTATATGAAATTGCCAGAAGACATCCTTACTTTTATGCCCCG
GAACTCCTTTTCTTTGCTAAAAGGTATAAAGCT**GCT**TTTACAGAATGTTGCCAAGCT
GCTGATAAAGCTGCCTGCCTGTTGCCAAAGCTCGATGAACTTCGGGATGAAGGGAAG
GCTTCGTCTGCCAAACAGAGACTCAAGTGTGCCAGTCTCCAAAAATTTGGAGAAAGA
GCTTTCAAAGCATGGGCAGTAGCTCGCCTGAGCCAGAGATTTCCCAAAGCTGAGTTT
GCAGAAGTTTCCAAGTTAGTGACAGATCTTACCAAAGTCCACACGGAATGCTGCCAT
GGAGATCTGCTTGAATGTGCTGATGACAGGGCGGACCTTGCCAAGTATATCTGTGAA
AATCAAGATTCGATCTCCAGTAAACTGAAGGAATGCTGTGAAAAACCTCTGTTGGAA
AAATCCCCTGCAATTGCCGAAGTGGAATGATGAGATGCCTGCTGACTTGCCTTCA
TTAGCTGCTGATTTTGTGAAAGTAAGGATGTTTGCAAAAACCTATGCTGAGGCAAAG
GATGTCTTCCTGGGCATGTTTTTGTATGAATATGCAAGAGCATGA

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

SVVLLLRLAKTYETTTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCSELFQQL
GEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSV
VLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHAD
ICTLSEKERQIKKQ TALVELVKHKPKATKEQLKAVMDDFAAFVEKCKADDKETCFA
EEGKKLVAAASQAALGL

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

TCTGTCGTGCTGCTGCTGAGACTTGCCAAGACATATGAAACCACTCTAGAGAAGTGC
TGTGCCGCTGCAGATCCTCATGAATGCTATGCCAAAGTGTTTCGATGAATTTAAACCT
CTTGTGGAAGAGCCTCAGAATTTAATCAAACAAAATTGTGAGCTTTTTGAGCAGCTT
GGAGAGTACAAATTCCAGAATGCGCTATTAGTTCGTTACACCAAGAAAGTACCCCAA
GTGTCAACTCCAACCTTGTAGAGGTCTCAAGAAACCTAGGAAAAGTGGGCAGCAAA

TGTTGTAAACATCCTGAAGCAAAAAGAATGCCCTGTGCAGAAGACTATCTATCCGTG
 GTCCTGAACCAGTTATGTGTGTTGCATGAGAAAACGCCAGTAAGTGACAGAGTCACC
 AAATGCTGCACAGAATCCTTGGTGAACAGGCGACCATGCTTTTCAGCTCTGGAAGTC
 GATGAAACATACGTTCCCAAAGAGTTTAATGCTGAAACATTACCTTCCATGCAGAT
 ATATGCACACTTTCTGAGAAGGAGAGACAAATCAAGAAACAAACTGCACTTGTGAG
 CTCGTGAAACACAAGCCCAAGGCAACAAAAGAGCAACTGAAAGCTGTTATGGATGAT
 TTCGCAGCTTTTGTAGAGAAGTGCTGCAAGGCTGACGATAAGGAGACCTGCTTTGCC
 GAGGAGGGTAAAAAACTTGTGCTGCAAGTCAAGCTGCCTTAGGCTTATGA

Generation and Expression of HA or HAA based heteromultimers

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

EFATMAVMAPRTLVLVLLSGALALTQTWAG. 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. Figures 16A and 16B depict the protein structure and symbolic rendering of ABH1 and ABH2 respectively. As the two figures demonstrate, both ABH constructs are composed of two transporter polypeptides A and B, sequences described in *Table 3*. 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 6A also demonstrates that formation of heterodimers in ABH2 is dependent on DNA transfection ratio, with the 1:1 producing the most heterodimeric product. Finally, 6A demonstrates that fragment A of ABH2, when expressed as stand-alone molecule, is more susceptible to forming homodimers and staying monomeric, a result also observed in Figure 6B. 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 heterocomplex. See *Table 5* below for details.

Table 5: Albumin based heteromultimer constructs

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

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 was carried out as described in 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 was 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) were added to the cultures. The cultures were 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-sterilized (clarified) using a 0.45 µm filter (Millipore). The supernatant was stored at 4 °C for short-term storage and at -80 °C for long-term 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.

Table 6: 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

Purification of ABH2, HSA and heteromultimers

As observed from Figure 6A, the 1:1 DNA transfection ratio of the two transporter polypeptides produced the highest level of heterodimeric ABH2 product. CHO cells transiently co-expressing this ratio of the two polypeptides were cultured as described above. Subsequent heterodimer purification was carried out as follows. The wild-type human Albumin was transfected into CHO cells and purified similarly to the ABH2.

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 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. Purification yields were comparable to wild-type full length HSA, at about 10mg/L. This result was also comparable to the 9mg/L generated by V1087 control performed in HEK cells. Yields were determined by nanodrop A280 measured after Blue Sepharose and size exclusion chromatography.

Stability determination of Albumin based Heteromultimers using Differential Scanning Calorimetry (DSC)

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 7 and Figure 7 for resulting melting temperature determined.

Table 7: Melting temperature for Albumin based heteromultimers

Molecule	Measured Mass (Da)	Theoretical MW (Da)	T _m ° C
HSA Wild Type	66620	66470	75
ABH2	66100	65880	63
ABH1	Not determined	Not determined	60

Evaluation of FcRn Binding of HSA and ABH2 using Surface Plasmon Resonance

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 8 illustrates FcRn binding data. Values in parenthesis refer to standard deviation.

Table 8: 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	
HSA	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
HSA			9.0E-07 (1.0E-07)	Equilibrium Fit
ABH2			9.0E-07 (1.0E-07)	Equilibrium Fit

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

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). The term 4D5 as used herein throughout refers to the humanized sequences of the 4D5 antibody and corresponds to the amino acid sequence of the variable domains in the trastuzumab antibody along with the linker region between the V_L and V_H domains in the scFv molecular format. These form a set of 8 base construct monomers based on transporter polypeptide 1 and 8 base construct monomers based on 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.

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 (GGG)4GG linker for all other termini in the other monomer.

Table 9 illustrates the 16 base constructs (Base construct #1-Base construct #16) that were generated by fusing the 4D5 and NM3E 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.

Table 9:

Single Fusions				
#	Fusion 1	Fusion 2	Fusion 3	SEQ ID NOs (nucleotide/amino acid):
1	NM3E2	F1		95/96
2	F1	NM3E2		97/98
3	NM3E2	F2		99/100
4	F2	NM3E2		101/102
5	4D5	F1		103/104
6	F1	4D5		105/106
7	4D5	F2		107/108
8	F2	4D5		109/110
Double Fusions				
9	NM3E2	F1	NM3E2	111/112
10	NM3E2	F2	NM3E2	113/114
11	4D5	F1	4D5	115/116
12	4D5	F2	4D5	117/118
13	NM3E2	F1	4D5	119/120
14	4D5	F1	NM3E2	121/122
15	NM3E2	F2	4D5	123/124
16	4D5	F2	NM3E2	125/126

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. Additional detail regarding the preparation and expression of these constructs are found in Example 11.

Purification was performed by application of the cellular supernatant with expressed 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 application 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 10 below. All constructs showed the expected molecular weight.

Table 10: 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.

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	Sequences (polypeptide)
513	NM3E				monovalent	SEQ ID NO: 96 SEQ ID NO: 10
514		NM3E			monovalent	SEQ ID No: 98 SEQ ID NO: 10
515			NM3E		monovalent	SEQ ID NO: 8 SEQ ID No: 100
516				NM3E	monovalent	SEQ ID NO: 8 SEQ ID No: 102
517	4D5				monovalent	SEQ ID No: 104 SEQ ID NO: 10
518		4D5			monovalent	SEQ ID No: 106 SEQ ID NO: 10
519			4D5		monovalent	SEQ ID NO: 8 SEQ ID No: 108
520				4D5	monovalent	SEQ ID NO: 8 SEQ ID No: 110
521	NM3E		NM3E		bivalent	SEQ ID No: 96 SEQ ID No: 100
522	NM3E			NM3E	bivalent	SEQ ID No: 96 SEQ ID No: 102
523		NM3E	NM3E		bivalent	SEQ ID No: 98 SEQ ID No: 100
524		NM3E		NM3E	bivalent	SEQ ID No: 98 SEQ ID No: 102
525	4D5		4D5		bivalent	SEQ ID No: 104 SEQ ID No: 108
526	4D5			4D5	bivalent	SEQ ID No: 104 SEQ ID No: 110
527		4D5	4D5		bivalent	SEQ ID No: 106 SEQ ID No: 108
528		4D5		4D5	bivalent	SEQ ID No: 106+108/110
529	NM3E	NM3E			bivalent	SEQ ID No:112 SEQ ID NO: 10
530			NM3E	NM3E	bivalent	SEQ ID NO: 8 SEQ ID No: 114
531	4D5	4D5			bivalent	SEQ ID No: 116 SEQ ID NO: 10

532			4D5	4D5	bivalent	SEQ ID NO: 8 SEQ ID No: 118
540	4D5	4D5		4D5	trivalent	SEQ ID No: 116 SEQ ID No: 110
542	4D5	4D5	4D5	4D5	tetravalent	SEQ ID No: 116 SEQ ID No: 118
543	NM3E		4D5		bispecific	SEQ ID No: 96 SEQ ID No: 108
544	NM3E			4D5	bispecific	SEQ ID No: 96 SEQ ID No: 110
545		NM3E	4D5		bispecific	SEQ ID No: 98 SEQ ID No: 108
546		NM3E		4D5	bispecific	SEQ ID No: 98 SEQ ID No: 110
547	4D5		NM3E		bispecific	SEQ ID No: 104 SEQ ID No: 100
548		4D5	NM3E		bispecific	SEQ ID No: 106 SEQ ID No: 100
549	4D5			NM3E	bispecific	SEQ ID No: 104 SEQ ID No: 102
550		4D5		NM3E	bispecific	SEQ ID No: 106 SEQ ID No: 102
551	NM3E	4D5			bispecific	SEQ ID No: 120 SEQ ID NO: 10
552	4D5	NM3E			bispecific	SEQ ID No: 122 SEQ ID NO: 10
553			NM3E	4D5	bispecific	SEQ ID NO: 8 SEQ ID No: 124
554			4D5	NM3E	bispecific	SEQ ID NO: 8 SEQ ID No: 126
593	4D5			NM3E	bispecific	SEQ ID No: 128
594	NM3E			4D5	bispecific	SEQ ID No: 130

SPR binding of monovalent ABH2 fused to a single antiCD16scFv

Purified heteromultimer ABH2 fused to a single antiCD16scFv to the N terminus of transporter polypeptide SEQ ID 10 (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.

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 Sigma-Aldrich (Oakville, ON). 10% Tween 20 solution was purchased from Teknova (Hollister, CA).

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

A 500nM 3-fold dilution series of V515 was injected over 3000 RUs CD16aWT (L6) compared to blank (L5). Flow rate 50 $\mu\text{L}/\text{min}$ 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 $\text{M}^{-1}\text{s}^{-1}$). All K_D values are reported as the mean and standard deviation from three independent runs.

As shown in Table 11, ABH2 heteromultimer fused to a single antiCD16scFv has full activity and binds its target with good reproducibility and K_D similar to the free anti CD16 scFv (NM3E).

Table 11: SPR data for monovalent ABH2 fused to a single antiCD16scFv.

	Injection #1			Injection #2			KD (M) Ave	KD SD
	k_a 1/Ms	k_d 1/s	KD M	k_a 1/Ms	k_d 1/s	KD 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	5.66E+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.

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.

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.

Sequence of Cargo molecule GLP-1

SEQ ID No: 12: HAEGTFTSDVSSYLEGQAAKEFIAWLVKGRG

Sequence of Cargo molecule Glucagon

SEQ ID NO: 13: HSQGTFTSDYSKYLDSSRAQDFVQWLMNT

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

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 shown in Figure 11. 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 Å. 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.

Human annexin WT Sequence

SEQ ID NO: 14:

GS AVSPYPTFNPSSDVAALHKAIMVKGVD EATIIDILTKRNNAQRQQIKAAYLQETGKPLD
ETLKKALTGHLEEVVLALLKTPAQFDADELRAAMKGLGTDEDTLIEILASRTNKEIRDINR
VYREELKRDLAKDITS DTSGDFRNALLSLAKGDRSEDFGVNEDLADSDARALYEAGERRK
GTDVNVFNTILTTRSYPQLRRVFQKYTKYSKHDMNKVLDLELKGDI EKCLTAIVKCATSKP
AFFAEKLHQAMKGVGTRHKALIRIMVSRSEIDMNDIKAFYQKMYGISLCQAILDETKGDY
EKILVALCGGN

Sequence of Annexin based transporter polypeptide-1:

SEQ ID NO: 15:

SAVSPYPTFNPSSDVAALHKAIMVKGVD EATIIDILTKRNNAQRQQIKAAYLQETGKPLDE
TLKKALTGHLEEVVLALLKTPAQFDADELRAAMKGLGTDEDTLIEILASRTNKEIRDINRV
YREELKRDLAKDITS DTSGDFRNALLSLAKG

Sequence of Annexin based transporter polypeptide-2:

SEQ ID NO: 16:

GVNEDLADSDARALYEAGERRKGTDVNVFNTILTTRSYPQLRRVFQKYTKYSKHDMNKV
LDLELKGDI EKCLTAIVKCATSKPAFFAEKLHQAMKGVGTRHKALIRIMVSRSEIDMNDIK
AFYQKMYGISLCQAILDETKGDYEKILVALCGGN

Figure 12 shows a plot of the buried solvent accessible surface area at the interface of Annexin based transporter polypeptide-1 (ABT-1), and Annexin based transporter 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

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 13 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.

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

Figure 13 provides structure of transferrin molecule based on the PDB structure 1H76. The two monomeric 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 14 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.

Sequence of Transferrin based transporter polypeptide-1:

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 DLLFKDSAAG FLKVPPRMDA KMYLGYEYVT AIRNLREG.

Sequence of Transferrin based transporter polypeptide-2:

SEQ ID NO: 18:ECKPVKWCALSHHE RLKCDEWSVN SVGKIECVSA ETTEDCIAKI
 MNGEADAMSL DGGFVYIAGK CGLVPVLAEN YNKSDNCEDT PEAGYFAVAV
 VKKSASDLTW DNLKGKKSCH TAVGRTAGWN IPMGLLYNKI NHCRFDEFFS
 EGCAPGSKKD SSLCKLCMGS GLNLCEPNK EGYGYTGAF RCLVEKGDVA
 FVKHQVTPQN TGGKNPDPWA KNLNEKDYEL LCLDGTRKPV EEYANCHLAR
 APNHAVVTRK DKEACVHKIL RQQQHLFGSN VTDCSGNFCL FRSETKDLLF
 RDDTVCLAKL HDRNTYEKYL GEEYVKA VGN LRKCSTSSLL EACTFRRP

Example 8: Design of disulfide stabilized HA or HAA based heteromultimer proteins

Albumin based transporter polypeptides were designed wherein as compared to albumin, specific positions were mutated to cysteine in each of the transporter polypeptides to enable the selective formation of a disulfide link between two transporter polypeptide, resulting in a stable heterodimer with a quasi-native albumin monomer structure.

The residues for the introduction of cysteines were selected taking into consideration the following factors:

- Form a covalent bond between the two transporter polypeptides, thereby increasing the stability of the heteromultimer and the resultant quasi-native monomeric form.
- must be within specific CA-CA and CB-CB limits to form a disulphide bond
- compatible local environment (generally preferring shorter hydrophobic side chains in partially solvent exposed regions) is not likely to interfere with existing polar contacts.

Mutagenesis was performed by QuickChange, and expression and purification were performed as presented in Examples 1 and 2 above.

Table 12 below identifies a number of albumin-based heteromultimers comprising introduced cysteines that were designed. ABH3-8 were designed based on the ABH2 design, while ABH9-12 were based on the ABH1 design.

Table 12: Albumin based heteromultimers comprising introduced cysteines

Hetero-multimer	Variant ID	Transporter polypeptide 1 (first-last	Transporter polypeptide 2 (first-last amino acid of the	New Cys in transporter	New CYS in transporter
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		amino acid of the N-terminal albumin segment are denoted)	C-terminal albumin segment are denoted)	polypeptide 1	polypeptide 2
ABH3	881	1-337 (SEQ ID NO 19)	342-585 (SEQ ID NO 20)	A217C	V343C
ABH4	882	1-337 (SEQ ID NO 21)	342-585 (SEQ ID NO 22)	L331C	A350C
ABH5	883	1-337 (SEQ ID NO 23)	342-585 (SEQ ID NO 24)	A217C	L346C
ABH6	884	1-337 (SEQ ID NO 25)	342-585 (SEQ ID NO 26)	W214C	V343C
ABH7	885	1-337 (SEQ ID NO 27)	342-585 (SEQ ID NO 28)	A335C	L346C
ABH8	886	1-337 (SEQ ID NO 29)	342-585 (SEQ ID NO 30)	L198C	V455C
ABH9	887	1-293 (SEQ ID NO 31)	304-585 (SEQ ID NO 32)	A217C	A335C
ABH10	888	1-293 (SEQ ID NO 33)	304-585 (SEQ ID NO 34)	A217C	L331C
ABH11	889	1-293 (SEQ ID NO 35)	304-585 (SEQ ID NO 36)	L198C	N458C
ABH12	890	1-293 (SEQ ID NO 37)	304-585 (SEQ ID NO 38)	A194C	V455C

Variants ABH3, ABH4, ABH5, ABH6, ABH7 and ABH8 were expressed with DNA ratio 1:1 (Fragment 1:Fragment 2) in small scale 2 mL CHO cultures. A gel of expression is shown in Figure 35. WT-Albumin (v225) along with previous variants AlbuCORE 1 and AlbuCORE 2 were also expressed as controls. Variant ABH3, ABH5 and ABH7 expressed similarly to ABH2, while the remaining variants displayed lower expression and possible disulfide scrambling, noticeable by multiple bands in non-reducing SDS PAGE, potentially because of the presence of the natural disulfide positions as well as the introduced cysteine mutations. Recovery of expression may be achieved by changing the cut site (see Example 9) or transfecting with different DNA ratios. No Linkers were present at the termini of these constructs. Introduction of the disulfide bonding cysteine mutations in variants such as ABH9, ABH10, ABH11 and ABH12 are expected to address this issue since they lack a natural disulfide bonding positions in the original interface..

Example 9: Design of HA or HAA based heteromultimer proteins based on different segmentation sites

Table 13 below provides heteromultimer constructs as described herein comprising transporter polypeptides designed by segmenting albumin at various sites as shown in Figures 15A-15P and listed in Table 1. Differently from previous constructs described in Example 8 and proceeding ones, all AlbuCORE constructs in Example 9 were created with a GGGGS linker fused to the C terminus of Fragment 1 and the N terminus of fragment 2. This was done for multiple reasons: 1) The presence of a short linker re-creates a typical fusion molecule and allows us to understand the compatibility of the cut site(s) with the presence of a linker. 2) The presence of a linker may also help stabilize the local environment surrounding the cut site, including shielding some exposed hydrophobic patches. 3) Presence of a linker reduces the number of variables in subsequent phases,

when a warhead is fused to the molecule. See Table 13 for expression results. Expression was initially tested in small scale CHO cultures with the two fragment in each AlbuCORE variant performed in different combinations of ratios. The gel in the Figures 35A and B illustrates the expression results.

Table 13: Albumin based heteromultimers constructs based on different albumin segments.

Heteromultimer	ID	Transporter polypeptide 1 (first-last amino acid of the N-terminal albumin segment are denoted)	Transporter polypeptide 2 (first- last amino acid of the C-terminal albumin segment are denoted)
ABH13	AlbuCORE_1A	1-339 (SEQ ID NO 39)	340-585 (SEQ ID NO 40)
ABH14	AlbuCORE_2A	1-300 (SEQ ID NO 41)	301-585 (SEQ ID NO 42)
ABH15	AlbuCORE_3	1-364 (SEQ ID NO 43)	365-585 (SEQ ID NO 44)
ABH16	AlbuCORE_4	1-441 (SEQ ID NO 45)	442-585 (SEQ ID NO 46)
ABH17	AlbuCORE_6	1-83 (SEQ ID NO 47)	85-585 (SEQ ID NO 48)
ABH18	AlbuCORE_7	1-171 (SEQ ID NO 49)	172-585 (SEQ ID NO 50)
ABH19	AlbuCORE_9	1-281 (SEQ ID NO 51)	282-585 (SEQ ID NO 52)
ABH20	AlbuCORE_13	1-114 (SEQ ID NO 53)	115-585 (SEQ ID NO 54)

Expression of selected variants was performed as described in Example 2 , while purification was carried out as described in Example 17. The characteristics of these molecules with respect to the linkers used, disulphide cross-links, and a summary of the expression results are shown in Table 14.

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Table 14: Results of expression of variants listed in Table 13:

SEQ ID (polypeptide)	Variant	Scaffold	Split /Deletion	frag1:frag2	frag1 MW	frag2 MW	Interface S-S [disulfide link between the two fragments forming AlbuCORE]	Optimal DNA ratio (Fragment 1:Fragment 2)	Expression (compared to AlbuCORE_1 and cloned WT HSA). Rough estimate based on band intensity (assessed by software).	Links
39+40	1634	AlbuCORE_1A	nick(339-340)	1-339:340-585	39.03 kDa	28.10 kDa	316.CYS - 361.CYS	1:1	~ 30% better than closely related AlbuCORE_1. Single fragments still visible in 1:0 and 0:1 DNA ratios. When in excess, <u>no clear signs of multimerization.</u>	Fragment 1: GGGGS (new C terminus) Fragment 2: GGGGS (new N terminus)
41+42	1635	AlbuCORE_2A	nick(300-301)	1-300:301-585	34.53 kDa	32.59 kDa	N/A	Only 1:1, 1:0 and 0:1 was tested	Expression is ~30 % better than AlbuCORE_1 but needs native gel for assessment of complex.	Fragment 1: GGGGS (new C terminus) Fragment 2: GGGGS (new N terminus)
43+44	1636	AlbuCORE_3	nick(364-365)	1-364:365	41.78 kDa	25.35 kDa	360.CYS -	1:2	~ 30-50 % better.	Fragment 1:

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				-585			369.CYS		Fragment 1 tends to multimerize when in excess (DNA ratio not optimized)	GGG GS (new C terminus) Fragment 2: GGG GS (new N terminus)
45+46	1637	AlbuCOR E_4	nick(441-442)	1-441:442-585	50.65 kDa	16.48 kDa	437.CYS - 448.CYS	1:2	~ 30-50 % better. Fragment 1 in excess when DNA ratio not optimized. No signs of multimerization.	Fragment 1: GGG GS (new C terminus) Fragment 2: GGG GS (new N terminus)
47+48	1638	AlbuCOR E_6	Δ (84)	1-83:85-585	9.68 kDa	57.29 kDa	75.CYS - 91.CYS	Only 1:1, 1:0 and 0:1 was tested	Faint dimer band present, 1:1 appears fine. Fragment 2 tends to multimerize when expressed in 0:1 DNA ratio. In 1:1 no clear signs of scrambling nor multimerization.	Fragment 1: GGG GS (new C terminus) Fragment 2: GGG GS (new N terminus)
49+50	1639	AlbuCOR E_7	nick(171-172)	1-171:172-585	20.18 kDa	46.95 kDa	168.CYS - 177.CYS	Weak expression	50 % or more lower.	Fragment 1: GGG GS (new

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										C terminus) Fragment 2: GGGGS (new N terminus)
51+52	1640	AlbuCORE_9	nick(281-282)	1-281:282-585	32.43 kDa	34.70 kDa	278.CYS - 289.CYS	2:1	~ 30-50 % better. Fragment 1 tends to multimerize when in access (DNA ratio not optimized). Results unclear for 0:1 lane.	Fragment 1: GGGGS (new C terminus) Fragment 2: GGGGS (new N terminus)
53+54	1641	AlbuCORE_13	nick(114-115)	1-114:115-585	13.31 kDa	53.82 kDa	N/A	Weak expression	50 % or more lower.	Fragment 1: GGGGS (new C terminus) Fragment 2: GGGGS (new N terminus)
This is ABH2	221	AlbuCORE_1 (CONTROL)	nick(338-341)	1-337:342-585	38.48 kDa	27.51 kDa	316.CYS - 361.CYS		Weak expression of Fragment 1 and Fragment 2 still visible in 1:0 and 0:1. The single fragments	Fragment 1: GGGGS (new C terminus) Fragment 2:

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									show sign of aggregation, likely due to disulfide scrambling. Awaiting reducing SDS-PAGE to confirm.	GGG GS (new N termin us)
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Example 10: Multiple Cargo Proteins

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.

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.

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.

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.)

Example 11. Preparation of anti-Her2, monospecific, mono- to tetra-valent cargo-loaded albumin-based polypeptides; anti- Her2 x Her3 bi-specific, bivalent cargo loaded albumin-based polypeptides; and various controls.

A number of different cargo-loaded albumin based transporter polypeptides were prepared as described in this example. The anti-HER2 scFvs described in Table 8 of Example 3 were prepared as follows. Briefly, ABH2 was loaded with anti-Her2 scFvs in various cis and trans configurations according to the methods described here. The final mono-specific polypeptide ranged from mono to tetra-valent. Details of the amino acid and nucleic acid sequences of the component polypeptides are also shown in Table 8. In a similar fashion, ABH2 was also loaded with anti-Her2 and anti-Her3 scFvs in both cis and trans configurations, producing bi-specific, bivalent molecules. As controls utilized in various Examples, V1087, single scFvs, one-arm Fcs and bivalent Fcs were also produced.

ABH2 loaded with anti-Her2 4D5 scFv. The different possible configurations of ABH2 loaded anti-Her2 are schematically rendered in Figure 17. As this figure shows, numerous cis and trans variations were prepared depending on whether the scFv is attached to the N or C termini of either fragment A or B of ABH2. A short GGSG linker was designed at the natural N terminus of ABH2 Fragment 1 and a longer (GGG)₄GG linker was designed at the C terminus of ABH2 Fragment 1 and N and C termini of ABH2 Fragment 2. 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 expressed 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 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 application 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, and in Figure 17. All constructs showed the expected molecular weight.

Monovalent ABH2 fused to one anti-Her2 4D5 scFv fusion are exemplified by variants 517, 518, 519, 520 as described in Table 8. Bivalent and trivalent ABH2 fused to two or three anti-Her2 4D5 scFv fusions are exemplified by variants 525, 526, 527, 528, 531, 532, 540 as described in Table 8. All of these variants were produced by co-expression of the two Albumin fragments as described in the following paragraph. Finally, attaching anti-Her2 scFvs to all four possible termini formed the tetravalent fusion protein, referred to as variant 542.

ABH2 loaded with anti-Her2 x anti-Her3 scFvs (variants 1377, 1378, and 1090).

Different versions of albumin-based heteromultimers fused to anti-HER2 and anti-HER3 were prepared. The anti-HER2 and anti-HER3 warheads that were used target the same receptors as MM-111, with the main difference being the anti-HER2 warhead, which was either the non-neutralizing B1D2 warhead used in MM-111 or the neutralizing 4D5 warhead.

AlbuCORE_1 (ABH2) fragment 1 was fused to anti-HER3 at the N terminus through a GGGS linker and complexed with fragment 2 fused to antiHER2 (4D5) through a (GGSG)4GG linker positioned at the N terminus of fragment 2 (yielding variant 1378, with warheads in trans) or the C terminus of fragment 2 (yielding v1377, with warheads in cis). A third type of AlbuCORE_1 (ABH2) molecule was formed by combining fragment 1 fused to antiHER3 at its N terminus through a GGGS linker and fragment 2 fused to antiHER2 (B1D2) at its C terminus through a GGGS linker (variant number 1090). This molecule has warheads in cis and is almost identical to MM-111. The main differences between the MM-111 polypeptide and the ABH2-based polypeptide are that 1) the linkers used in the MM-111 polypeptide were more hydrophobic, while the linkers used on the ABH2-based polypeptides were polyGLY(S) and 2) The ABH2-based polypeptides lack the C34S/N504Q mutation originally introduced by Merrimack.

MM-111 and derivatives (variant 1087, 1088, and 1089)

The MM-111 molecule is a single polypeptide fusion protein of two scFvs, anti-Her2 (B1D2) and anti-Her3 (H3), linked to the C and N termini, respectively, of a modified human serum albumin protein, and is produced by Merrimack. The resulting molecule is bispecific and bivalent. As a control, a variant of the MM-111 molecule was constructed in which the anti-Her3 (H3) warhead was fused to the N terminus of albumin by a short AAS linker, while anti-Her2 (B1D2) was fused to the C terminus through an AAAL linker to create the benchmark control variant 1087. This control

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variant lacks the C34S/N504Q mutation originally introduced by Merrimack in their MM-111 molecule. Additional MM-111-related molecules were constructed: 1) albumin fused to only the anti-Her3 (H3) warhead at the N terminus (v1088) and 2) the anti-Her2 (B1D2) fused to the C terminus (v1089). In both these cases the linkers used were identical to MM-111. All molecules were expressed in a 1:1 ratio in CHO cells and purified as described for the multimeric 4D5 fusions.

Single anti-Her2 scFvs

Free Anti-Her2 human scFv (v218) was expressed in 1L CHO3E7 cells (v218). A (His)₆ tag was fused to the C terminus through a cleavable linker containing a TEV protease site. The protein was purified by metal (Co²⁺) affinity (column volume=1 mL) coupled to a fractogel system followed by Superdex 200 size exclusion chromatography. Post purification yields were 3.6 mg of 4D5 scFv after Co²⁺ affinity and 1.38 mg after size exclusion.

Figure 31A is a SDS-PAGE depicting the purification of v218 after Co²⁺ affinity. The expression product post-washes, column elution and desalting are provided. Figure 31B is the chromatography profile of the variant following the second purification through the size exclusion column.

Control Fc molecules (878, 628 and 630):

A single anti-Her2 (4D5) human scFv was fused to an Fc fragment to produce a monovalent, monospecific antibody product. In order to ensure formation of the heterodimeric Fc, heterodriver mutations were included in the Fc chain.

V878: OA4-scFv-B1D2, a monovalent anti-Her2 antibody, where the Her2 binding domain is a scFv on chain A, and the Fc region is a heterodimer having the mutations L351Y_F405A_Y407V in Chain A, and T366L_K392M_T394W in Chain B.

V628: a bivalent anti-Her2 antibody, where both Her2 binding domains are in the scFv format, and the Fc region is a heterodimer having the mutations L351Y_S400E_F405A_Y407V in Chain A, and T366I_N390R_K392M_T394W in Chain B.

V630: a monovalent anti-Her2 antibody, where the Her2 binding domain is an scFv, and the Fc region is a heterodimer having the mutations L351Y_S400E_F405A_Y407V in Chain A, and T366I_N390R_K392M_T394W in Chain B.

Table 15 provides a listing of the polypeptide sequences for the variants described in this section.
Table 15:

Variant	Polypeptide A SEQ ID No: (nt/aa)	Polypeptide B SEQ ID No: (nt/aa)
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1377	161/162	163/164
1378	165/166	167/168
1090	169/170	171/172
1087	155/156	
1088	157/158	
1089	159/160	
218	93/94	
878	145/146	
628	151/152	149/150
630	153/154	149/150
1092	137/138	139/140
1093	141/142	143/144
1094	133/134	
1095	135/136	

Example 12. Target binding to SKOV cells by ABH2 fused to anti-Her2 4D5 scFvs in multivalent configurations.

The affinity of ABH2 anti-Her2 fusion proteins to Her2⁺ cells was examined in this Example. The various scFv attachment configurations are provided in Figure 17. The ABH2 (AlbuCORE™) alone, the anti-Her2 scFv alone, a monovalent one-armed Fc molecule and a bivalent two-armed Fc molecule were utilized as controls. These are summarized in the “Controls” panel of Figure 17. The ABH2 protein was constructed as per Example 2 and the fusion proteins were created as per Example 11.

FACS Binding Assessment

The binding affinity of the various anti-Her2 ABH2 constructs and controls to SKOV cells expressing Her2 was assessed via FACS. Detection was performed using an anti-HSA polyclonal (FITC labeled). All K_D determined are apparent.

Whole Cell Binding by FACS Protocol:

Once cells have reached sufficient viability, (ie 2x10⁶ cells/ml cells (> 80% viability)) the media was aspirated from the T75 culture flask and washed with cold PBS, which was subsequently aspirated. 3mL dissociation buffer per flask was added and cells counted. The suspension was then centrifuged at 1050 rpm for 5 minutes and cells resuspended in RPMI media containing 5% FBS @ 1X10⁵ cells/ 100μl. 100 ul of cell suspension was added to eppendorf tubes with the subsequent addition of 10μl/ tube of the primary antibody (goat anti human HSA). The cells were then incubated for 2 hours at 4°C on a rotating support. Tubes were then centrifuged for two minutes at

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2000 rpm at room temperature. The media was aspirated, and the pellet washed with 500 ul RPMI media containing 5% FBS and re-centrifuged. The secondary antibody (Alexa Fluor 488-conjugated anti goat IgG) was added to final concentration of 2ug/sample. The pellet was resuspended in 100 ul solution, incubated for 1hour at 4C on rotating support and centrifuged 2 minutes at 2000 rpm. The cells were then washed with 500 ul RPMI media containing 5% FBS re-centrifuged and re-suspended in 500 ul per pellet for flow cytometric analysis.

The K_D values for each construct are provided below the representative rendering in Figure 17. All K_D determined are apparent and values represent an average of six concentrations tested per molecule, up to 3 μ M. As the figure demonstrates, increasing the valency tends to increase binding affinity, indicated by the decrease in apparent K_D value. Generally, anti-Her2 scFv attached to the N termini of either fragment polypeptide exhibits greater affinity for Her2 than one attached to the C terminus. In some embodiments, this property of the multispecific multivalent species to reduce effective affinity on fusion at specific positions can be employed to attenuate the affinity and activity of some fusion partner species.

Example 13. Bi-specific binding of ABH2 fused to anti-Her2 and anti-Her 3 scFvs.

The binding affinity of an ABH2 loaded in a cis configuration with anti-Her2 and anti-Her3 scFvs to MALME-3M cells were assessed and compared to the v1087 control. Both the fusion protein and control were constructed as per Example 11.

FACS analysis of bi-specific binding

The binding affinity of an exemplary albumin-based heteromultimer variant 1090 (anti-Her2 x anti-Her3 ABH2) to MALME-3M and SKOV cells was evaluated using FACS with FITC-labeled anti-HSA antibodies, as described in Example 12. As described in Example 11, v1090 comprises B1D2/H3 fusions, Merrimack's linker length, and the less hydrophobic linker polyGLY)(S).

Figure 18A depicts the binding of variants 1088, 1089 and 1090 compared to the control 1087 in SKOV cells. Figure 18B depicts the binding of variants 1088, 1089 and 1090 compared to the control 1087 in MALME-3M cells. Table 16 below provides data regarding characterization of the binding of these variants in both cell types. All units in Table 16 are nM unless otherwise indicated. Binding to MALME-3M cells for 1087 and 1090 was 5-55 nM and 200-10,000 nM, respectively. Binding to SKOV cells for 1087 and 1090 was 10 nM and 9.4 nM, respectively.

Table 16: Binding data for variants in SKOV and MALME-3M cells.

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Variant	warhead at natural N terminus (pos. 1)	warhead at natural C terminus (pos. 2)	K _d (MALME 20120529)	B _{max} (MALME 20120529)	K _d (SKOV 20120603)	HillSlope (MALME 20120529)	B _{max} (MALME 20120603)
1087 (MM- 111)	HER3	HER2	12 μ M	1469	10.69	0.97	8583
1088 (Unsplit HSA)	HER3	--	0.8 μ M	74	5 μ M	1.0	
1089 (Unsplit HSA)	--	HER2	80 μ M	1149	1.6	1.8	1572
1090 (AlbuCORE)	HER3	HER2	0.2 μ M	323	8.4	0.7	2658

Example 14. Serum stability of ABH2 fused to anti-Her2.

The stability of ABH2 loaded with an anti-Her2 in human sera was performed to assess potential proteolytic cleavage of the linker. Five variants were tested: v517, v518, v519, and v520

Human sera incubation and subsequent FACS analysis.

The anti-Her2 ABH2 fusion proteins described above was tested for binding to SKOV cells and then incubated in previously frozen human sera at 100 μ g/ml (Sigma #H4522) for 24 hours at room temperature, before being re-tested for binding to the same stock of SKOV cells. Detection was performed using goat FITC labeled polyclonal anti-HSA antibody. The fusion protein's binding affinity to SKOV-3 cells was assessed using FACS, with pre-incubation proteins used as a control. Figures 19A to D depicts the binding curve of both the pre-incubation and post-incubation samples of the variants tested.

As shown in Figures 19A to D, all fusions appeared stable and capable of binding to the target antigen after serum incubation.

Example 15. FcRn binding of ABH2 fused to anti-Her2 and anti-Her3 scFvs.

The affinity of ABH2 loaded in a trans configuration with anti-Her2 and anti-Her3 scFvs was assessed for binding to FcRn and compared to both wild-type HSA and the 1087 control.

For this experiment the benchmark control v1087 and one of the AlbuCORE HER2/HER3 fusions (v1378) was used. Figure 20A illustrates the trans configuration of the v1378 fusion.

FcRn Binding.

Human FcRn (hFcRn) was immobilized on the SPR surface and purified anti-Her3 x Her2 ABH2 (v1378) and v1087 were flowed over at pH 6.0. Methods are identical to Example 2, except the directionality of the assay was reversed.

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Figure 20A and 20B show the different binding curves of Anti-Her2 x Her3 ABH2 and V1087 respectively. The Anti-Her2 x Her3 ABH2 bound hFcRn with a K_D of 0.97 μM , while the V1087 molecule bound hFcRn with a K_D of 2.76 μM . From the figures, it can be seen that both the fusion ABH2 and v1087 are comparable in FcRn binding affinity to wild-type-HSA ($K_D = 1.4\mu\text{M}$ at pH 5.5, as described in Chaudhury, C et al. *Biochemistry* 2006, 45, 4983-4990).

Example 16. Bench-top stability of ABH2 and monovalent anti-Her2 ABH2.

The bench-top stability of both the ABH2 scaffold and a monovalent ABH2 anti-Her2 fusion protein (v517) was assessed over a five-day period at three different temperatures.

Benchtop Stability

Samples from each molecule were divided into nine tubes, with each tube containing $\sim 10\ \mu\text{g}$ of protein. An initial amount of material corresponding to 10 μg of protein was placed in PBS at 1mg/mL and incubated at 37°C (bath), room temperature (RT) or 4°C (fridge); protein stability was assessed over the course of five days. At each time point an aliquot was extracted and mixed 1:1 with Commassie Blue loading buffer with subsequent freezing. After the last time step all aliquots were thawed and loaded on a native gel, along with reducing and non-reducing SDS-PAGE. Figure 21 is a native gel of the ABH2 scaffold and anti-Her2 ABH2 fusion on days 1, 3 and 5. As the figure demonstrates, both the ABH2 scaffold and the anti-Her2 ABH2 remained stable over the 5 day period. The doublet band visible for the fusion protein likely represents two different reduction states of the disulfide. In presence of reducing agent the two bands in fact collapse into one.

Example 17. Purification optimization using the AlbuPure® Process

As an alternate to the Blue Sepharose purification described in Example 2, which leads to some amount of residual contaminants in the elution and the need for high salt elution, a AlbuPure® based affinity purification method was employed. Optimization procedures consisted in testing binding to the Blue Sepharose matrix in both batch and direct mode, different elution protocols were tried, along with different pH and temperatures. Finally a specific resin (i.e. AlbuPure®) tailored towards the purification of albumin and albumin fusions was tested. Different binding and elution conditions were tested using AlbuPure®, involving binding in presence of different salts and elution using a pH gradient or competing detergents.

Purification of Wild-type HSA: Comparison of Blue Sepharose to AlbuPure®

Blue Sepharose purification was performed on wild-type HSA as per methods described in Example 2. Figure 22A is a non-reducing SDS-PAGE gel profile of the total, flowthrough, wash and elution fractions obtained during the Blue Sepharose purification. Figure 22B represents the LC analysis of the Blue Sepharose elution fraction after gel filtration (SEC). As can be seen from the numerous peaks in the chromatogram, there was significant protein and ion contamination even after the purification.

AlbuPure® purification was performed on wild-type HSA as follows. The cellular supernatant containing wild-type HSA was solubilized in PBS. Prepacked AlbuPure® columns (column volume: 1 mL) were equilibrated in 50 mM sodium acetate at pH 5.3. After loading the supernatant the column was washed three times with solutions containing 50 mM sodium acetate but increasing pH (i.e. 1st was pH 6; 2nd wash pH 7). The final wash was performed in ammonium acetate, pH 8.0. The protein of interest was then eluted using a solution containing 50 mM ammonium acetate, pH 7.0 supplemented with 10 mM sodium octanoate. The AlbuPure® process only required one step to obtain a comparable amount of protein to that of Blue Sepharose. Protein yields from 500 mL initial CHO culture are shown in Table 17 below.

Table 17: Protein yields after purification

Variant ID	Blue Sepharose purification (pre SEC)	Blue Sepharose purification (post SEC)	AlbuCORE (octanoate elution)	Initial cell culture	Comments
V221 (naked HSA)	8.6 mg	2.79 mg	10.34 mg	500 mL	
V519	3.3 mg	1.8 mg	8.16 mg	500 mL	HER2 fusion. Warhead appears to be proteolytically cleaved
V520	4 mg	0.93 mg	4.3 mg	500 mL	HER2 fusion. Warhead appears to be proteolytically cleaved

Figure 22C represents a LC analysis of the AlbuPure affinity column elution fraction after gel filtration (SEC). A comparison of Figures 22B and 22C demonstrate that the AlbuPure process results in a final solution with a much higher level of purity than that from Blue Sepharose along with lower amount of higher MW aggregating protein.

Purification of engineered AlbuCOREs: Comparison of Wild-type HSA and Engineered AlbuCOREs using AlbuPure®

The two fragment peptides of AlbuCORE-1A, 3, 6 and 9 described in Table 11 were transiently co-expressed in CHO cells using separate vectors. The optimal DNA ratio for expression of the two fragments constituting the different AlbuCORE molecules were (fragment1:fragment 2) AlbuCORE-1= 1:1; AlbuCORE-3=1:2; AlbuCORE-6=1:1; AlbuCORE-9=2:1. All expressions were transient and performed in identical fashion to the previous AlbuCORE molecules. Wild-type HSA was also transiently expressed in CHO cells. Expression yields of the fusion proteins were within 75% of the WT-HSA (approx. 50-70mg/L). Amount of material was determined by A280 after albuPure purification.

After co-expression, the AlbuCORE scaffolds were purified using the AlbuPure® protocol described above. To assess purity, the elute from the AlbuPure® affinity column was run through a gel filtration system (SEC) and the flowthrough analyzed using LC. The same was performed for wild-type HSA. Figure 23A represents the chromatogram of the wild-type HSA, while Figures 23B, 23C, 23D, and 23E are chromatograms of AlbuCORE 1, 3, 6 and 9 respectively. As these figures demonstrate, AlbuPure® purification yields are comparable results between wild-type and engineered AlbuCORE. The yields in terms of mg of purified protein from 500 mL initial CHO culture after AlbuPure® purification were determined to be: AlbuCORE-1=16 mg ; AlbuCORE-3=25 mg; AlbuCORE-6=27; AlbuCORE-9=25 mg.

Scale-up Expression and Purification of AlbuCORE-1

AlbuCORE-1 was transiently co-expressed in CHO using the pTT5 vector at a ratio of 1:1 fragment A:B, see example 2. Purified yields of AlbuCORE-1 in CHO cells were approx. 10mg/L, which is comparable to the 9mg/L yield of v1087 in CHO cells, calculated from the same amount of starting material.

AlbuCORE-1 was purified using the AlbuPURE protocol described above. The affinity column elute was applied to a gel filtration-LC analysis system. Figure 24A represents the chromatogram of the AlbuPure purification. The main peak represents the protein of interest.

The purified samples were also analyzed via reducing and non-reducing SDS-PAGE gels, depicted in Figure 24B. As the figure demonstrates, in non-reducing conditions, the key elution product is the whole AlbuCORE-1 protein; in reducing conditions, the AlbuCORE-1 protein separates into the two

fragments previous described. Significantly, there is little contamination, especially when compared with Figure 22A.

SDS-PAGE and LC/MS Analysis of AlbuCORE-1 and WT HSA

The total, flowthrough, wash and elute of the wild-type HSA and AlbuCORE-1 purifications were collected and analyzed via non-reducing SDS-PAGE. As shown in Figure 25A, representing the aforementioned gel profiles, demonstrate, there is little contamination after the AlbuPure process (compare Figure 25A and Figure 22A) and there is also no homodimer formation.

The purified protein was then analyzed using LC/MS. The protein samples were analyzed by LC-MS using an Agilent 1100 HPLC system coupled to an LTQ-Orbitrap XL hybrid mass spectrometer (ThermoFisher Scientific) via a high-flow electrospray interface. The samples (2.5 µg) were injected onto a 2.1 x 10 mm Poros R2 column (Applied Biosystems) and eluted using a 2 mL/min gradient of 20-90% ACN, 0.1% FA over 3 minutes. The flow was split post-column to direct 100 µL/min into the electrospray interface. The column and solvent were heated to 80°C to improve protein peak shape. The LTQ-Orbitrap XL was calibrated using ThermoFisher Scientific's LTQ Positive Ion ESI calibration solution (caffeine, MRFA and Ultramark 1621), and tuned using a 25ug/uL solution of BSA. The cone voltage (source fragmentation setting) was 40 V, the FT resolution was 7,500 and the scan range was m/z 400-4,000. The protein spectra were deconvoluted using ThermoFisher's Promass software (input range: m/z 2500-3050; output mass range: 100,000–160,000 Da; Peak width: 1.5; Merge width: 0.5; Baseline removal: 0.5 (low)). The abundances of the hetero- and homodimer antibody species were determined directly from the resulting molecular weight profiles. The linearity of response was confirmed using defined mixtures of antibodies. Limits of detection were approximately 2%. Figure 25B provides the spectrograms of purified AlbuCORE-1 and WT-HSA. As the spectrogram for AlbuCORE-1 demonstrates, there is no homodimer formation (such as A:A or B:B). Variation from the theoretical molecular weight may be due to ion binding, miscellaneous ligands, or glycation.

Example 18. Spatial organization of termini of albumin-based polypeptides

Albumin-based polypeptides provide an alternative scaffold to antibodies for delivery of cargo molecules. As the difference between Figures 26A and 26B demonstrate, engineered albumin-based polypeptides have a greater degree of positional freedom compared to regular antibodies, as the spatial separation between the four termini varies significantly more. Distances were determined

after modeling the AlbuCORE molecule onto one of the available crystal structures: PDBID= 4EMX. Furthermore, the four termini are also oriented at different planar angles.

Finally, the distances and angles between the termini can be subject to further modulation by choosing different segmentations sites, binding domains and/or linkers. Figure 26C demonstrates alternative AlbuCORE scaffolds available to vary the inter-termini distances for antigen binding optimization in multivalent binding modes.

Example 19. Stability of additional albumin-based polypeptides as measured via DSC

To determine the stability and melting points of additional AlbuCORE polypeptides, DSC experiments were performed on AlbuCORE-3, 6 and 9 as well as controls WT-Albumin and AlbuCORE-1. DSC experiments were performed as set out in Example 2. Figure 27 shows the melting curve of four scaffolds, as compared to that of the WT-HSA. As the Figure shows, scaffolds 3, 6 and 9 possess melting points very similar to the 75°C demonstrated by the wild-type. Furthermore, AlbuCORE-6 was found to be more thermally stable than wild-type HSA, at 76°C. Additional DSC experiments were performed on AlbuCORE 1A and 4. The T_m of AlbuCORE 1A was 66°C and the T_m of AlbuCORE-4 was 70°C. AlbuCORE-1A narrows the gap between the two fragments in AlbuCORE 1 (ABH1) to one residue only and adds a GGGGS linker to both the N and C non natural termini. The same applies to AlbuCORE 2 (ABH2) and AlbuCORE-2A.

Example 20: Preparation of Anti-CD3 x CD19 heteromultimer constructs.

Anti-CD3 and anti-CD19 scFvs were attached to several different antibody platforms as follows. The albumin-based polypeptide platform was AlbuCORE-1; controls included WT-HSA, the Azymetric scaffold (v873) and the anti-CD3 x CD19 BiTE control v891.

ABH2 loaded with anti-CD19 x anti-CD3 scFvs (1092, 1093, 1094, and 1095): The sequences for the anti-CD19 and anti-CD3 scFvs were chosen from two molecules that are currently in clinical trials and are well documented and tested for stability and production. The anti-CD19 and anti-CD3 scFv were directly adopted from the BiTE molecule blinatumomab. The antiCD3 scFv was chosen in the VH-VL orientation, consistent with what used in BiTE. The benchmark molecule was BiTE. AlbuCORE_1 (ABH2) CD3/CD19 fusions were created by attaching the antiCD3 warhead to the natural N terminus of fragment 1 and the antiCD19 to the C terminus of fragment 2 (v1092). A second molecule was created where the warheads were reversed (i.e. anti-CD19 warhead at the natural N terminus of fragment 1 and the anti-CD3 at the C terminus of fragment 2, v1093). The linkers used were identical to the ones used for the multivalent HER2 AlbuCORE experiments:

GGGS at the N terminus of fragment 1 and (GGSG)₄GG at the C terminus of fragment 2.

Expression and purification were performed as previously described for the multivalent HER2.

Molecules 1093 and 1094 were designed to accommodate two different fusions at their natural termini. The scFv fusions were linked to the albumin molecule through a GGS linker at the N terminus and a GGSG linker at the C terminus. The length of the linkers reflect the ones used in the MM-111 molecule, despite having a different sequence type. Schematic representations of these molecules are shown in Figure 29.

Additional monovalent and multivalent CD19 heteromultimers and monovalent CD3 heteromultimers based on ABH2 in which the relative positions of the cargo polypeptides were changed were designed with schematic representations of some of these heteromultimers shown in Figure 28B. These heteromultimers were 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 antiCD19scFv (CD19) and/or anti-CD3 scFv (CD3). 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. Table 18 illustrates the base constructs (that were generated by fusing the CD3 and/or CD19 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.

Table 18: Base constructs for CD3 and CD19 monovalent and multivalent constructs

#	Single Fusions			
	Fusion 1	Fusion 2	Fusion 3	Polypeptide SEQ ID NOs:
1	CD3	F1		137/138
2	F1	CD3		119/120
3	F1	CD3 (short linker)		181/182
4	CD3	F2		179/180
5	CD3 (short linker)	F2		177/178
6	F2	CD3		141/142
7	CD19	F1		143/144
8	F1	CD19		183/184
9	F1	CD19 (short linker)		189/190
10	CD19	F2		187/188
11	CD19 (short linker)	F2		185/186

12	F2	CD19		139/140
Double Fusions				
13	CD19	F1	CD19	191/192
14	CD19	F1	CD19 (shorter linker)	193/194
15	CD19	F2	CD19	195/196
16	CD19 (shorter linker)	F2	CD19	197/198
17	CD3	HSA	CD19	133/134
18	CD19	HSA	CD3	135/136

As shown in Figure 28B, 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 (GGG)₄GG linker for all other termini in the other monomer. In addition a version of each fusion protein was created with a shorter linker at their non-natural terminus (ie C' of Fragment 1 and N' of Fragment 2) formed by the sequence GGSGGSG, replacing (GGG)₄GG.

A summary of the heteromultimers that were prepared are shown in Table 19.

Table 19: Monovalent, multivalent, and multispecific constructs that were generated by fusing the CD3 and CD19 cargo polypeptides to either N or C terminus of transporter polypeptide 1 or transporter polypeptide 2 of ABH2.

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	Sequences (Polypeptide)
1096	CD3				monovalent	SEQ ID No: 138 SEQ ID NO: 10
1097		CD3			monovalent	SEQ ID No: 120 SEQ ID NO: 10
1101 (shorter linker)		CD3			monovalent	SEQ ID No: 182 SEQ ID NO: 10
1098			CD3		monovalent	SEQ ID NO: 8 SEQ ID No: 180
1100 (shorter linker)			CD3		monovalent	SEQ ID NO: 8 SEQ ID No: 178
1099				CD3	monovalent	SEQ ID NO: 8

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						SEQ ID No: 142
1102	CD19				monovalent	SEQ ID No: 144 SEQ ID NO: 10
1103		CD19			monovalent	SEQ ID No: 184 SEQ ID NO: 10
1107		CD19			monovalent	SEQ ID No: 190 SEQ ID NO: 10
1104			CD19		monovalent	SEQ ID NO: 8 SEQ ID No: 188
1106			CD19		monovalent	SEQ ID NO: 8 SEQ ID No: 186
1105				CD19	monovalent	SEQ ID NO: 8 SEQ ID No: 140
1108	CD19			CD19	bivalent	SEQ ID No: 144 SEQ ID No: 140
1109	CD19	CD19		CD19	trivalent	SEQ ID No: 192 SEQ ID No: 140
1110	CD19	CD19	CD19	CD19	tetravalent	SEQ ID No: 192 SEQ ID No: 196
1111 (shorter linker at non- natural terminus)	CD19	CD19		CD19	trivalent	SEQ ID No: 194 SEQ ID No: 140
1112 (shorter linker at non- natural termini)	CD19	CD19	CD19	CD19	tetravalent	SEQ ID No: 194 SEQ ID No: 198
1092	CD3			CD19	bispecific	SEQ ID No: 138 SEQ ID No: 140
1093	CD19			CD3	bispecific	SEQ ID No: 144 SEQ ID No:

						142
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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 as described in Example 2.

The expression results are shown in Figure 28C. The monovalent CD3 and CD19 fusions express at similar levels. The expression of monovalent CD3 and CD19 have the same pattern (in terms of MW) as the one observed with HER2 fusions, indicating that variations in migration on non-reducing gel are independent of the warhead type.

Example 21: Ability of anti-CD3 x CD19 loaded albumin-based polypeptide to bind to B-cells and T-cells.

The ability of Anti-CD3 x CD19 loaded AlbuCORE-1 (v1092 and 1093) to CD3⁺ and CD19⁺ cells was assessed using FACS and compared to WT-HSA loaded with the same anti-CD scFvs (1094 and 1095). The AlbuCORE-1 constructs were additionally tested against the AzymetricTM and the BiTE MT-103 for B-cell and T-cell binding as follows. The method was carried out as described in Example 13, but in MALME-3M cells and SKOV cells.

The results are shown in Figure 29 which shows the K_D and Hill Slope data for the tested variants in Jurkat and Raji cells.

Example 22: Ability of anti-CD13 x CD19 loaded albumin-based polypeptide to bind to B-cells and T-cells.

The ability of variants 1092, and 1093 to bridge B- and T-cells was determined as follows.

Whole Cell Bridging by FACS

1 x 10⁶ cells/ml suspended in RPMI were labeled with 0.3 μM of the appropriate □CellTrace label and mixed and incubated at 37°C in a water bath for 25 minutes. Pellets were resuspended in 2 ml of L10 + GS1 + NaN₃ to a final concentration 5x x10⁶ cells/ml. Cell suspensions were analyzed (1/5 dilution) by flow cytometry to verify the appropriate cell labeling and laser settings. Flow-check and flow-set Fluorospheres were used to verify instrument standardization, optical alignment and fluidics.

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After flow cytometry verification, and prior to bridging, each cell line was mixed together at the desired ratio, at a final concentration of 1×10^6 cells/ml.

T:T bridging was assessed with Jurkat-violet + Jurkat-FarRed, B:B was assessed with RAJI-violet + RAJI-FarRed and T:B bridging was assessed with Jurkat-violet + RAJI-FarRed.

Antibody variants were diluted to 2x in L10+GS1+NaN3 at room temperature then added to cells followed by gentle mixing and a 30 min incubation.

Following the 30 min incubation 2 μ l of propidium iodide was added and slowly mixed and immediately analyzed by flow cytometry.

Bridging % was calculated as the percentage of events that are simultaneously labeled violet and Far-red.

Figure 30A compares the ability of v1093, a bi-specific CD3/CD19 antibody (v873) and the BiTE-like control v891 to bridge Jurkat CD3 T cells (bottom right quadrant) with Raji CD19 B cells (top left quadrant) by FACS. Bridged T-B cells appear in top right quadrant. This result demonstrates that at concentrations of as low as 0.3 nM v1093 is able to specifically bridge Jurkat T cells and Raji B cells to a similar extent (31% of total cells) as BiTE (21% of total cells) and a bi-specific CD3/CD19 antibody with an heterodimeric Fc (25%).

Figure 30B compares the ability of v1092, to the controls v891, v873, and v221. This figure shows that v1092 is able to bridge Jurkat T cells and Raji B cells to a similar extent to v891 (BiTE).

All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference.

What is claimed:

1. A heteromultimer comprising:
 - a first polypeptide construct that comprises (i) a first transporter polypeptide; and
 - a second polypeptide construct that comprises (ii) a second transporter polypeptide;
 - wherein each of said first and second transporter polypeptide comprises an amino acid sequence with at least 90% identity to a segment of an albumin polypeptide; and
 - wherein said first and second transporter polypeptides are obtained by segmentation of said albumin polypeptide at a segmentation site, such that the segmentation results in a deletion of zero to 3 amino acid residues at the segmentation site,
 - wherein said transporter polypeptides self-assemble to form a quasi-native structure of the monomeric form of said albumin polypeptide.
2. The heteromultimer of claim 1, wherein a) the segmentation site resides on a loop of the albumin polypeptide that has a high solvent accessible surface area (SASA) and limited contact with the rest of the albumin structure, b) the segmentation results in a complementary interface between the transporter polypeptides, wherein the interface is apolar, extensive and interdigitate.
3. The heteromultimer of claim 1 or 2, wherein the segmentation results in a deletion of zero to 2 amino acid residues at the segmentation site.
4. The heteromultimer of claim 1 or 2, wherein the segmentation results in a deletion of zero or 1 amino acid residues at the segmentation site.
5. The heteromultimer of any one of claims 1 to 4, wherein at least one of the first and second transporter polypeptides further comprises a linker.
6. The heteromultimer of claim 5, wherein the linker is a GGGGS linker.

7. The heteromultimer of any one of claims 1 to 6, wherein the quasi-native structure of the monomer form of said albumin polypeptide has a melting temperature greater than 65°C.
8. The heteromultimer of any one of claims 1 to 6, wherein the quasi-native structure of the monomer form of said albumin polypeptide has a melting temperature greater than 70°C.
9. The heteromultimer of any one of claims 1 to 6, wherein the quasi-native structure of the monomer form of said albumin polypeptide has a melting temperature greater than 75°C.
10. The heteromultimer of any one of claims 1 to 9, wherein at least one disulphide bond is formed across residues from the first and second transporter polypeptide and the disulphide bond is also observed in said albumin polypeptide.
11. The heteromultimer of any one of claims 1 to 10, wherein said albumin polypeptide is human serum albumin having a sequence of SEQ ID NO: 1 or analog or variant thereof.
12. The heteromultimer of claim 1, wherein said first transporter polypeptide has a sequence comprising SEQ ID NO:39 or analog or variant thereof, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:40 or analog or variant thereof.
13. The heteromultimer of claim 1, wherein said first transporter polypeptide has a sequence comprising SEQ ID NO:41 or analog or variant thereof, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:42 or analog or variant thereof.
14. The heteromultimer of claim 1, wherein said first transporter polypeptide has a sequence comprising SEQ ID NO:43 or analog or variant thereof, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:44 or analog or variant thereof.
15. The heteromultimer of claim 1, wherein said first transporter polypeptide has a sequence comprising SEQ ID NO:45 or analog or variant thereof, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:46 or analog or variant thereof.
16. The heteromultimer of claim 1, wherein said first transporter polypeptide has a sequence comprising SEQ ID NO:47 or analog or variant thereof, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:48 or analog or variant thereof.

17. The heteromultimer of claim 1, wherein said first transporter polypeptide has a sequence comprising SEQ ID NO:49 or analog or variant thereof, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:50 or analog or variant thereof.
18. The heteromultimer of claim 1, wherein said first transporter polypeptide has a sequence comprising SEQ ID NO:51 or analog or variant thereof, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:52 or analog or variant thereof.
19. The heteromultimer of claim 1, wherein said first transporter polypeptide has a sequence comprising SEQ ID NO:53 or analog or variant thereof, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:54 or analog or variant thereof.
20. The heteromultimer of claim 1, wherein at least one of said first and second transporter polypeptides comprises at least one mutation of an amino acid residue to cysteine or an analog thereof such that said cysteine forms a disulfide bond with a corresponding cysteine residue on the other transporter polypeptide.
21. The heteromultimer of claim 20, said first and second transporter polypeptide comprising at least one mutation of an amino acid residue to cysteine such that said cysteine forms a disulfide bond with a cysteine residue on another transporter polypeptide.
22. The heteromultimer of claim 21 said first transporter polypeptide comprising at least one mutation selected from A194C, L198C, W214C, A217C, L331C and A335C.
23. The heteromultimer of claim 21 said second transporter polypeptide comprising at least one mutation selected from L331C, A335C, V343C, L346C, A350C, V455C, and N458C.
24. A heteromultimer comprising:
 - a first polypeptide construct that comprises (i) a first transporter polypeptide; and
 - a second polypeptide construct that comprises (ii) a second transporter polypeptide;
 - wherein each of said first and second transporter polypeptide comprises an amino acid sequence with at least 90% identity to a segment of an albumin polypeptide; and

wherein said first and second transporter polypeptides are obtained by segmentation of said albumin polypeptide at a segmentation site, and

wherein at least one of said first and second transporter polypeptides comprises at least one mutation of an amino acid residue to cysteine or an analog thereof such that said cysteine forms a disulfide bond with the other transporter polypeptide, such that said transporter polypeptides self-assemble to form a quasi-native structure of the monomeric form of said albumin polypeptide.

25. The heteromultimer of claim 24, wherein the segmentation site a) resides on a loop of the albumin polypeptide that has a high solvent accessible surface area (SASA) and limited contact with the rest of the albumin structure, b) results in a complementary interface between the transporter polypeptides, wherein the interface is apolar, extensive and interdigitate.
26. The isolated heteromultimer of claim 24 or 25, wherein said first transporter polypeptide comprises at least one mutation selected from A194C, L198C, W214C, A217C, L331C and A335C.
27. The isolated heteromultimer of claim 24 or 25 said second transporter polypeptide comprising at least one mutation selected from L331C, A335C, V343C, L346C, A350C, V455C, and N458C.
28. The heteromultimer of any one of claims 24 to 27, wherein at least one of the first and second transporter polypeptides further comprises a linker.
29. The heteromultimer of claim 28, wherein the linker is a GGGGs linker.
30. The heteromultimer of any one of claims 24 to 29, wherein said albumin polypeptide is human serum albumin having a sequence of SEQ ID NO: 1 or analog or variant thereof.
31. The heteromultimer of claim 24, wherein said first transporter polypeptide has a sequence comprising SEQ ID NO:19 or analog or variant thereof, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:20 or analog or variant thereof.

32. The heteromultimer of claim 24, wherein said first transporter polypeptide has a sequence comprising SEQ ID NO:21 or analog or variant thereof, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:22 or analog or variant thereof.
33. The heteromultimer of claim 24, wherein said first transporter polypeptide has a sequence comprising SEQ ID NO:23 or analog or variant thereof, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:24 or analog or variant thereof.
34. The heteromultimer of claim 24, wherein said first transporter polypeptide has a sequence comprising SEQ ID NO:25 or analog or variant thereof, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:26 or analog or variant thereof.
35. The heteromultimer of claim 24, wherein said first transporter polypeptide has a sequence comprising SEQ ID NO:27 or analog or variant thereof, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:28 or analog or variant thereof.
36. The heteromultimer of claim 24, wherein said first transporter polypeptide has a sequence comprising SEQ ID NO:29 or analog or variant thereof, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:30 or analog or variant thereof.
37. The heteromultimer of claim 24, wherein said first transporter polypeptide has a sequence comprising SEQ ID NO:31 or analog or variant thereof, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:32 or analog or variant thereof.
38. The heteromultimer of claim 24, wherein said first transporter polypeptide has a sequence comprising SEQ ID NO:33 or analog or variant thereof, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:34 or analog or variant thereof.
39. The heteromultimer of claim 24, wherein said first transporter polypeptide has a sequence comprising SEQ ID NO:35 or analog or variant thereof, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:36 or analog or variant thereof.
40. The heteromultimer of claim 24, wherein said first transporter polypeptide has a sequence comprising SEQ ID NO:37 or analog or variant thereof, and wherein said second transporter polypeptide has a sequence comprising SEQ ID NO:38 or analog or variant thereof.

41. The heteromultimer of any one of claims 1 to 40, wherein said first polypeptide construct further comprises at least one first cargo polypeptide.
42. The heteromultimer of claim 41, wherein the first polypeptide construct further comprises two first cargo polypeptides.
43. The heteromultimer of claim 41 or 42, wherein the at least one first cargo polypeptide is an antigen-binding polypeptide construct.
44. The heteromultimer of claim 43, wherein the antigen binding polypeptide construct binds CD3, CD19, CD20, HER2, or HER3.
45. The heteromultimer of any one of claims 41 to 44, wherein said second polypeptide construct further comprises at least one second cargo polypeptide.
46. The heteromultimer of claim 45, wherein the second polypeptide construct further comprises two second cargo polypeptides.
47. The heteromultimer of claim 45 or 46, wherein the at least one second cargo polypeptide is an antigen-binding polypeptide construct.
48. The heteromultimer of claim 47, wherein said at least one second cargo polypeptide is selected from an antigen-binding polypeptide construct that binds CD3, CD19, CD20, HER2, or HER3.
49. The heteromultimer of any one of claims 1 to 48, wherein said first polypeptide construct further comprises at least one first cargo polypeptide, and said second polypeptide construct further comprises at least one second cargo polypeptide.
50. The heteromultimer of claim 49, wherein said first cargo polypeptide is an antigen-binding polypeptide construct that binds CD3 and said second cargo polypeptide is an antigen-binding polypeptide construct that binds to CD19 or CD20.
51. The heteromultimer of claim 50, wherein said first cargo polypeptide is and antigen-binding polypeptide construct that binds HER2 and said second cargo polypeptide is an antigen-binding polypeptide construct that binds HER3.

52. The heteromultimer of any one of claims 41 to 51, wherein said at least one first cargo polypeptide is fused to said first transporter polypeptide with a linker.
53. The heteromultimer of any one of claims 41 to 51, wherein said at least one second cargo polypeptide is fused to said second transporter polypeptide with a linker.
54. A heteromultimer comprising:
- a first polypeptide construct that comprises (i) a first transporter polypeptide; and (ii) at least one first cargo polypeptide that is an antigen-binding polypeptide construct that binds to CD3, CD19, CD20, HER2 or HER3, and
 - a second polypeptide construct that comprises (iii) a second transporter polypeptide; and (iv) at least one second cargo polypeptide
- wherein each of said first and second transporter polypeptide comprises an amino acid sequence with at least 90% identity to a segment of an albumin polypeptide; and
- wherein said first and second transporter polypeptides are obtained by segmentation of said albumin polypeptide at a segmentation site,
- wherein said transporter polypeptides self-assemble to form a quasi-native structure of the monomeric form of said albumin polypeptide.
55. The heteromultimer according to claim 54, wherein said first polypeptide construct comprises two first cargo polypeptides.
56. The heteromultimer according to claim 55, wherein the two first cargo polypeptides bind to CD3, HER2, HER3, or CD19.
57. The heteromultimer according to claim 55 or 56, wherein the two first cargo polypeptides are different from each other.
58. The heteromultimer according to claim 57, wherein one of said two first cargo polypeptides binds to CD3 and the other binds to CD19.

59. The heteromultimer according to claim 58, wherein one of said two first cargo polypeptides binds to HER2 and the other binds to HER3.
60. The heteromultimer according to any one of claims 54 to 59, wherein said second polypeptide construct comprises at least one second cargo polypeptide that is an antigen-binding polypeptide construct that binds to CD3, CD19, CD20, HER2 or HER3.
61. The heteromultimer according to claim 60, wherein said second polypeptide construct comprises two second cargo polypeptides.
62. The heteromultimer according to claim 61, wherein the two second cargo polypeptides bind to CD3, HER2, HER3, or CD19.
63. The heteromultimer according to claim 61, wherein the two second cargo polypeptides are different from each other.
64. The heteromultimer according to claim 63, wherein one of said two second cargo polypeptides binds to CD3 and the other binds to CD19.
65. The heteromultimer according to claim 63, wherein one of said two second cargo polypeptides binds to HER2 and the other binds to HER3.
66. The heteromultimer of any one of claims 54 to 65, wherein said albumin polypeptide is human serum albumin having a sequence of SEQ ID NO: 1 or analog or variant thereof.
67. The heteromultimer of any one of claims 54 to 65, wherein said at least one first cargo polypeptide is fused to said first transporter polypeptide with a linker.
68. The heteromultimer of any one of claims 54 to 65, wherein said at least one second cargo polypeptide is fused to said second transporter polypeptide with a linker.
69. The heteromultimer of claim 68, wherein the linker is a GGGGs linker.
70. The heteromultimer of any one of claims 1 to 69, wherein said heteromultimer binds to FcRn.
71. The heteromultimer of any one of claims 1 to 69, wherein at least one transporter polypeptide is an allo-albumin derivative.

72. The heteromultimer according to claim 1, 24, or 25, wherein the transporter polypeptides are derived from the same type of albumin polypeptide.
73. The heteromultimer according to claim 1, wherein the transporter polypeptides are derived from different types of albumin polypeptides.
74. The heteromultimer of any one of claims 1 to 40, wherein the first polypeptide construct further comprises at least one first cargo polypeptide that binds a target antigen, and the second polypeptide construct further comprises at least one second cargo polypeptide that is a toxin moiety.
75. The heteromultimer of any one of claims 1 to 40, wherein the first polypeptide construct further comprises at least one first cargo polypeptide that binds a target antigen, and wherein said target antigen is at least one of a-chain (CD25) of IL-2R, Amyloid beta, anti-EpCAM, anti-CD3, CD16, CD20, CD22, CD23, CD3, CD4, CD52, CD80, CTLA-4, EGFR, EpCAM, F protein of RSV, G250, glycoprotein IIB/IIIa R, HER2, HER3, IGF1R, EGFR, HSP90, IgE antibody, IL-12, IL-23, IL-1b, IL-5, IL-6, RANKL, TNF alpha, TNFR, TRAIL, VEGF-A, glucagon receptor, GLP receptor, and LDL receptor.
76. The heteromultimer of any one of claims 1 to 40 wherein at least one cargo polypeptide is an enzyme, hormone, therapeutic polypeptide, antigen, chemotoxin, radiotoxin, cytokine or variant or fragment thereof.
77. The heteromultimer of any one of claims 1 to 76, wherein said transporter polypeptides are derived from a protein by segmentation of said protein, each transporter polypeptide comprising an amino acid sequence with at least 95% identity to a segment of said protein.
78. The heteromultimer of any one of claims 1 to 76, wherein said transporter polypeptides are derived from a protein by segmentation of said protein, each transporter polypeptide comprising an amino acid sequence with at least 99% identity to a segment of said protein.
79. A host cell comprising nucleic acid encoding the heteromultimer of any one of claims 1 to 75.

80. The host cell of claim 79, wherein the nucleic acid encoding the first polypeptide construct and the nucleic acid encoding the second polypeptide construct are present in a single vector.
81. The host cell of claim 79, wherein the nucleic acid encoding the first polypeptide construct protein and the nucleic acid encoding the second polypeptide construct protein are present in separate vectors.
82. A pharmaceutical composition comprising the heteromultimer of claim 41-78 and an adjuvant.
83. A method of treating cancer comprising providing to a patient in need thereof an effective amount of the pharmaceutical composition of claim 82.
84. A method of inhibiting growth of a tumor, comprising contacting the tumor with an effective amount of the heteromultimer of any one of claims 41-78.
85. A method of shrinking a tumor, comprising contacting the tumor with an effective amount of the heteromultimer of any one of claims 41-78.
86. A method of designing self-associating polypeptides from a protein of interest comprising: segmenting said protein at at-least one segmentation site to obtain at least two polypeptide segments such that said polypeptide segments self-assemble to form a heteromultimer, wherein said heteromultimer forms a quasi-native monomeric structure of said protein, the method comprising the steps of
- a. Selecting at least one loop of the protein of interest that has a high solvent accessible surface area and limited contact with the rest of the structure of said protein, and
 - b. Introducing one segmentation site per selected loop, resulting in a complementary interface between the at least two polypeptide segments, wherein the interface is apolar, extensive and interdigitate.
87. The method of claim 86, comprising introduction of at least one disulfide bond between the polypeptide segments.
88. The method of claim 86, wherein the monomeric protein is albumin.

89. The method of claim 86, wherein the segmentation is performed such that the heteromultimer comprises no covalent bond between the polypeptides.
90. The method of claim 86, wherein the segmentation is performed such that the heteromultimer comprises at least one covalent bond between the polypeptides.
91. A heteromultimer designed by the method of claim 86.
92. A therapeutic scaffold comprising a heteromultimer designed by the method of claim 86.
93. The heteromultimer of any one of claims 24 to 78, wherein the quasi-native monomeric structure has a thermal stability that is comparable to that of albumin.
94. The heteromultimer of claim 93, wherein the thermal stability is measured by determining the melting temperature (T_m) of the quasi-native monomeric structure.
95. The heteromultimer of claim 94, wherein the thermal stability of the quasi-native monomer is within approximately 20°C of the thermal stability of albumin.
96. The heteromultimer of claim 41-78, wherein the activity of the cargo polypeptide is selectively altered on fusion to the transporter polypeptide relative to its unfused format.
97. The heteromultimer of claim 41-78, wherein the activity of the cargo polypeptide is selectively attenuated on fusion to the transporter polypeptide relative to its unfused format.
98. The heteromultimer of claim 41-78, wherein the activity of the cargo polypeptide is selectively attenuated 2-100 fold on fusion to the transporter polypeptide relative to its unfused format.
99. The heteromultimer of claim 1 wherein the C- and N- termini introduced at the segmentation site is fused to GGGGS peptide sequence.
100. The method of claim 86, further comprising the step of fusing a GGGGS peptide sequence to the C- and N- termini introduced at the segmentation site.

Figure 1

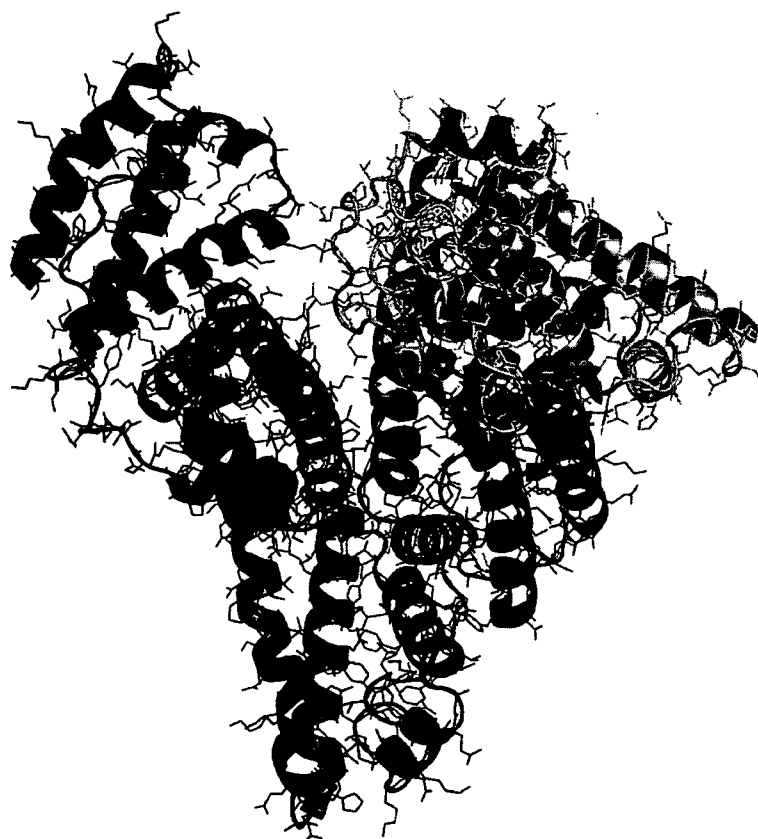


Figure 2

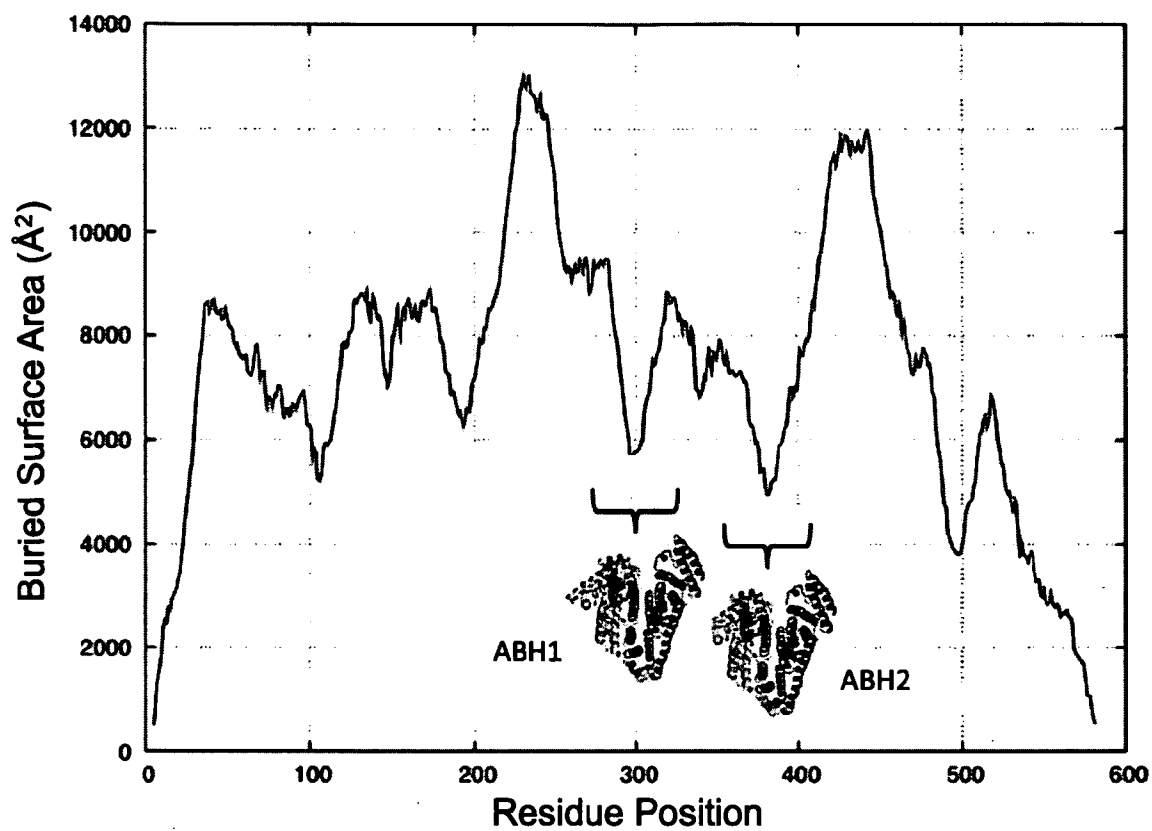


Figure 3

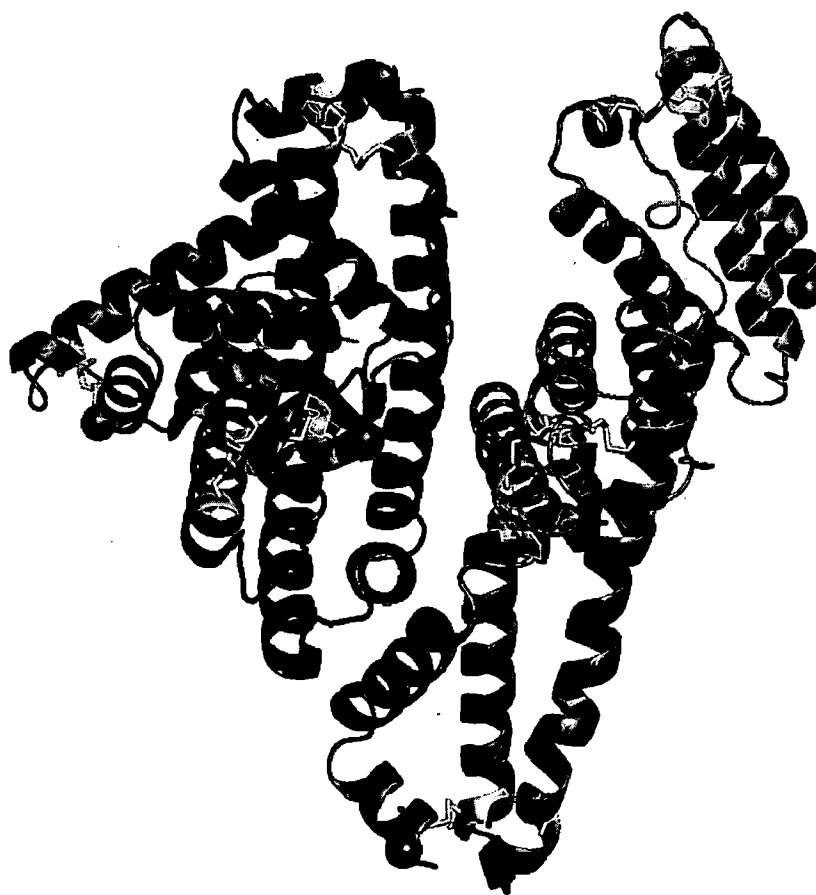


Figure 4

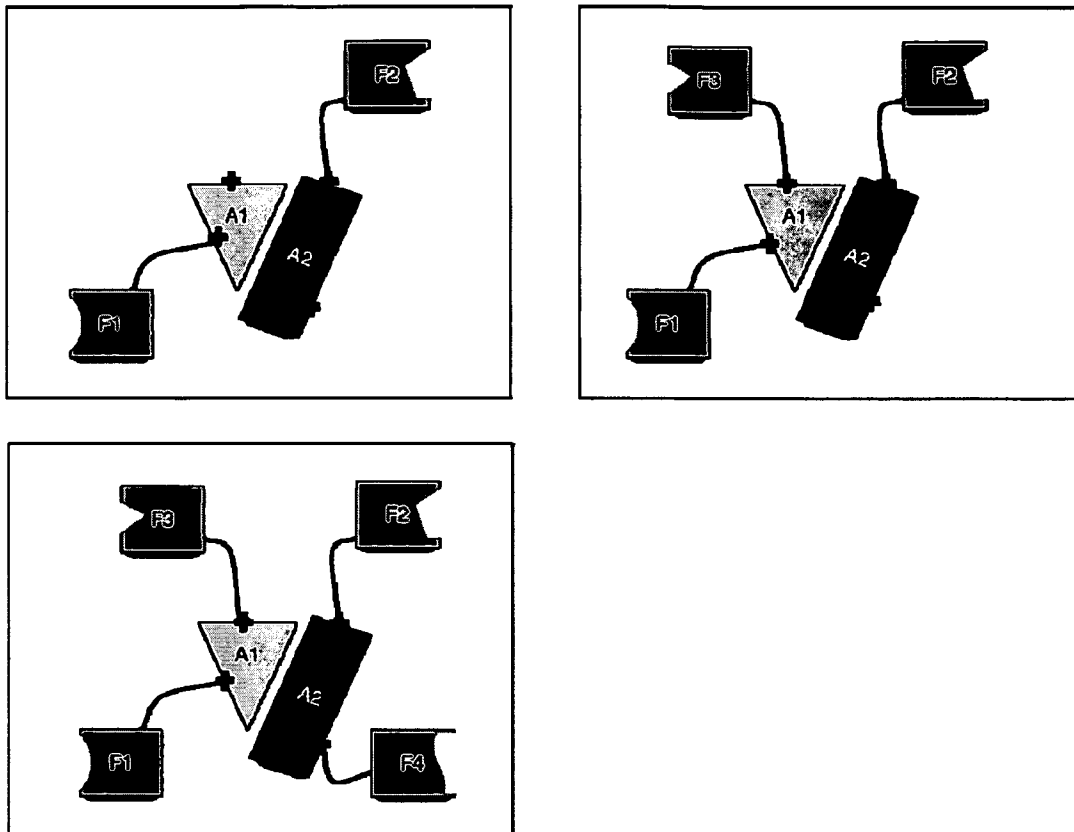


Figure 5

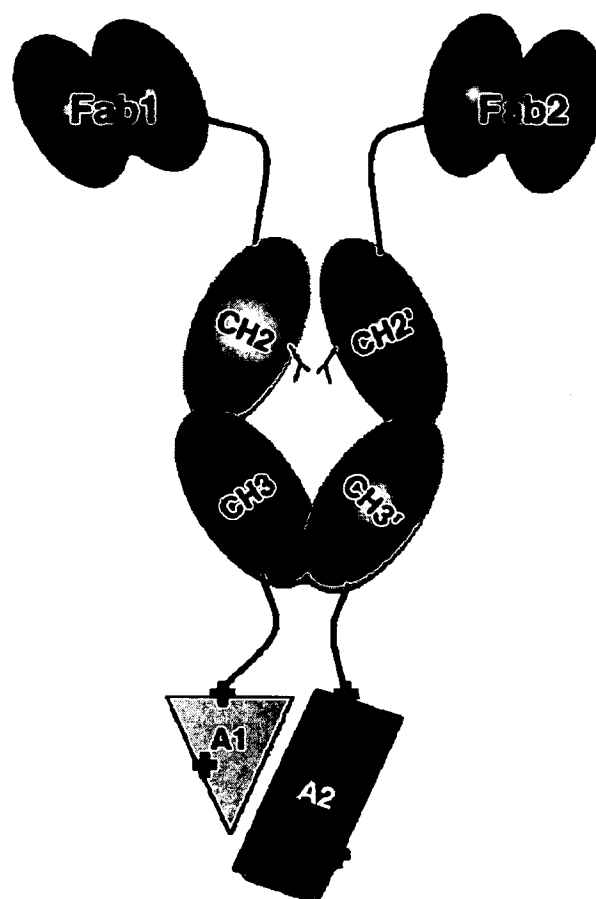


Figure 6A

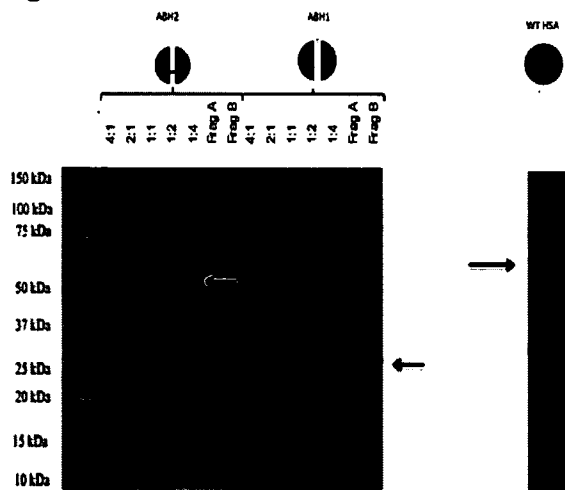


Figure 6B

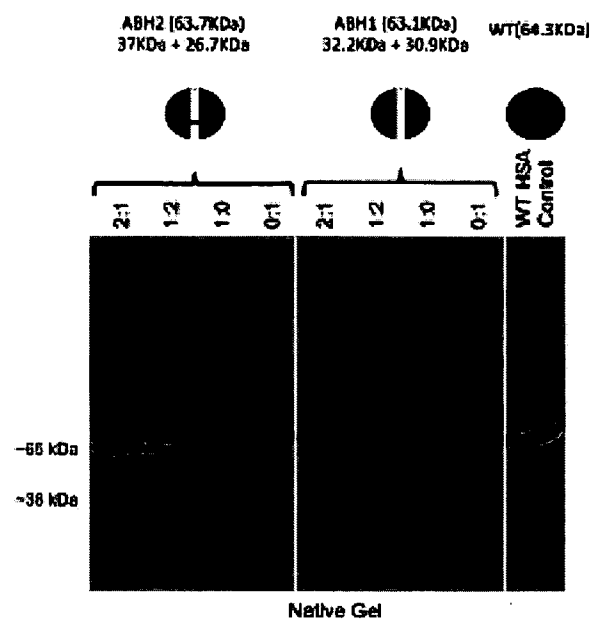


Figure 6C

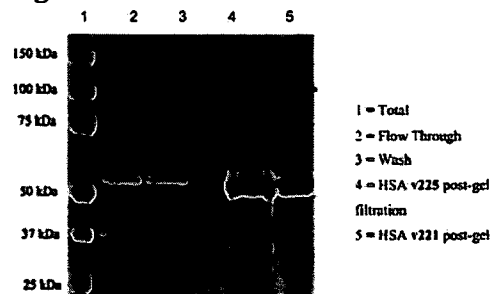


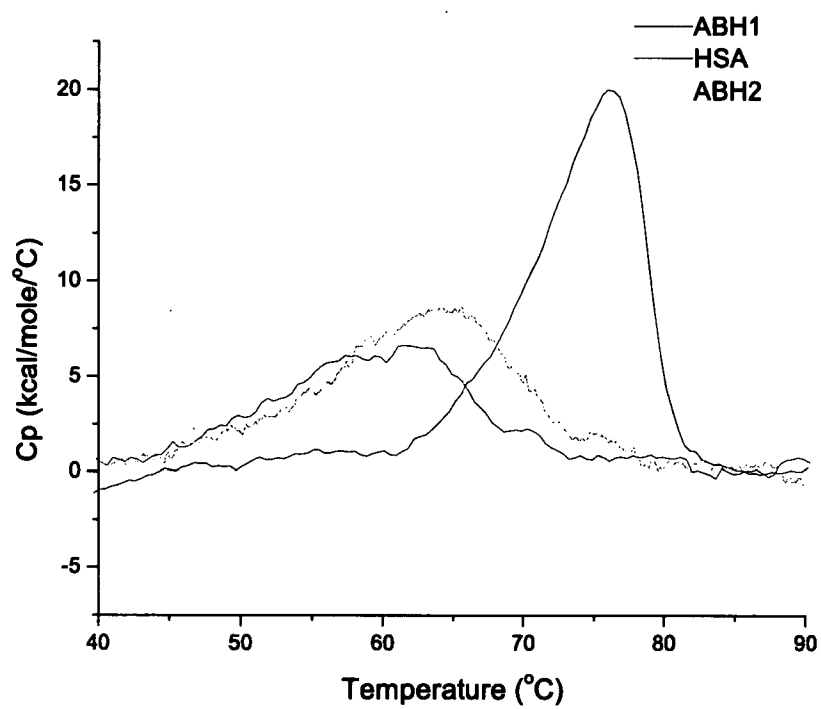
Figure 7

Figure 8A

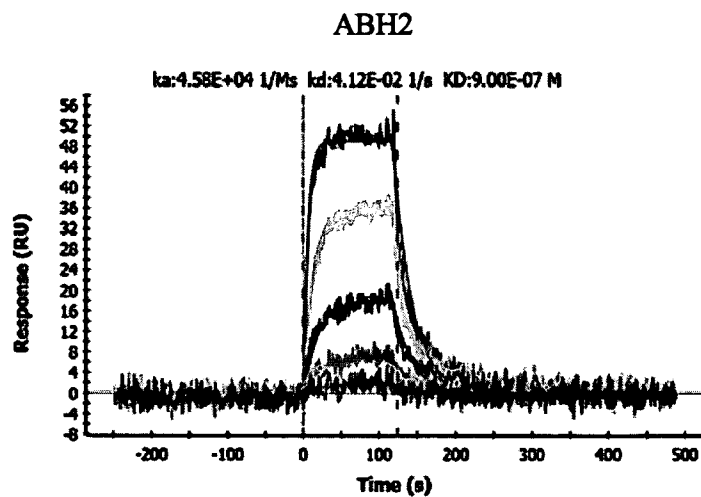


Figure 8B

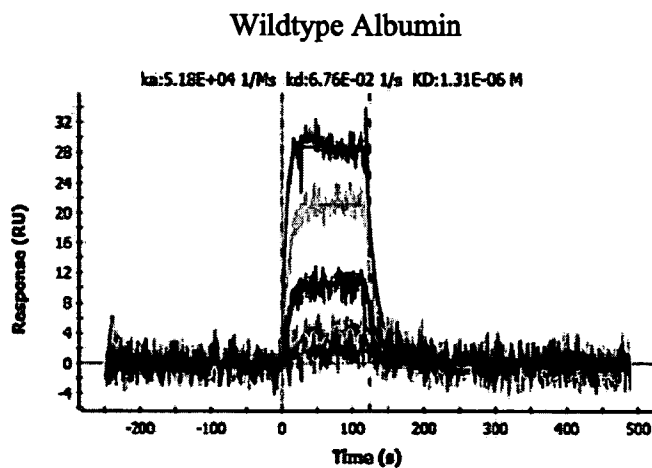


Figure 9

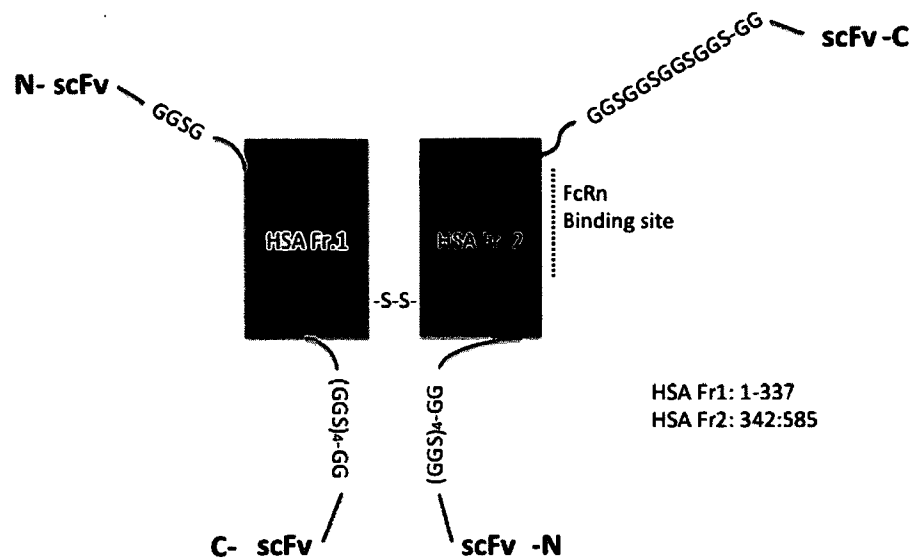


Figure 10A

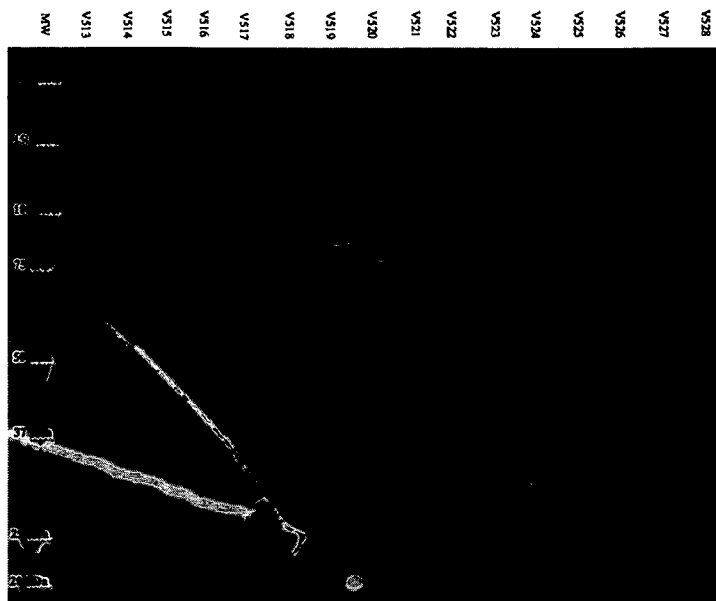


Figure 10B

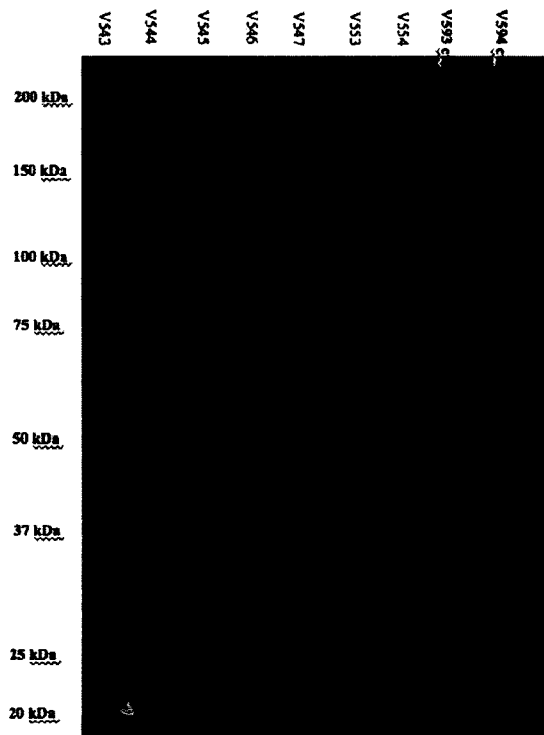
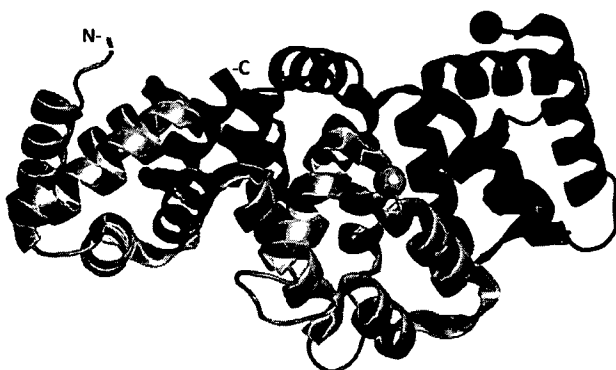


Figure 11

Annexin A1 (PDB-ID: 1MCX)



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

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

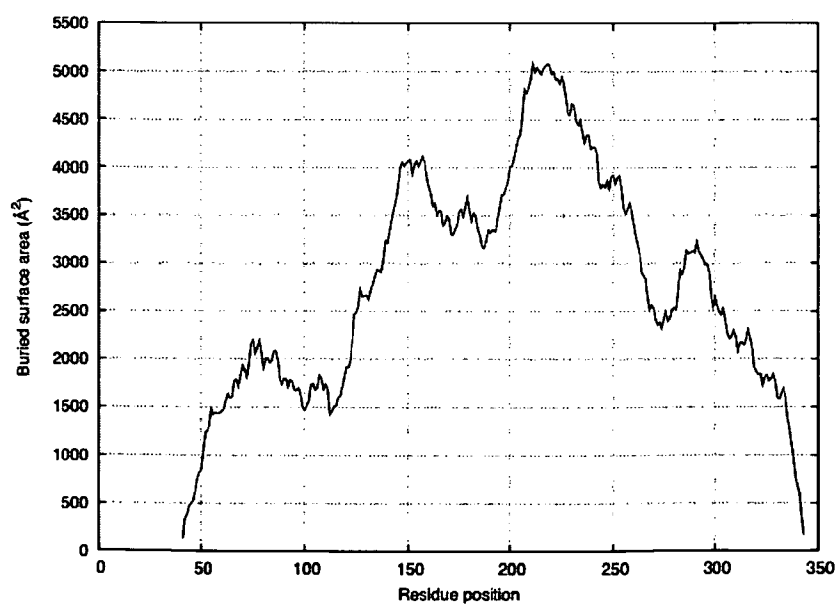
Figure 12

Figure 13

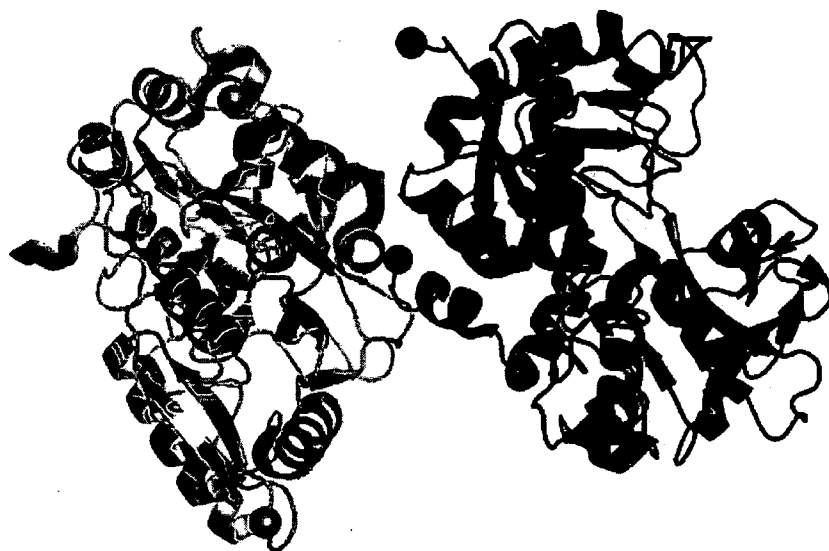
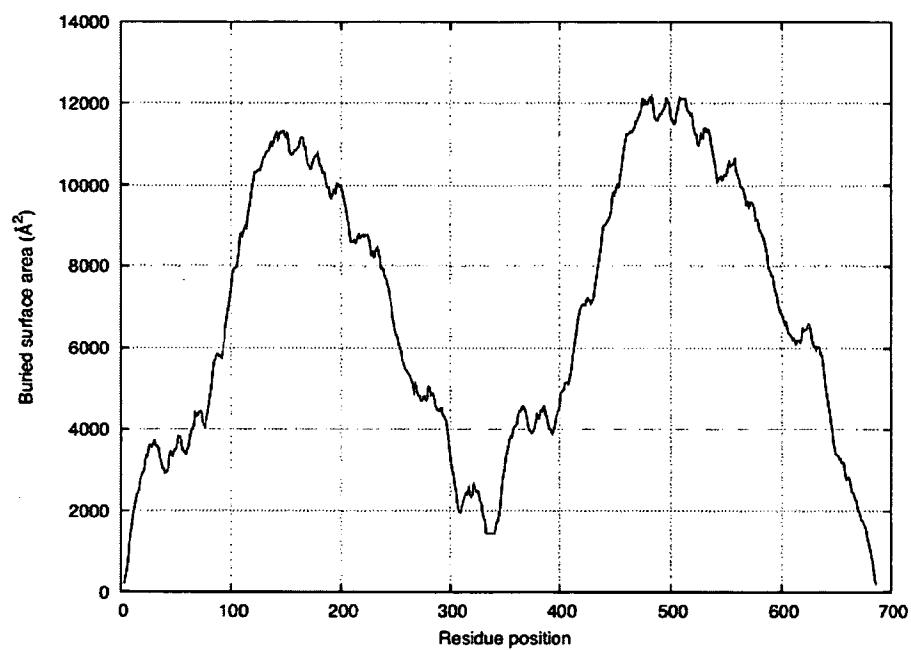
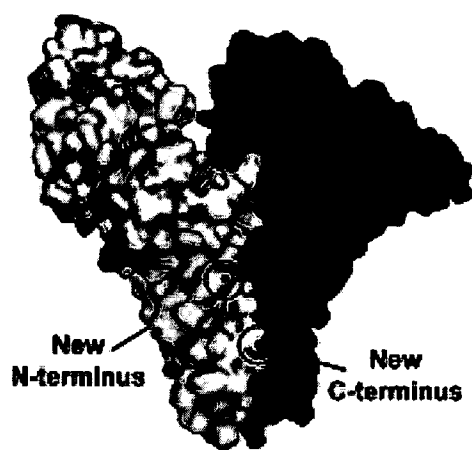


Figure 14

AlbuCORE_1**AlbuCORE_2****Figure 15A****Figure 15C****Figure 15B****Figure 15D**

AlbuCORE_3

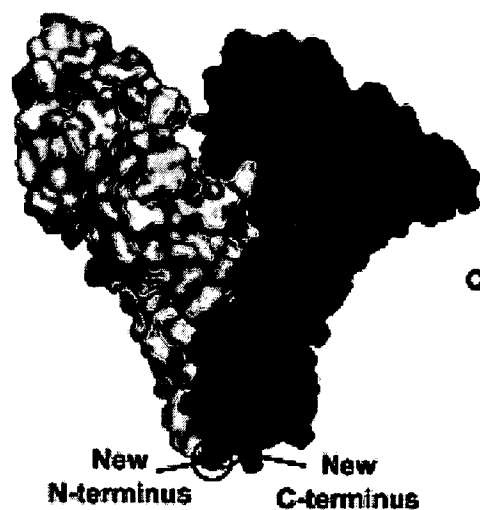


Figure 15E

AlbuCORE_4

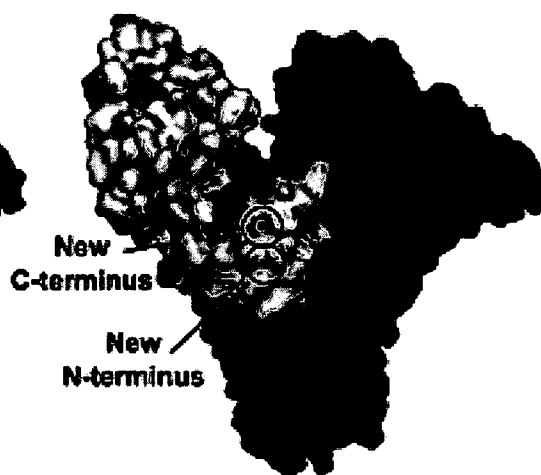


Figure 15G



Figure 15F

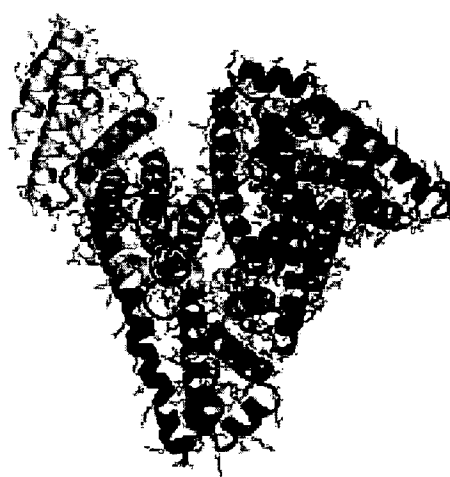


Figure 15H

AlbuCORE_6

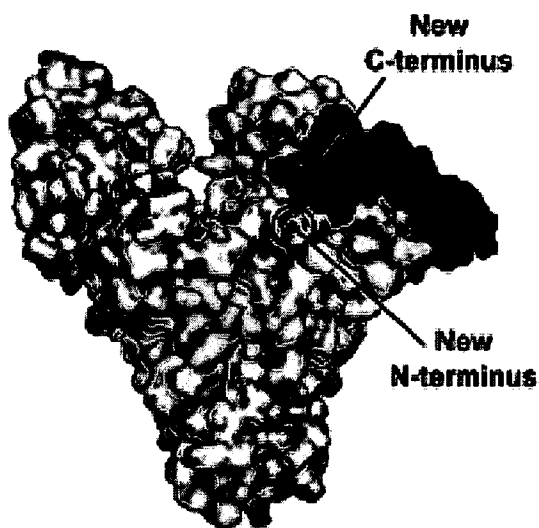


Figure 15I

AlbuCORE_7

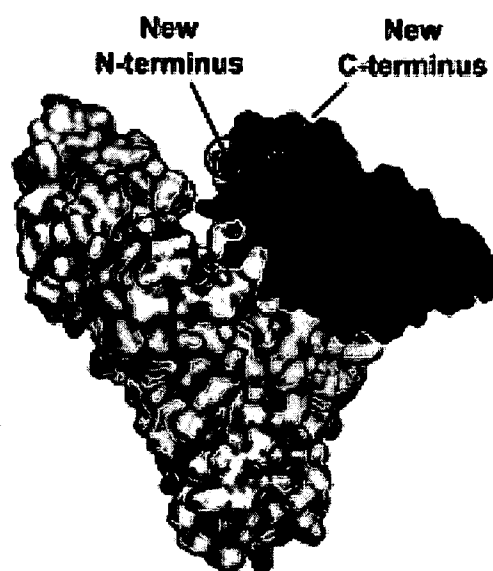


Figure 15K



Figure 15J



Figure 15L

AlbuCORE_9

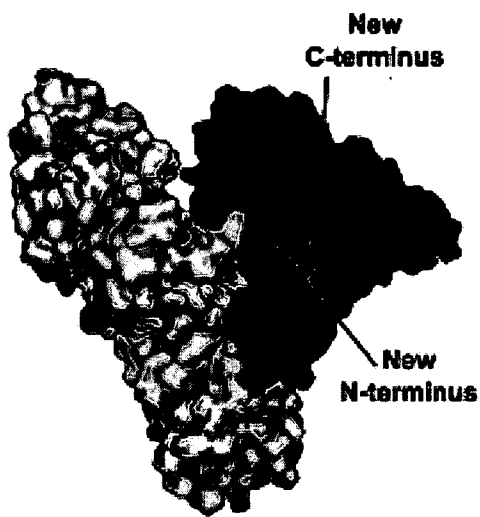


Figure 15M

AlbuCORE_13

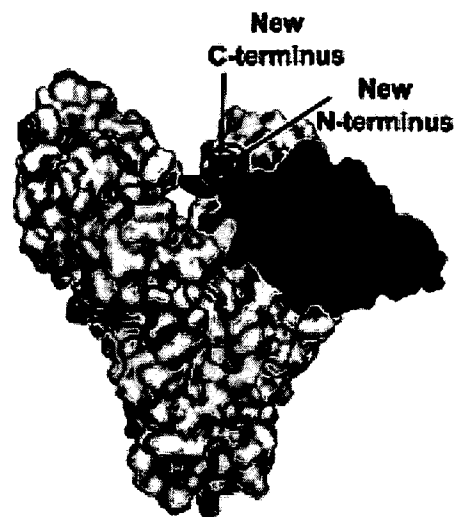


Figure 15O



Figure 15N

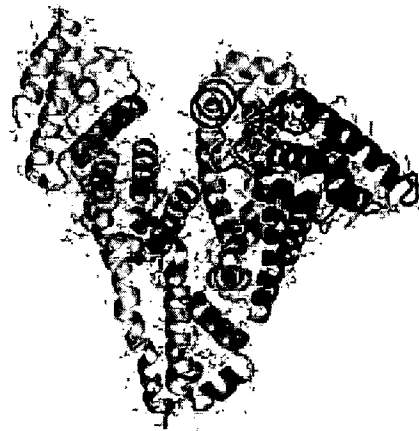


Figure 15P

Figure 16A
ABH2

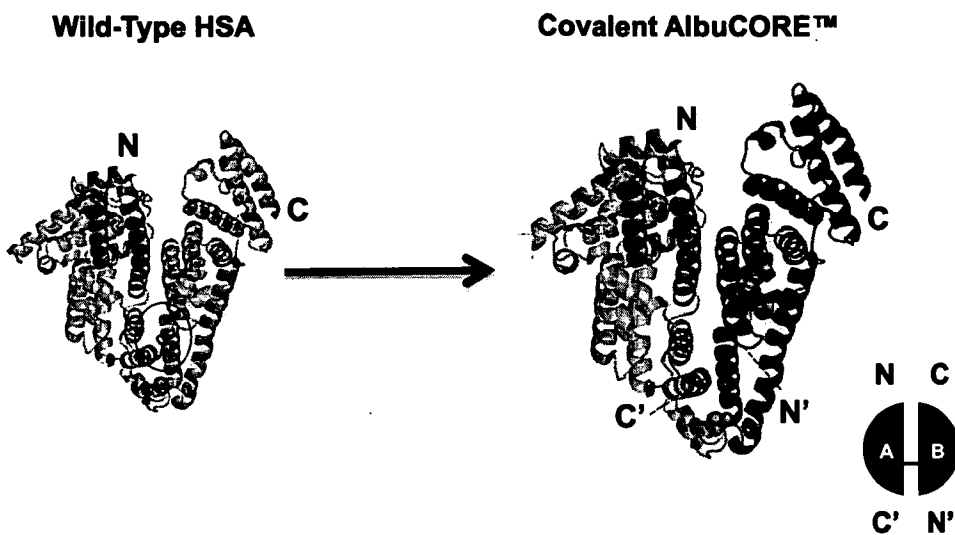


Figure 16B
ABH1

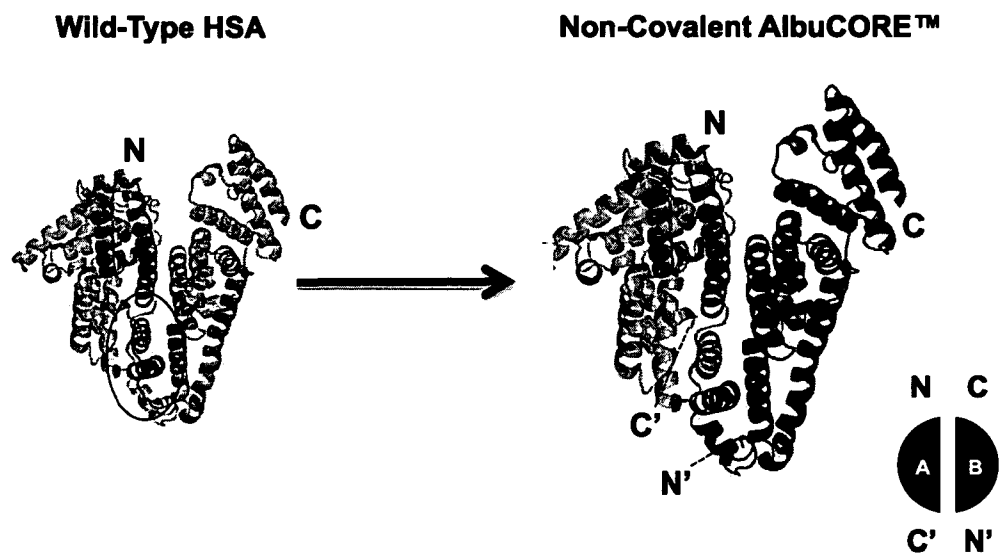
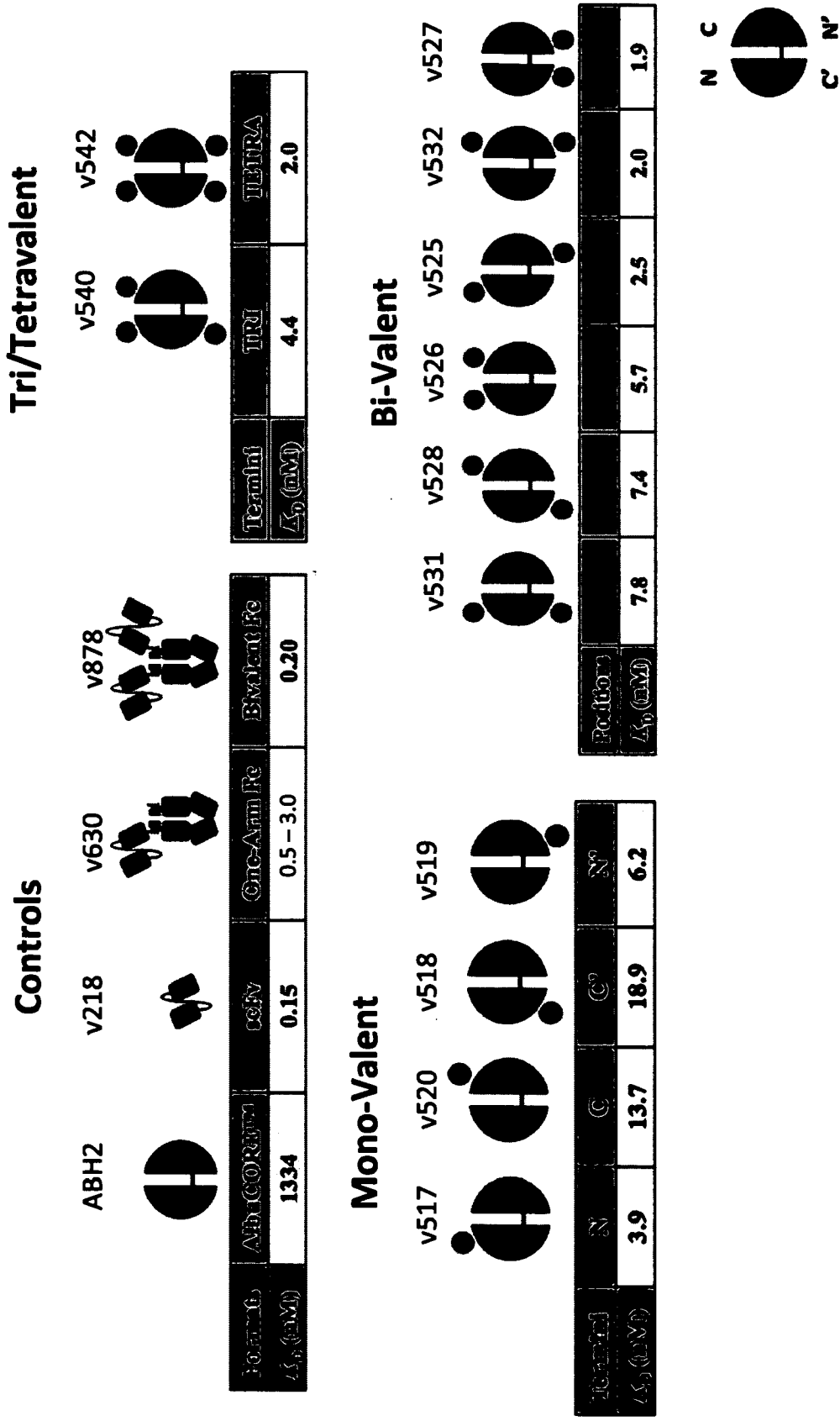


Figure 17



Mono-Valent

v517



v520



v518



v519



N	C	C'	N'	
ΔG (mM)	3.9	13.7	18.9	6.2

Bi-Valent

v531



v528



v526



v525



v532



v527



Position						
ΔG (mM)	7.8	7.4	5.7	2.5	2.0	1.9

N

C



C'

N'

Figure 18A

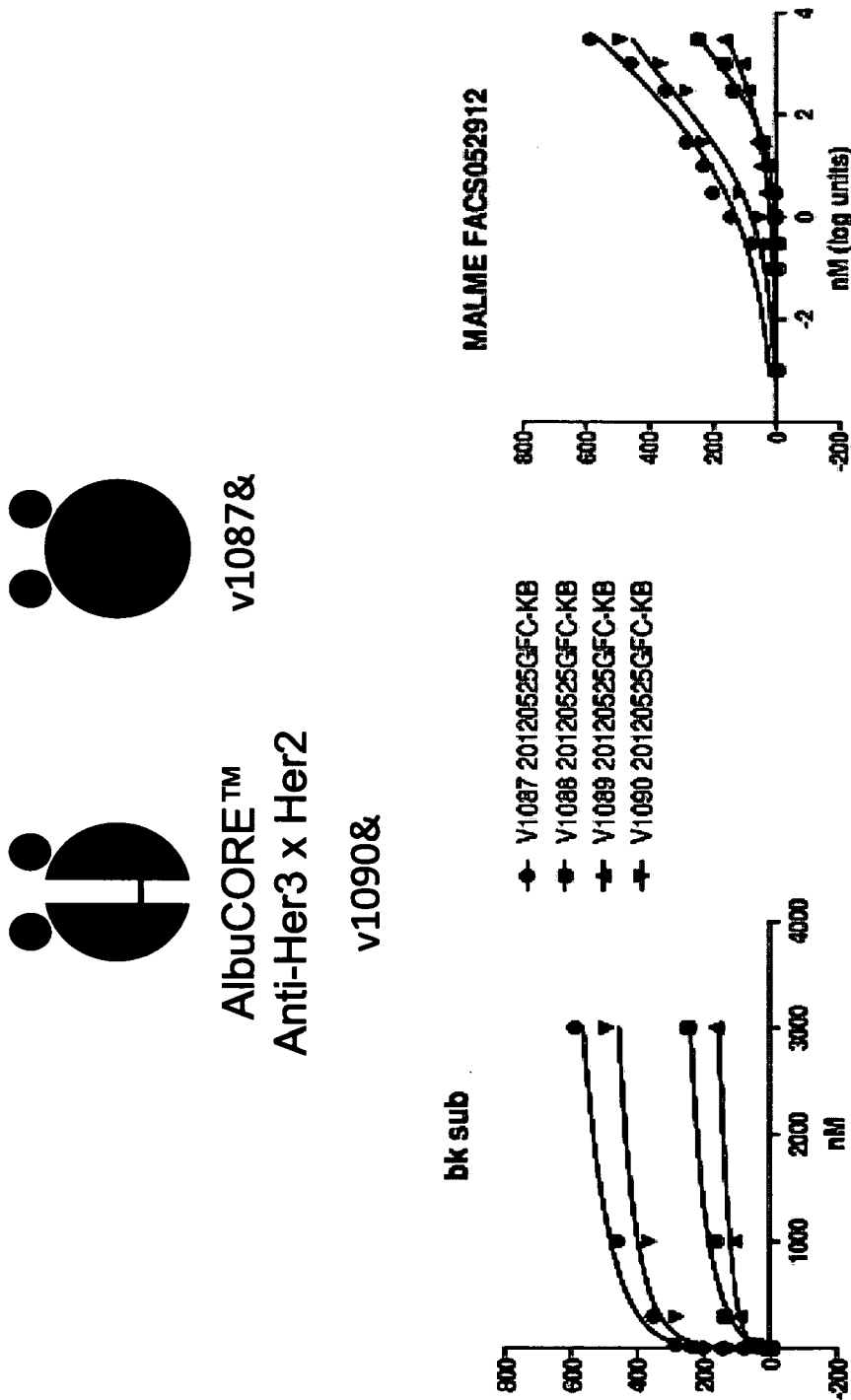


Figure 18B

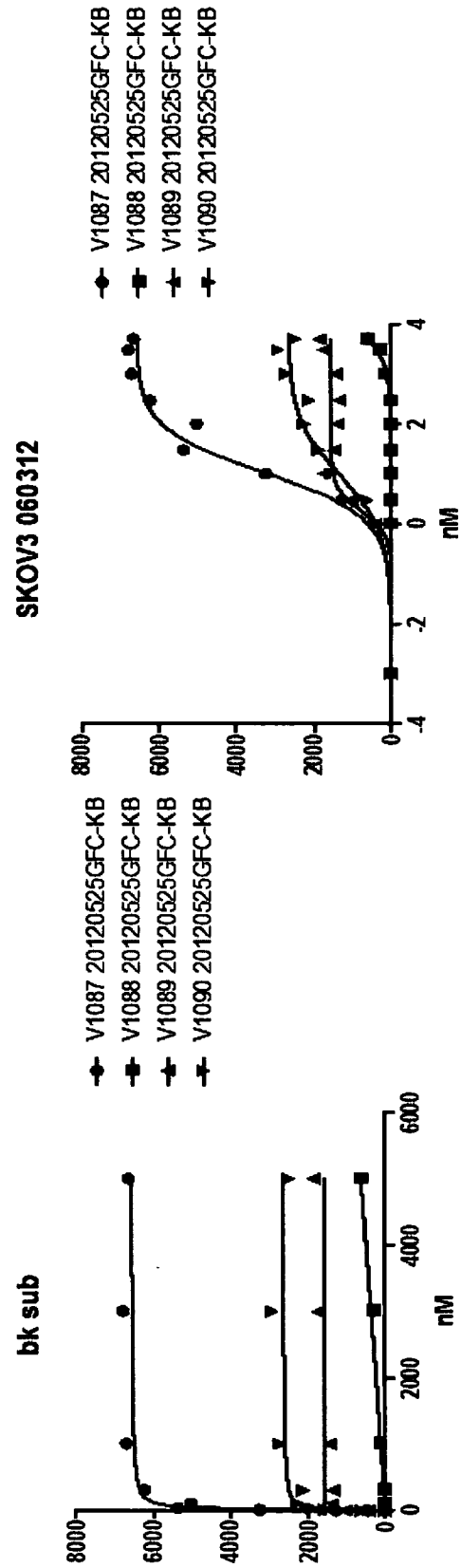
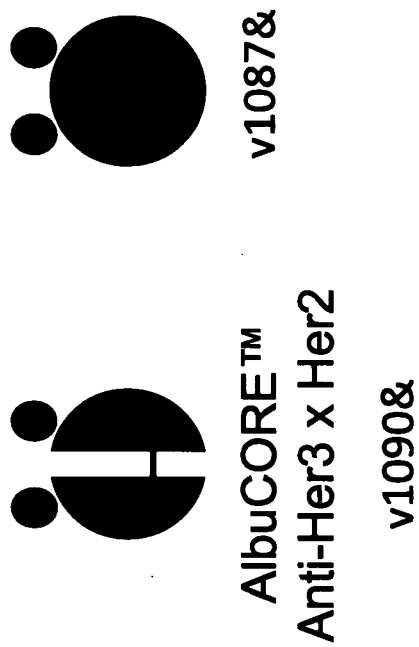


Figure 19A

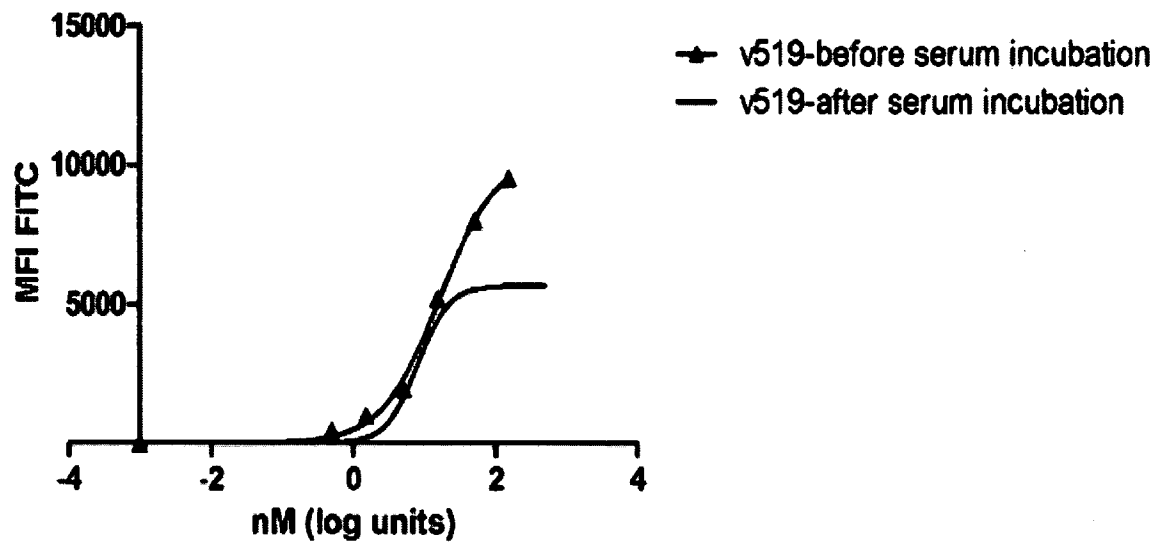


Figure 19B

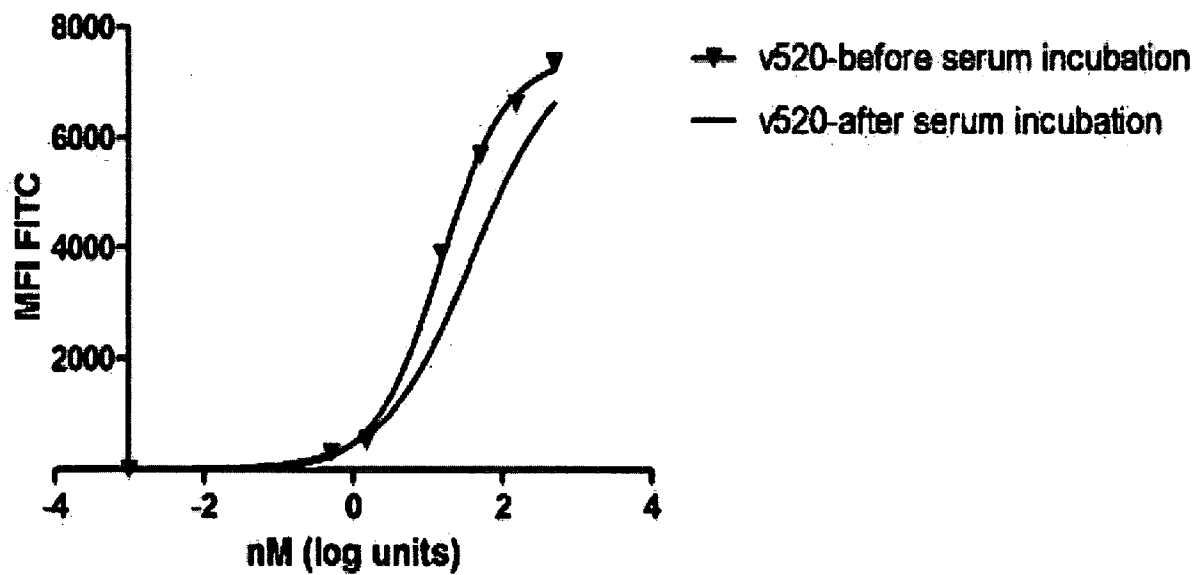


Figure 19C

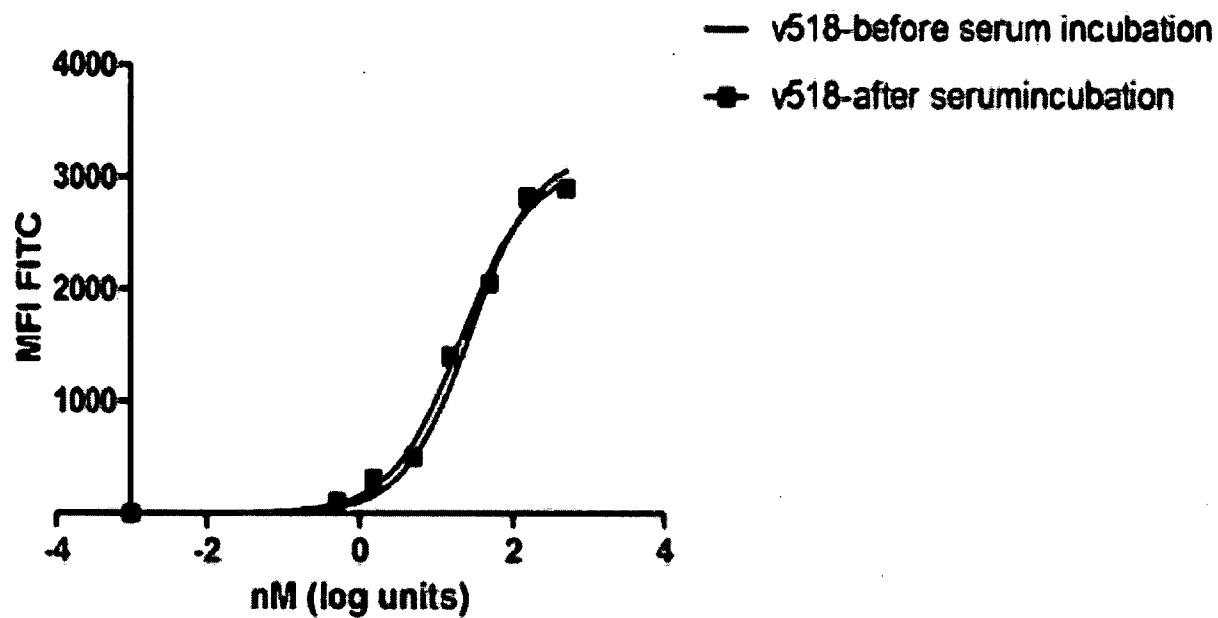


Figure 19D

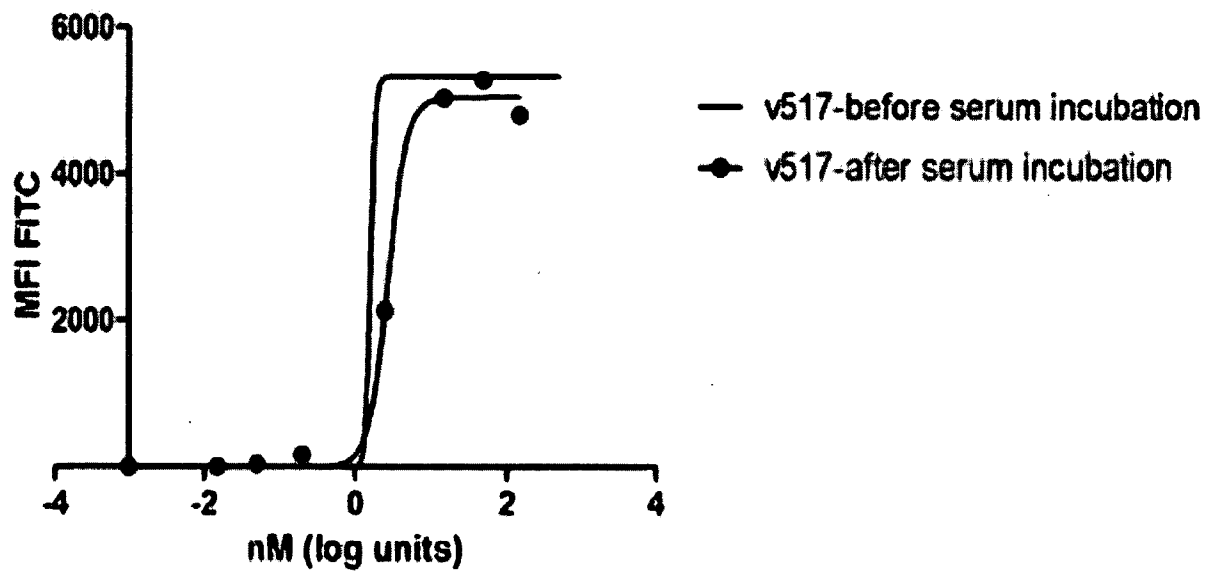


Figure 20A

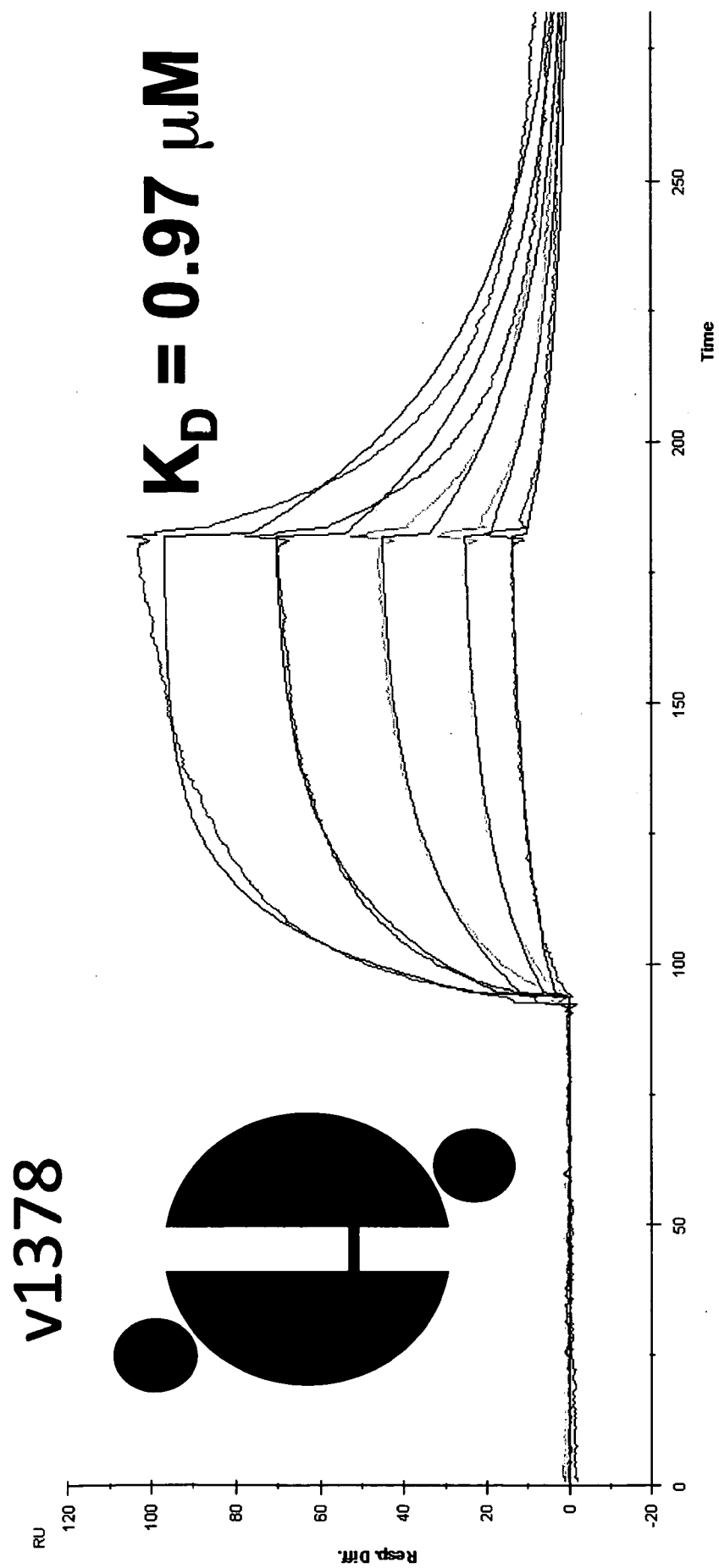


Figure 20B

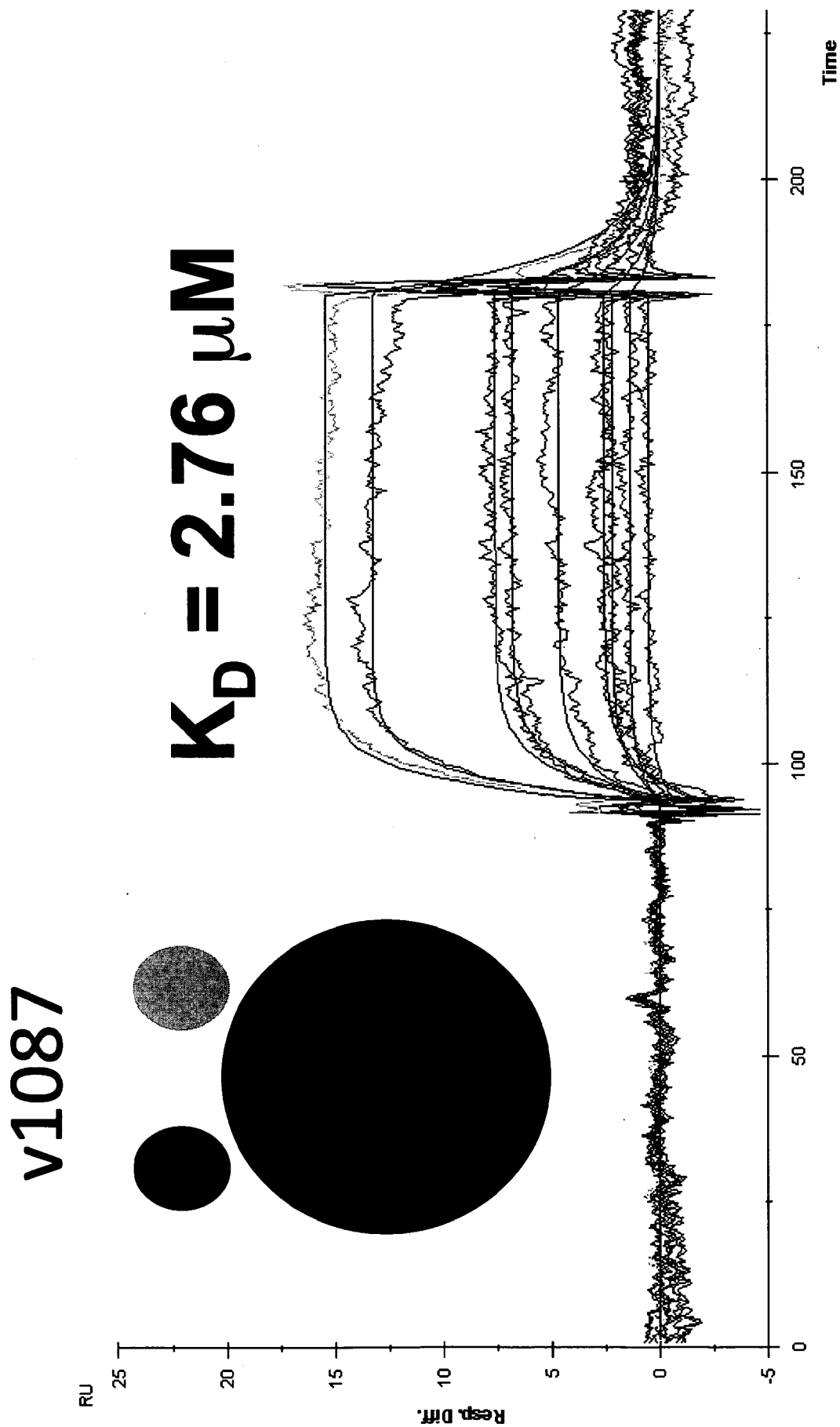
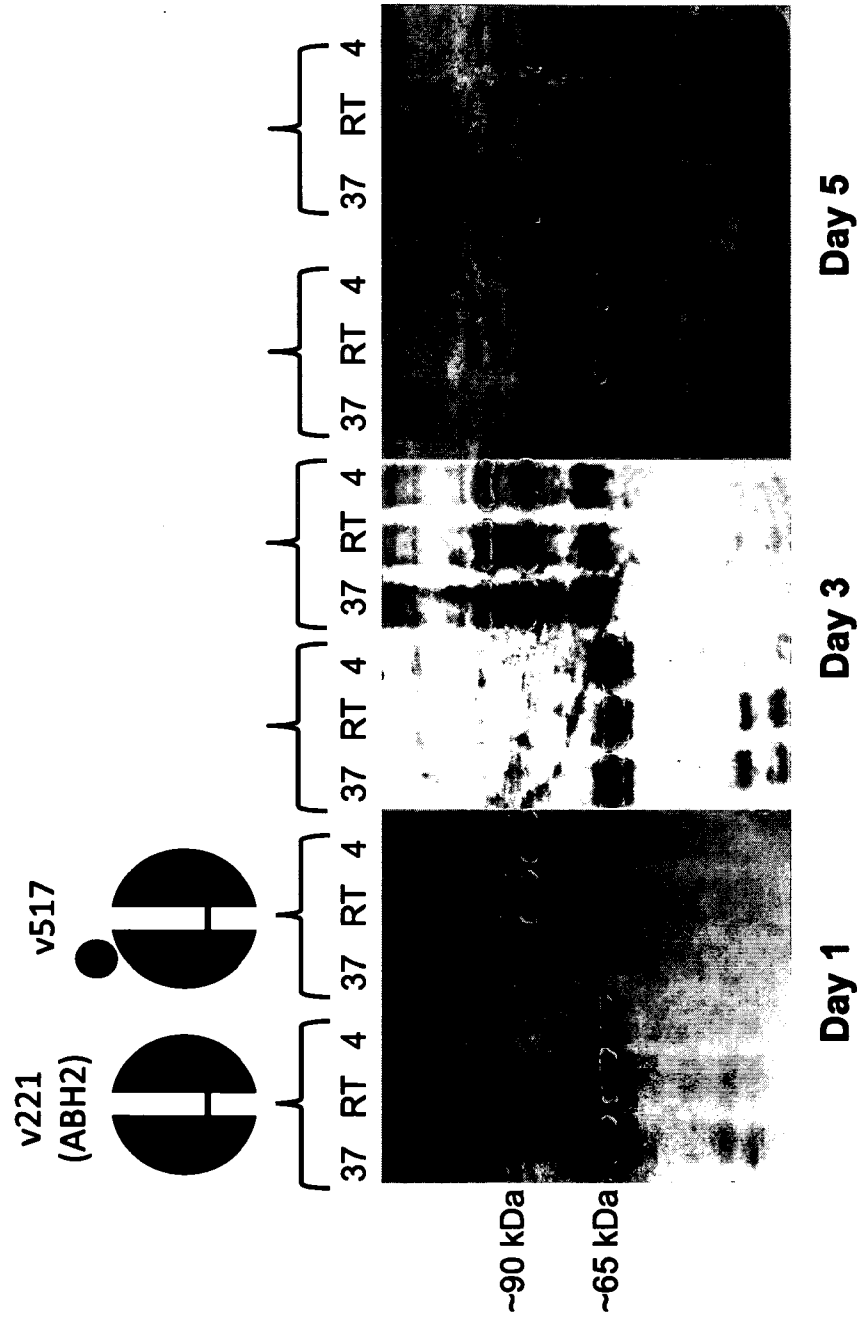


Figure 21



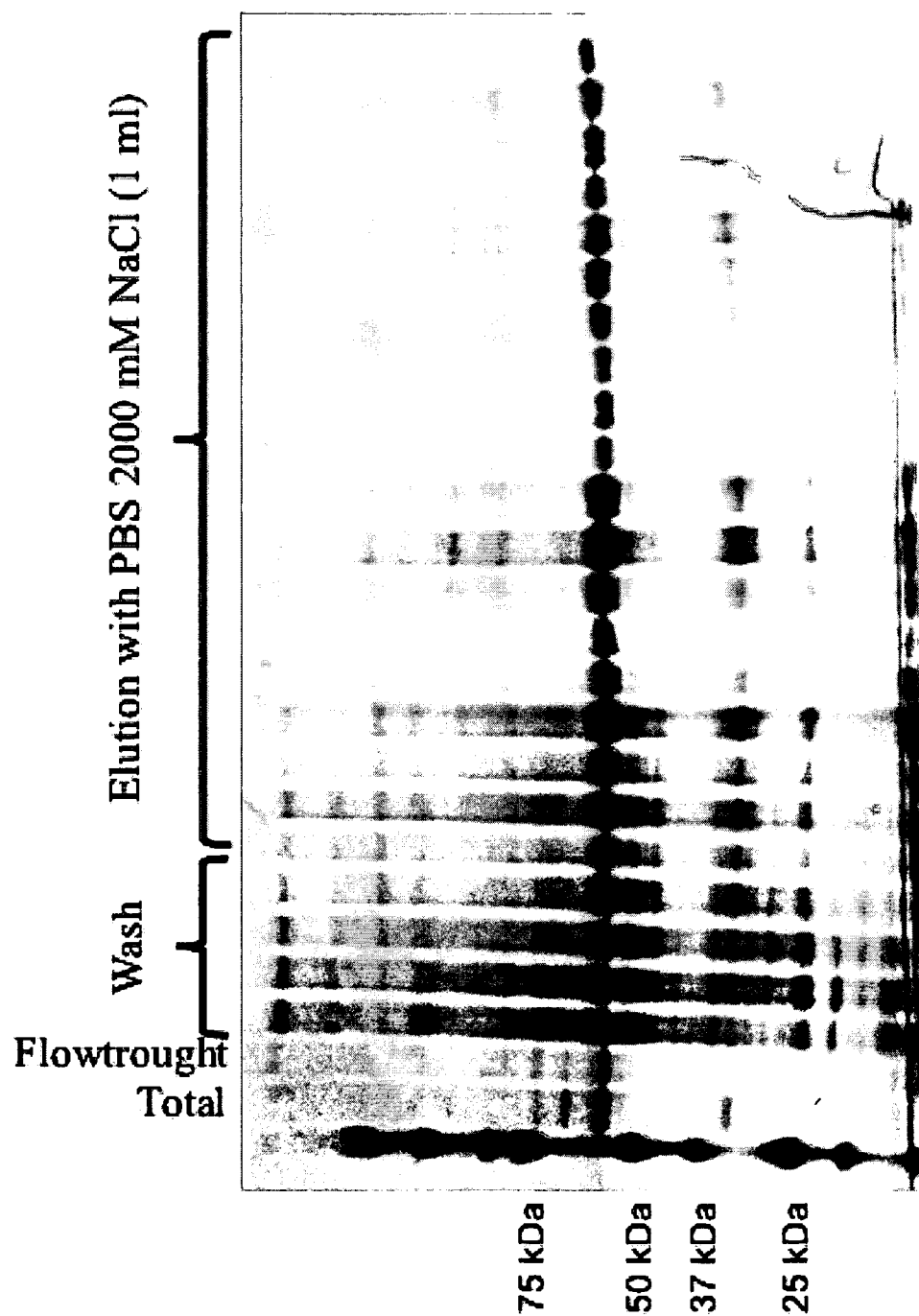


Figure 22A

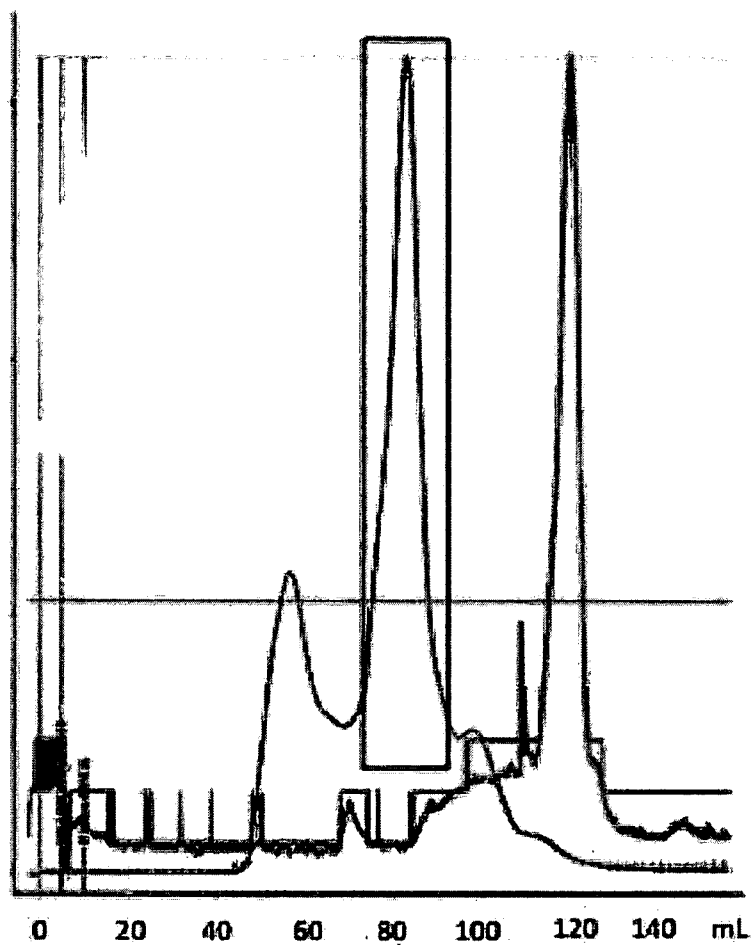
Figure 22B**POST-BLUE SEPHAROSE**

Figure 22C

Post AlbuPure®

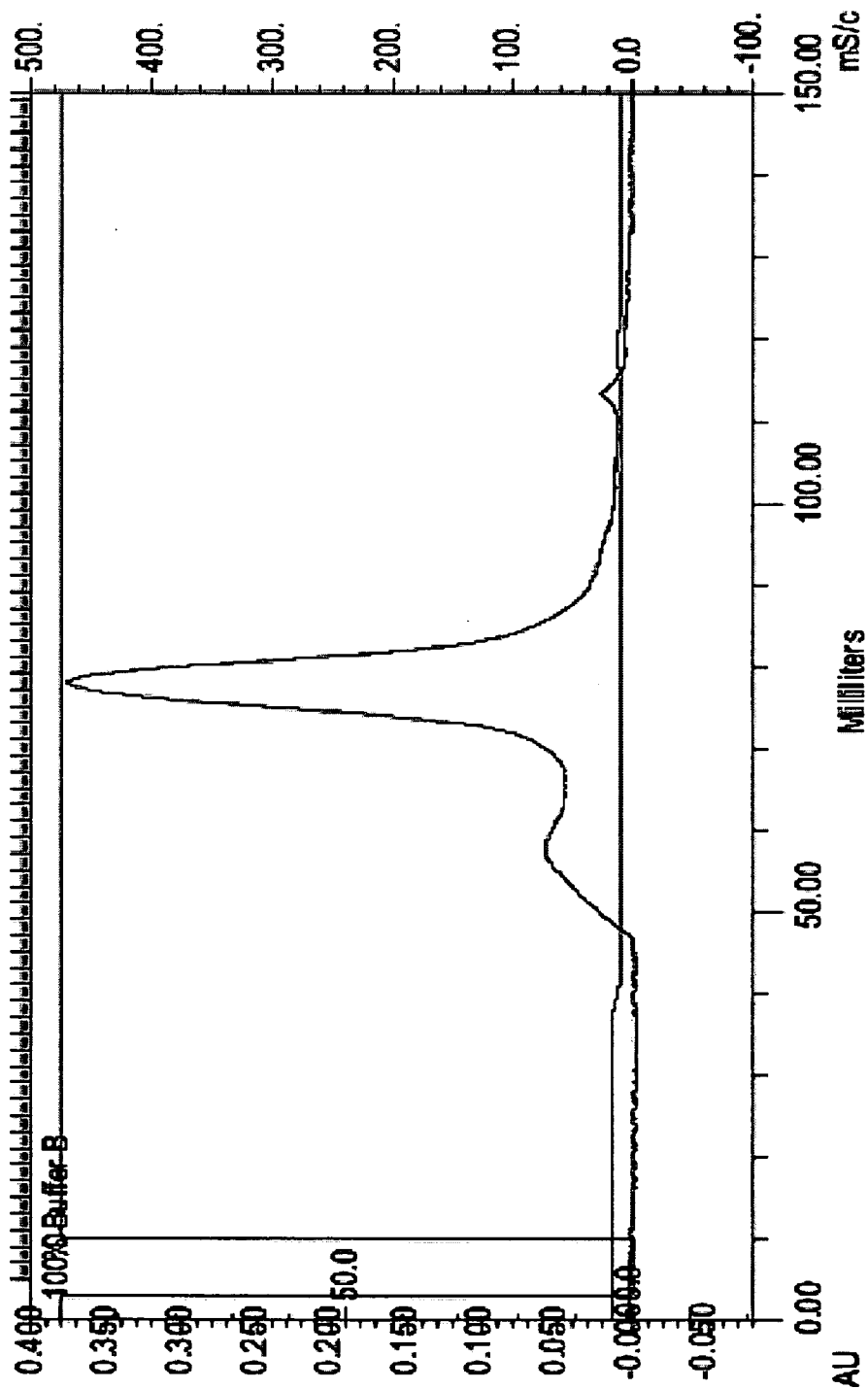


Figure 23A

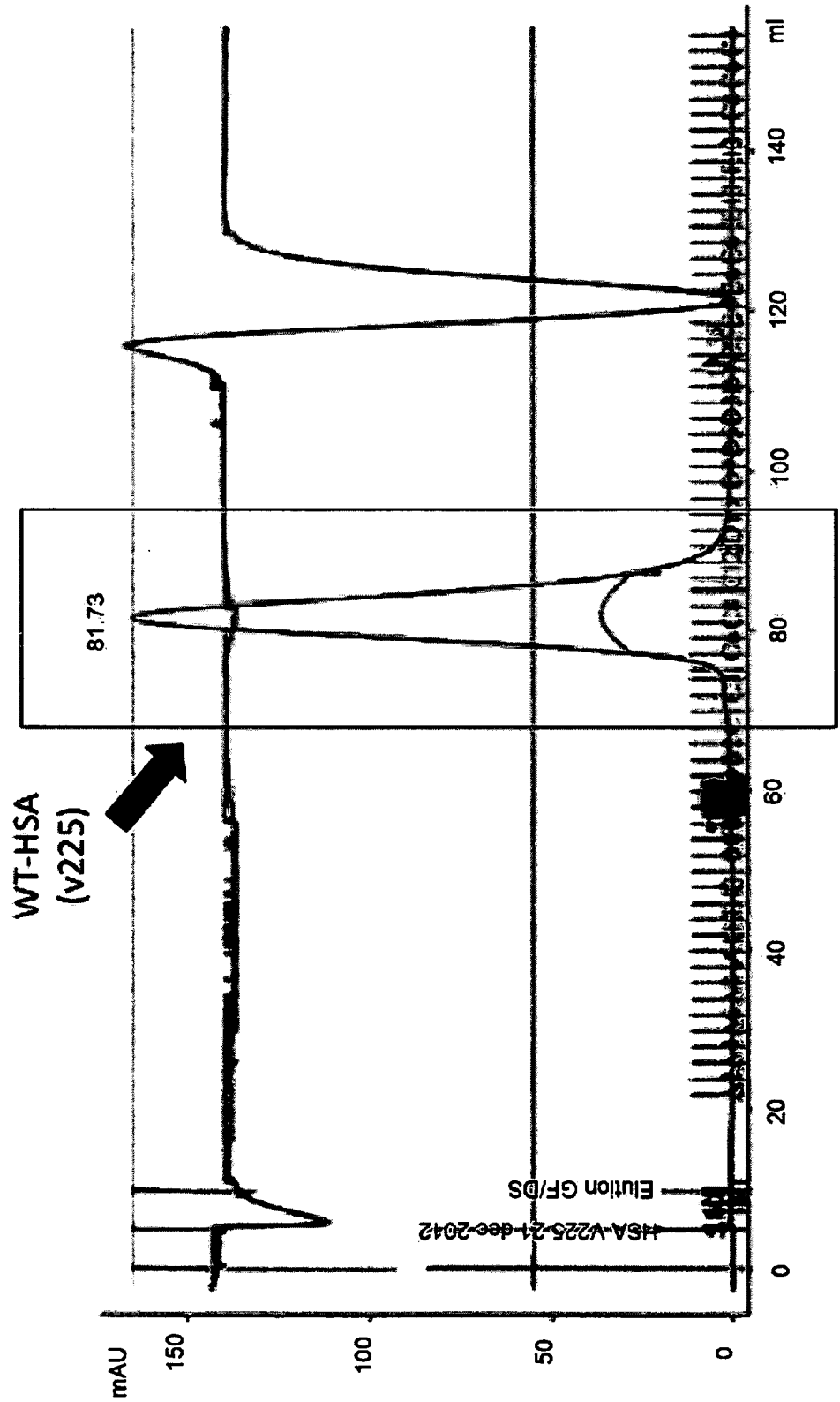


Figure 23B

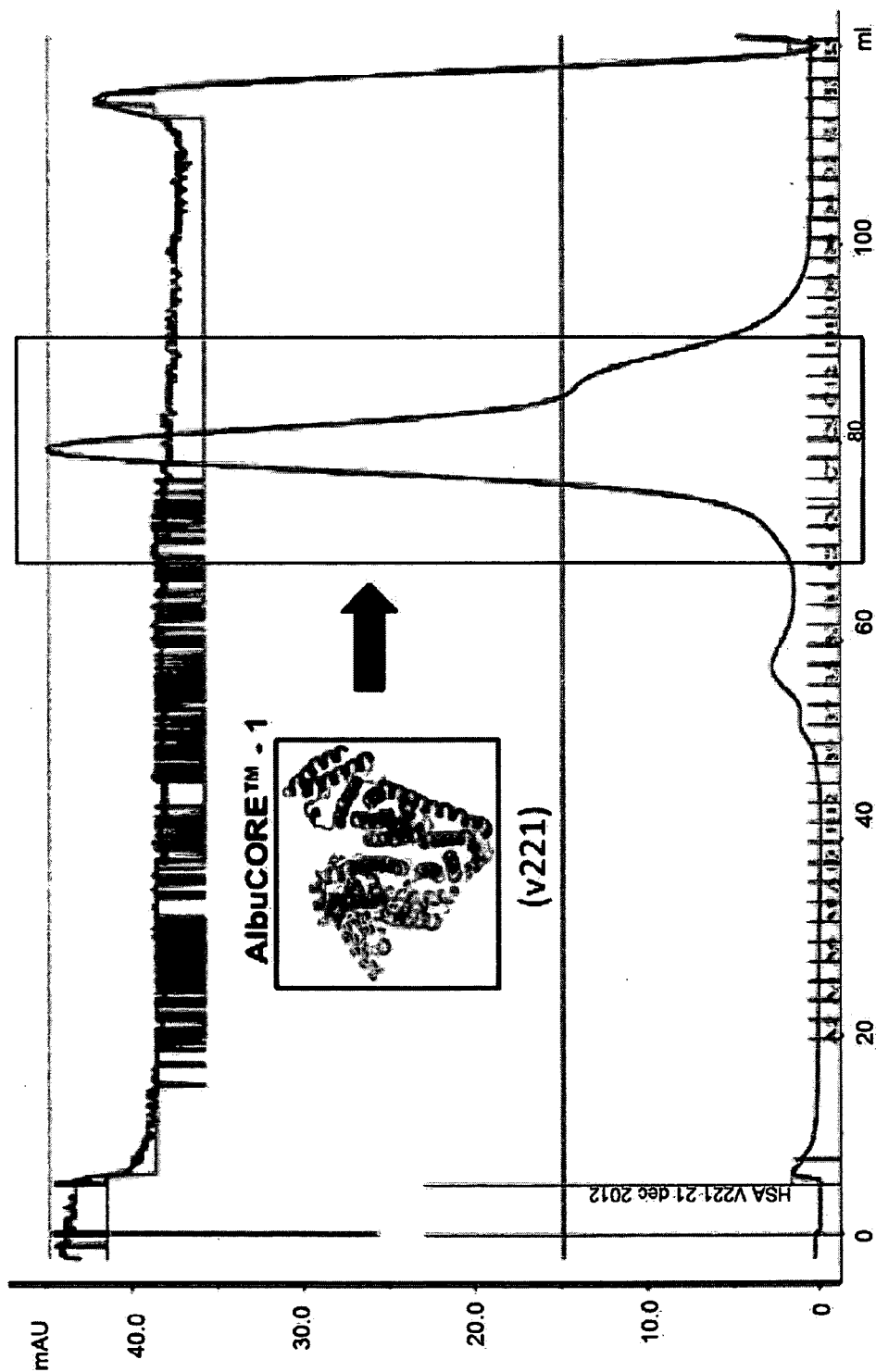


Figure 23C

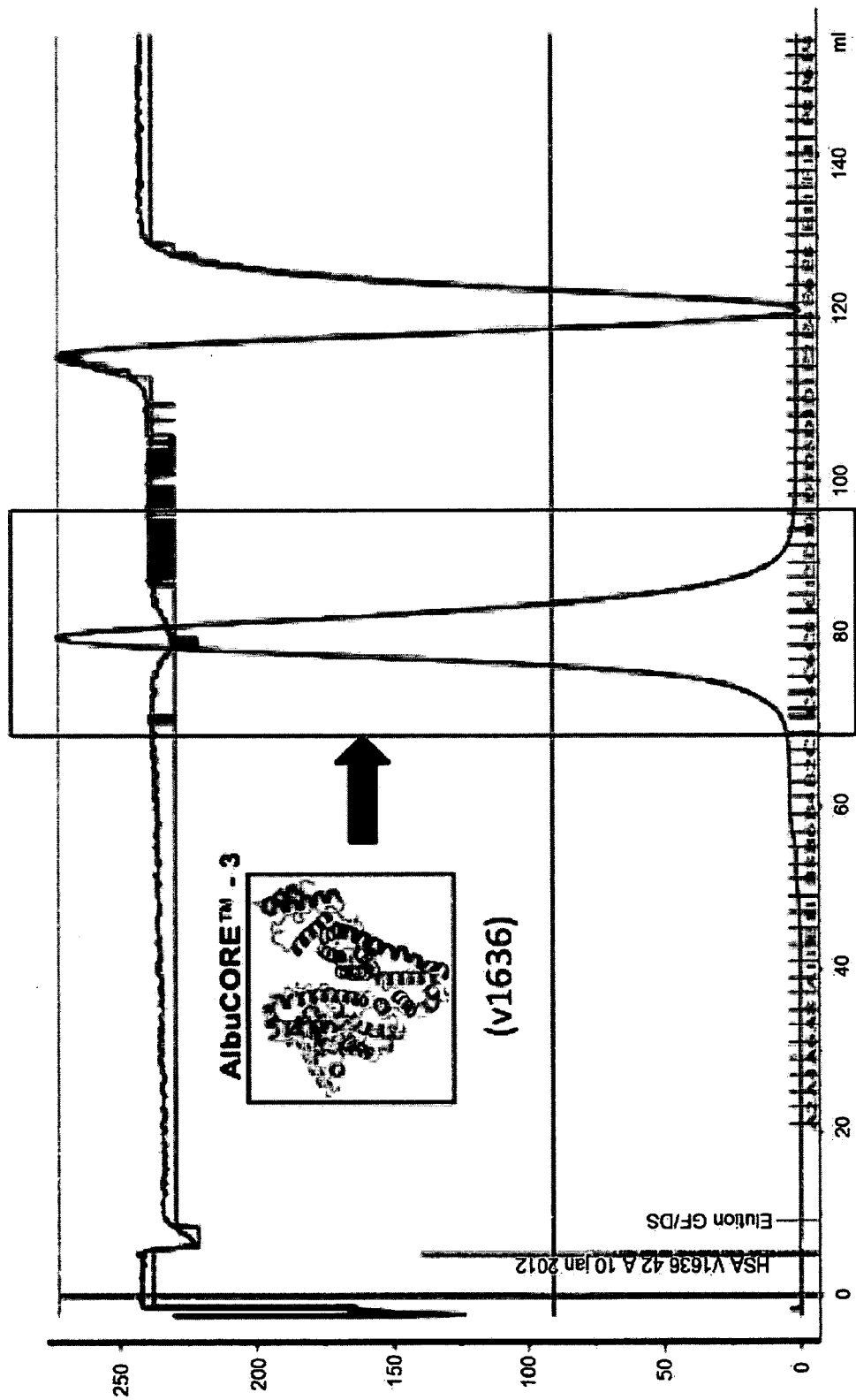


Figure 23D

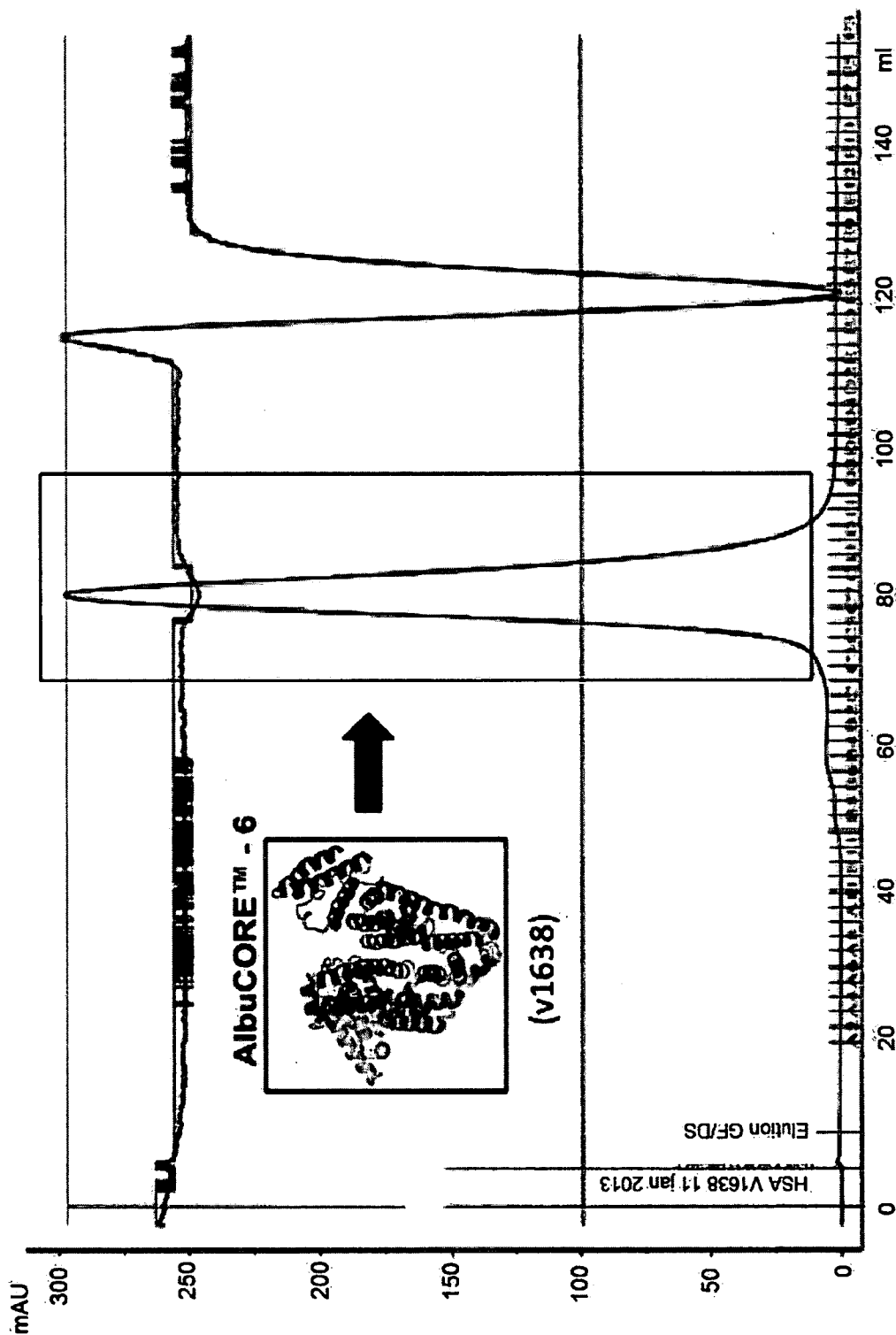


Figure 23E

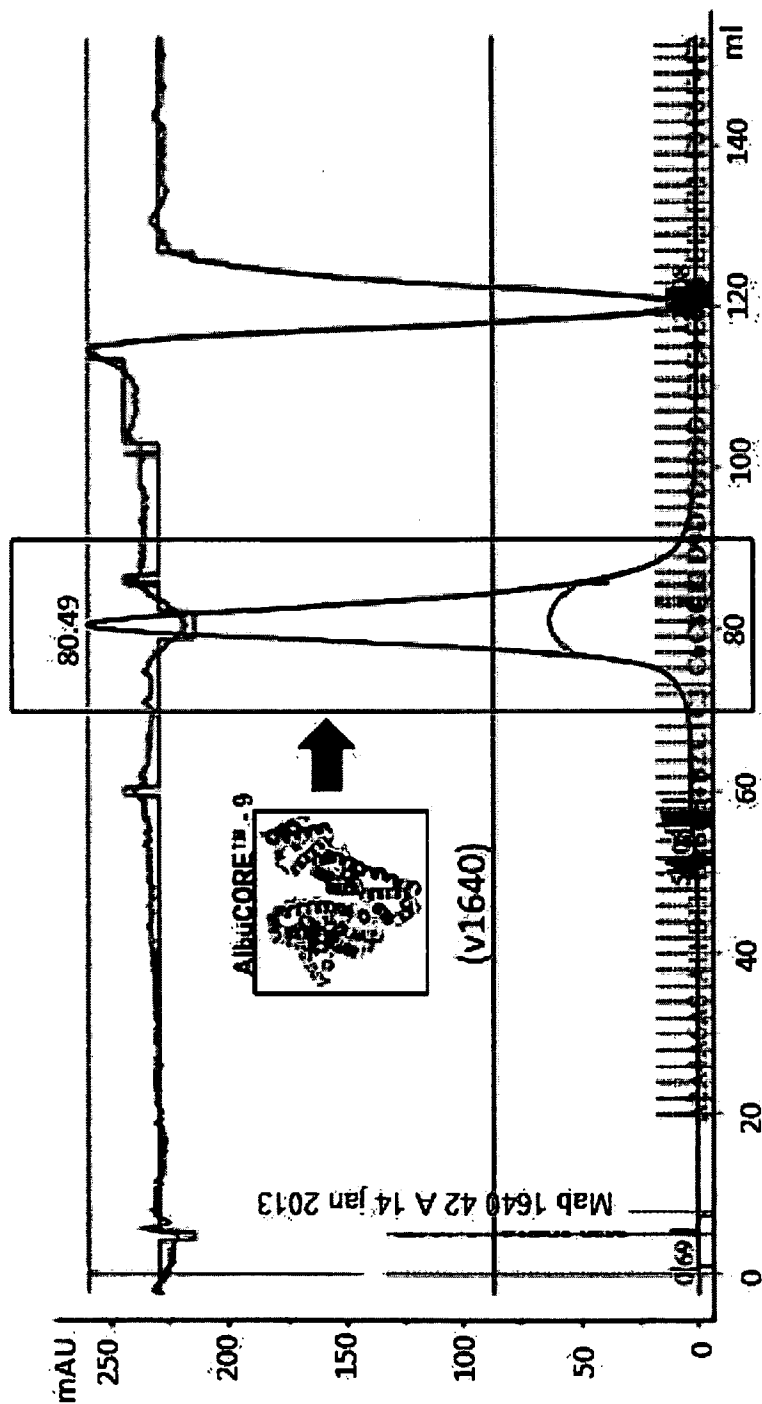


Figure 24A

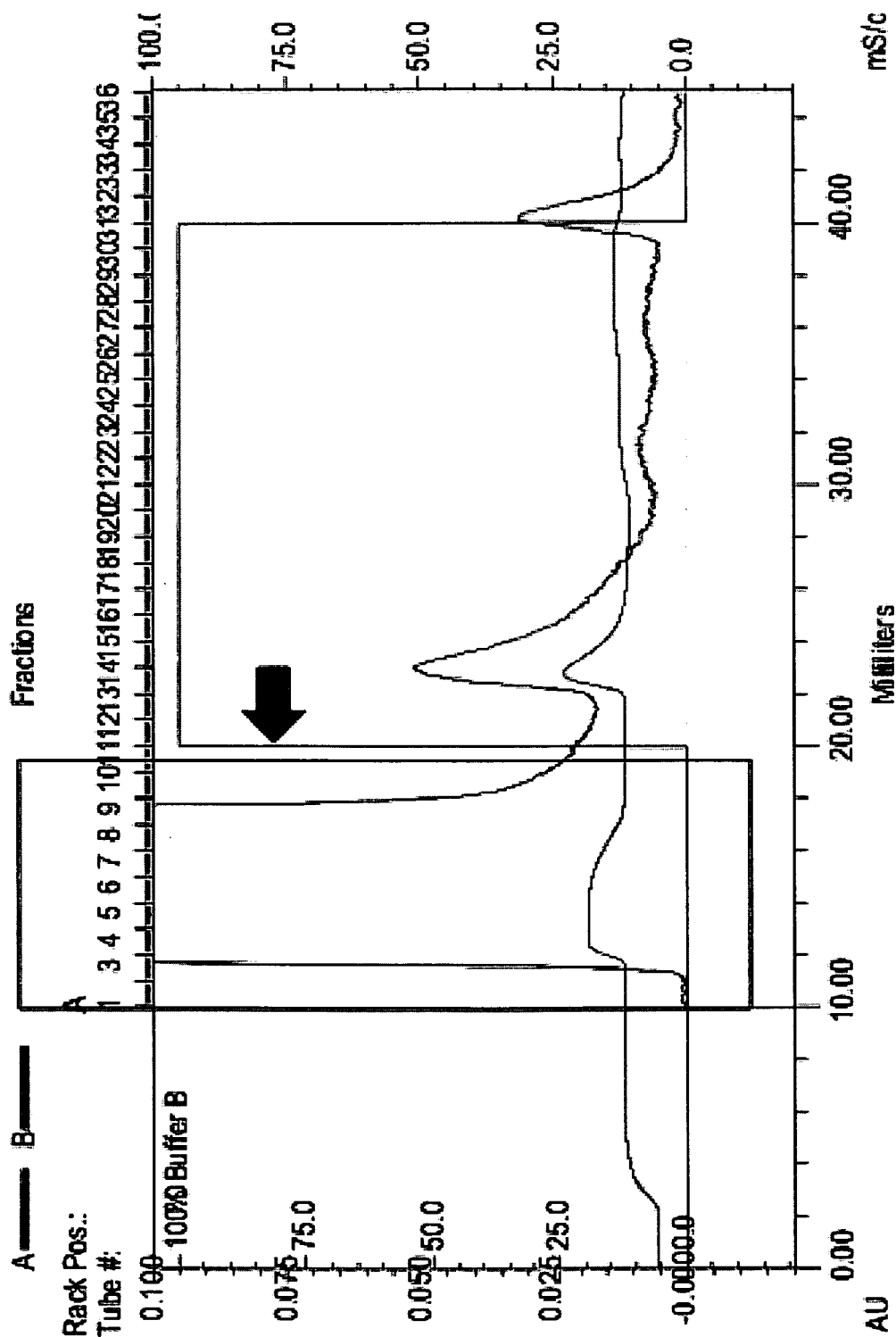


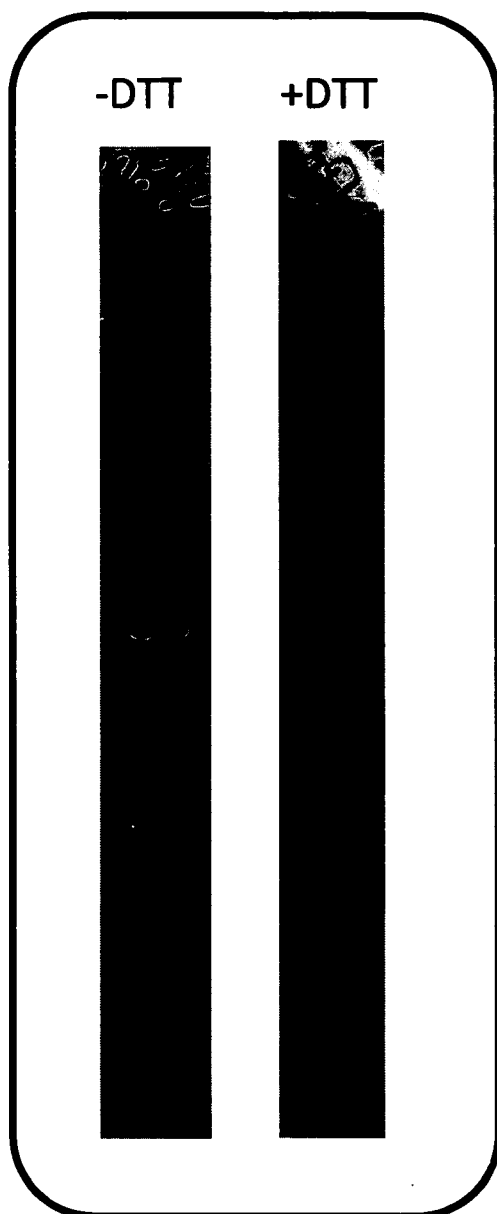
Figure 24B

Figure 25A

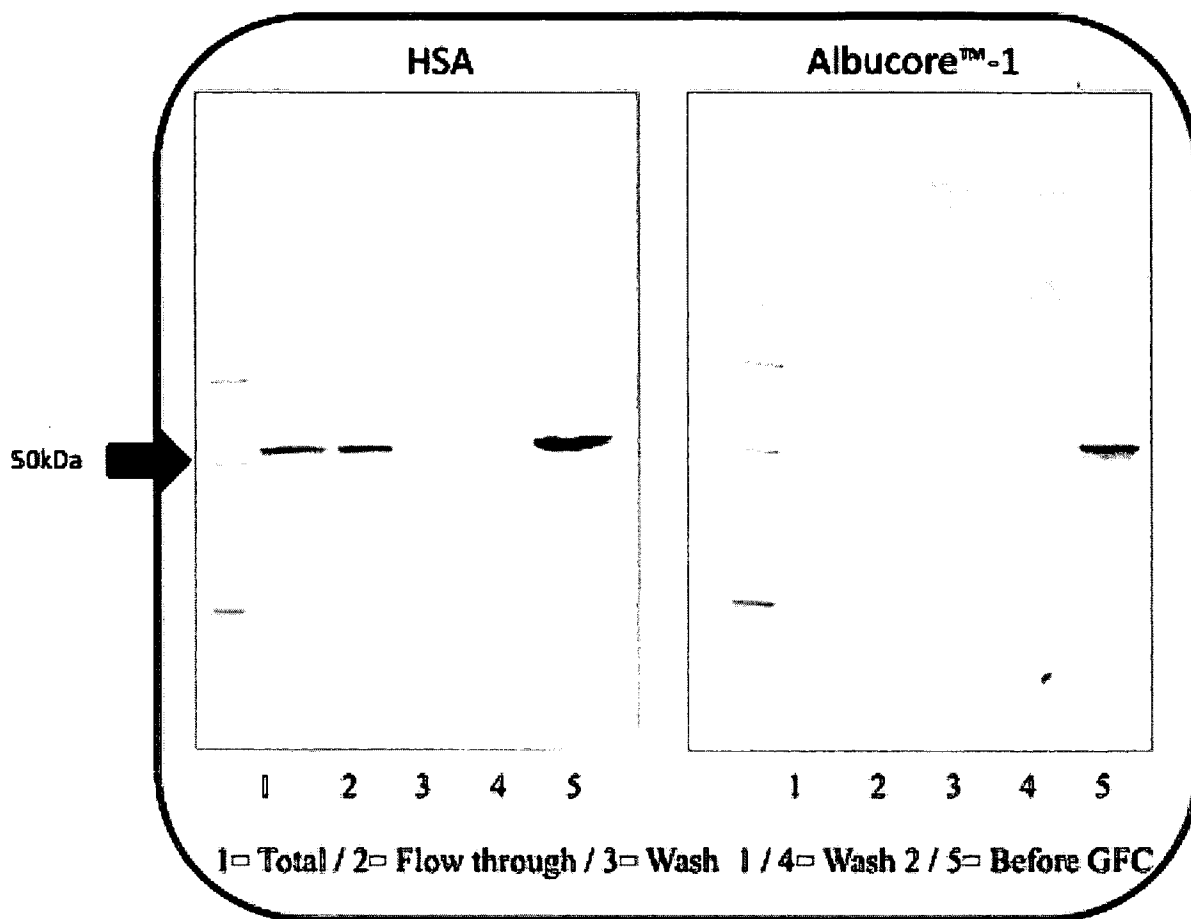
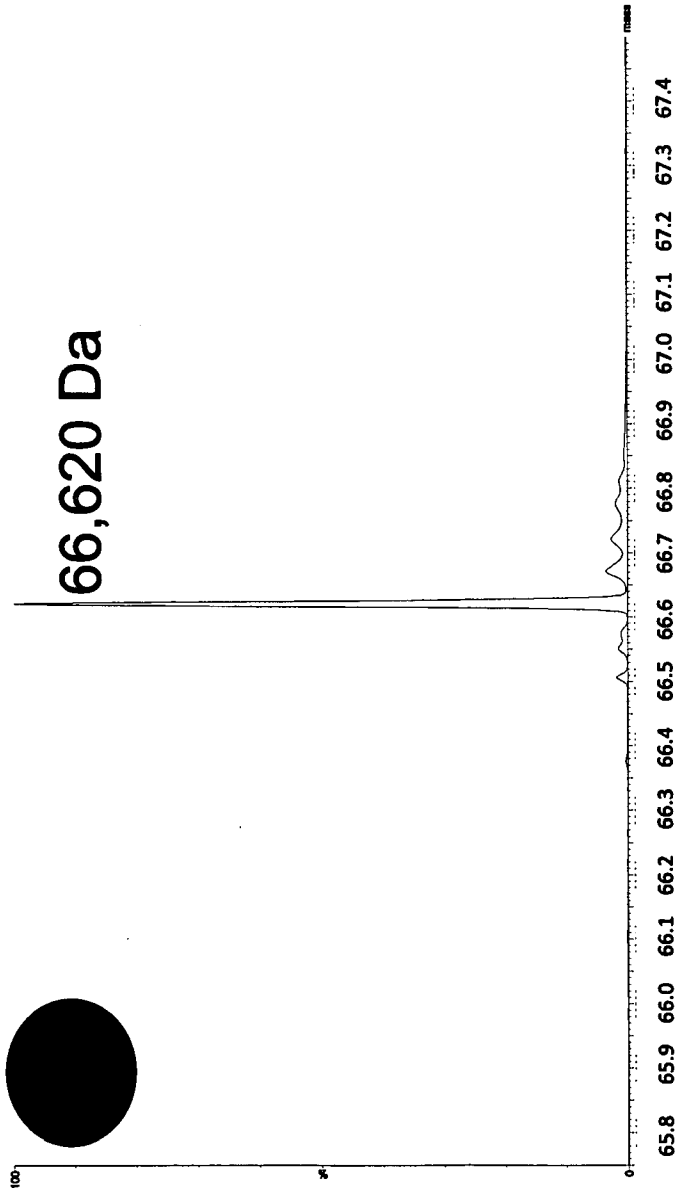
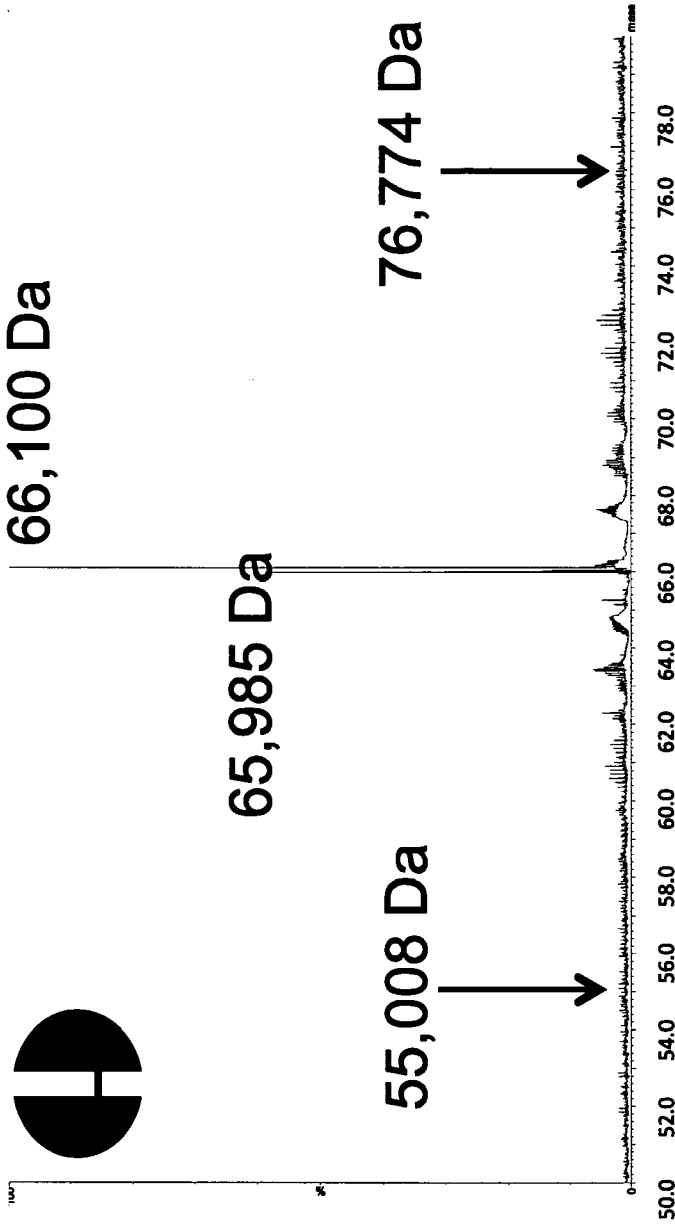


Figure 25B



Measured (Da)	Theoretical (Da)	Delta (Da)
66,620	66,470	+150

Figure 25B cont.



Measured (Da)	Theoretical (Da)	Delta (Da)
66,100	65,880	+220
65,985	65,880	+105

Figure 26A

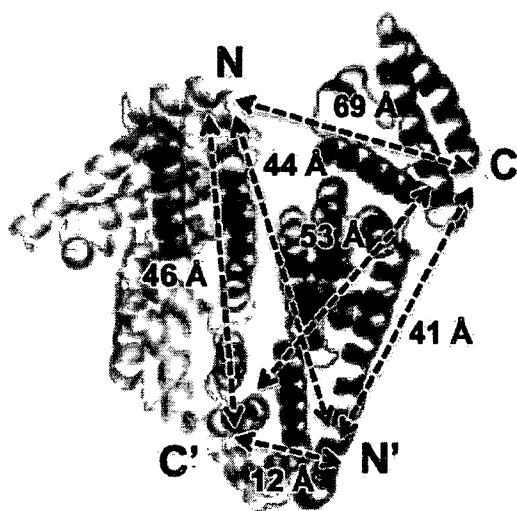


Figure 26B

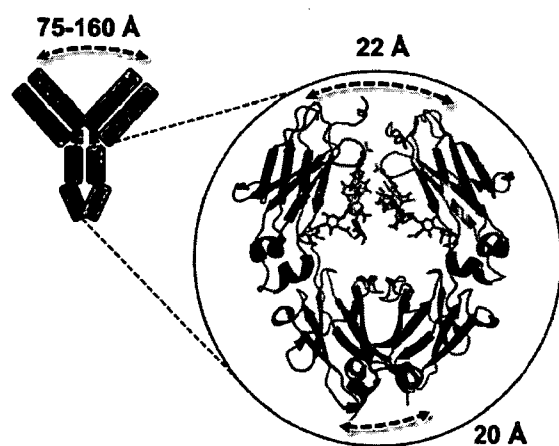


Figure 26C



Figure 27

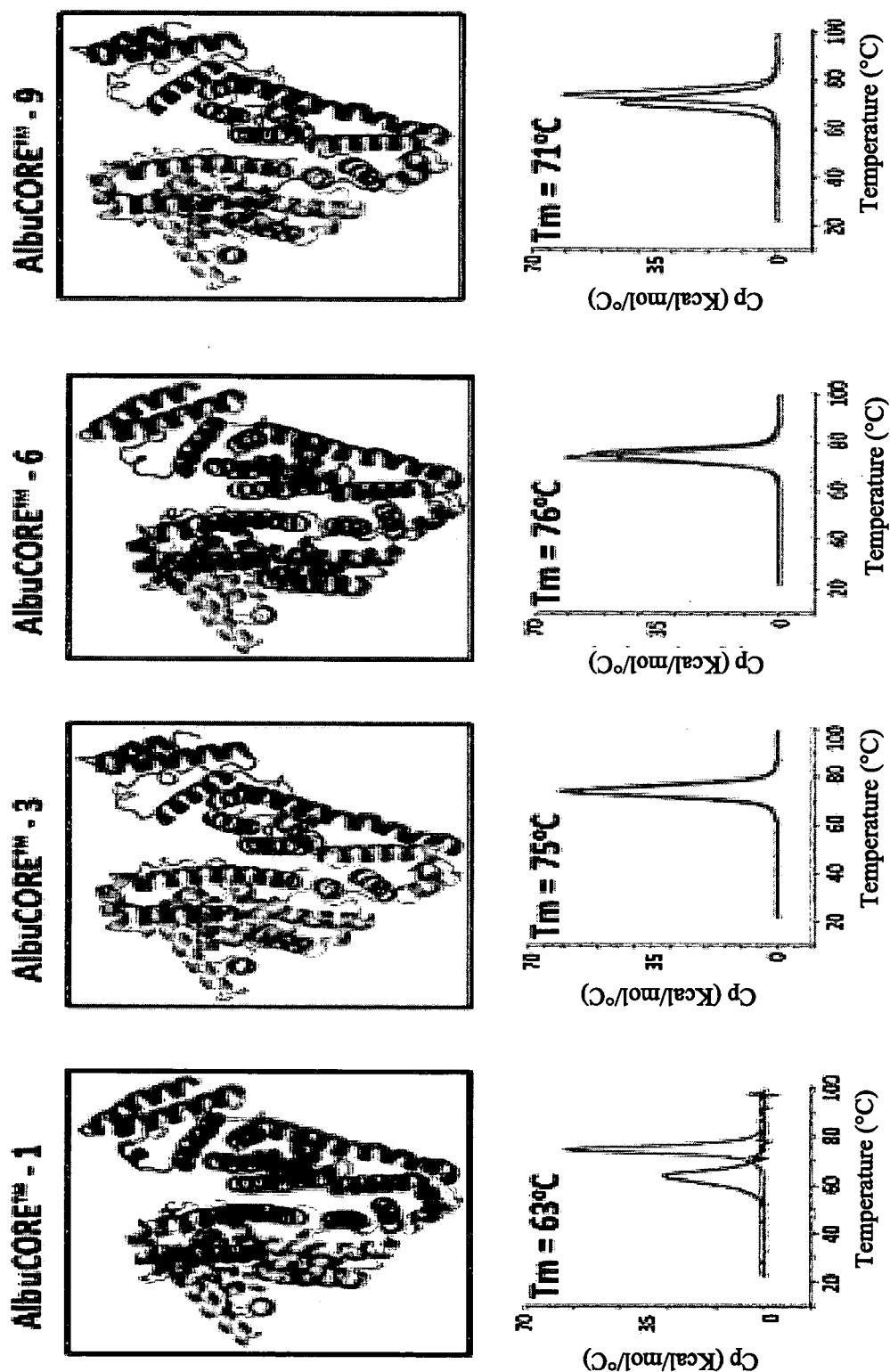


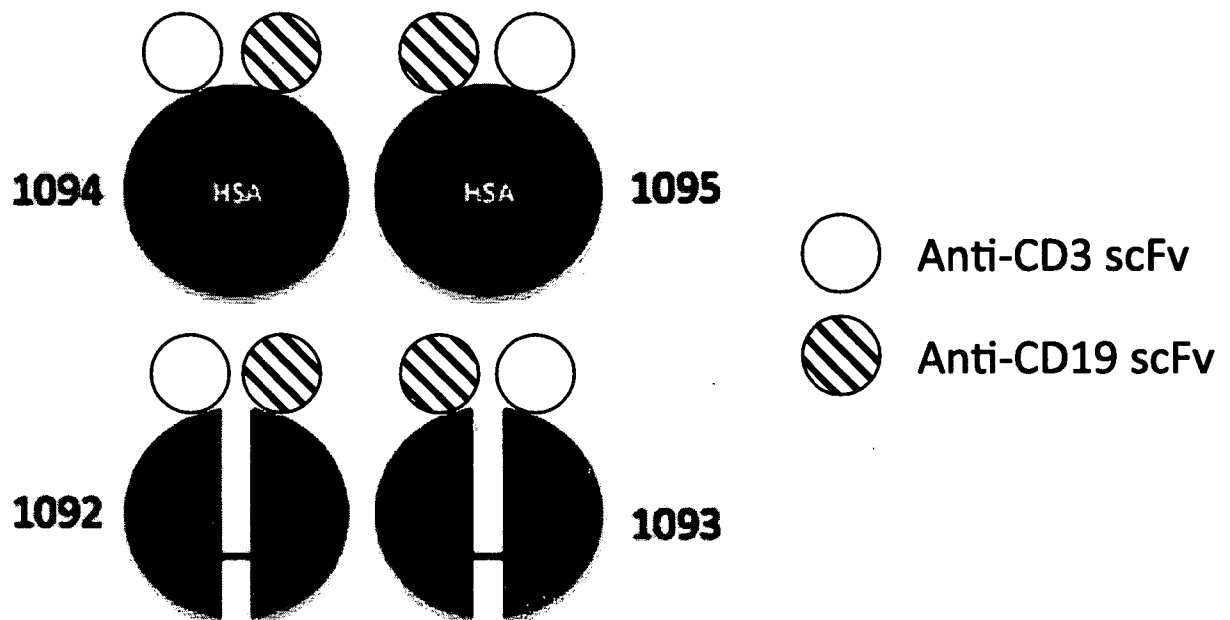
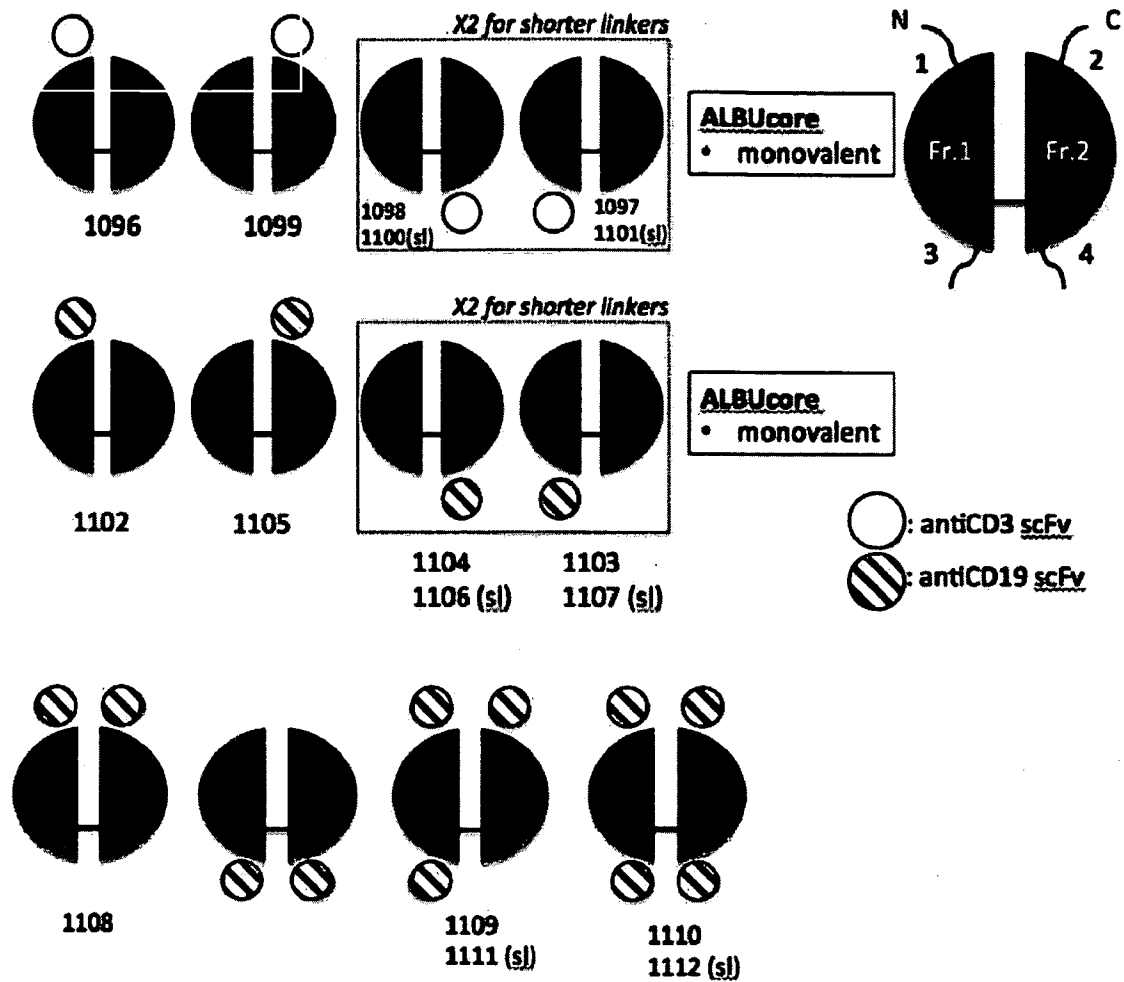
Figure 28A

Figure 28B



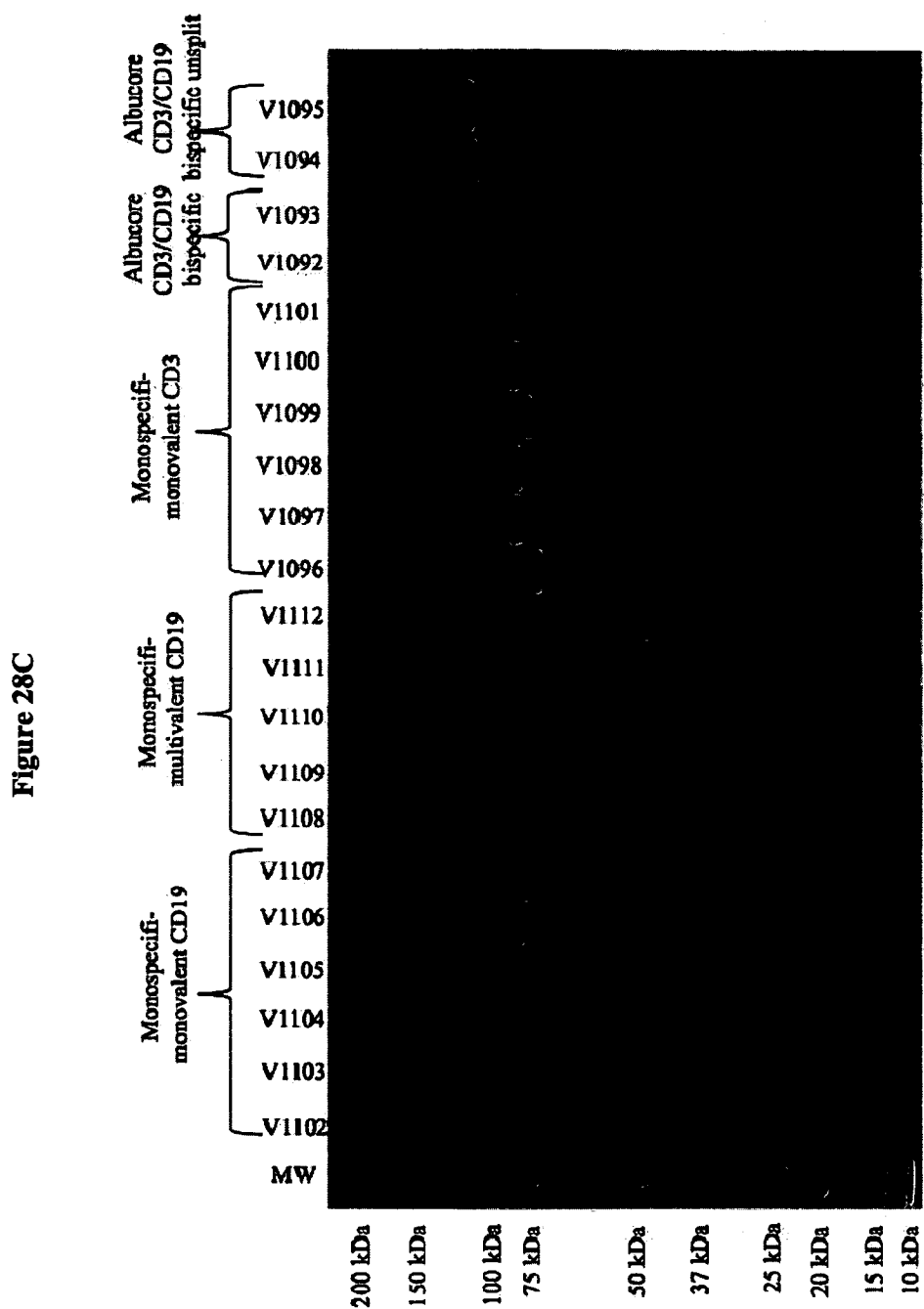


Figure 29

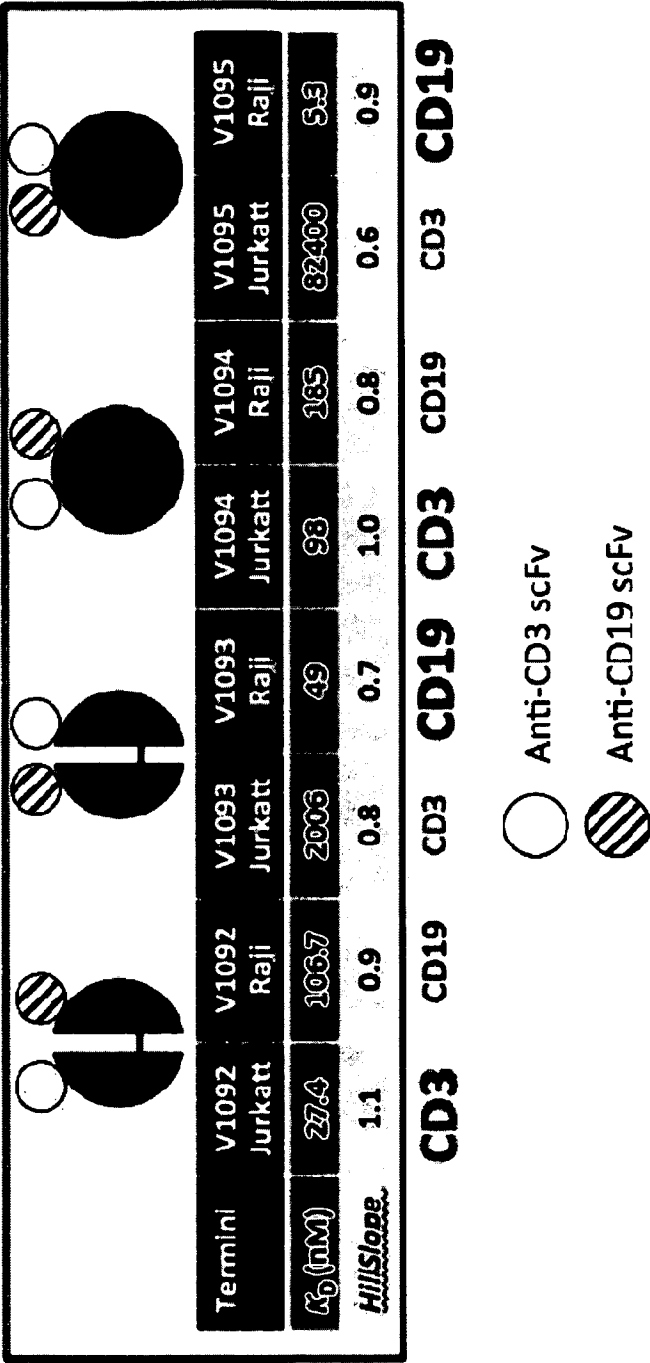


Figure 30A

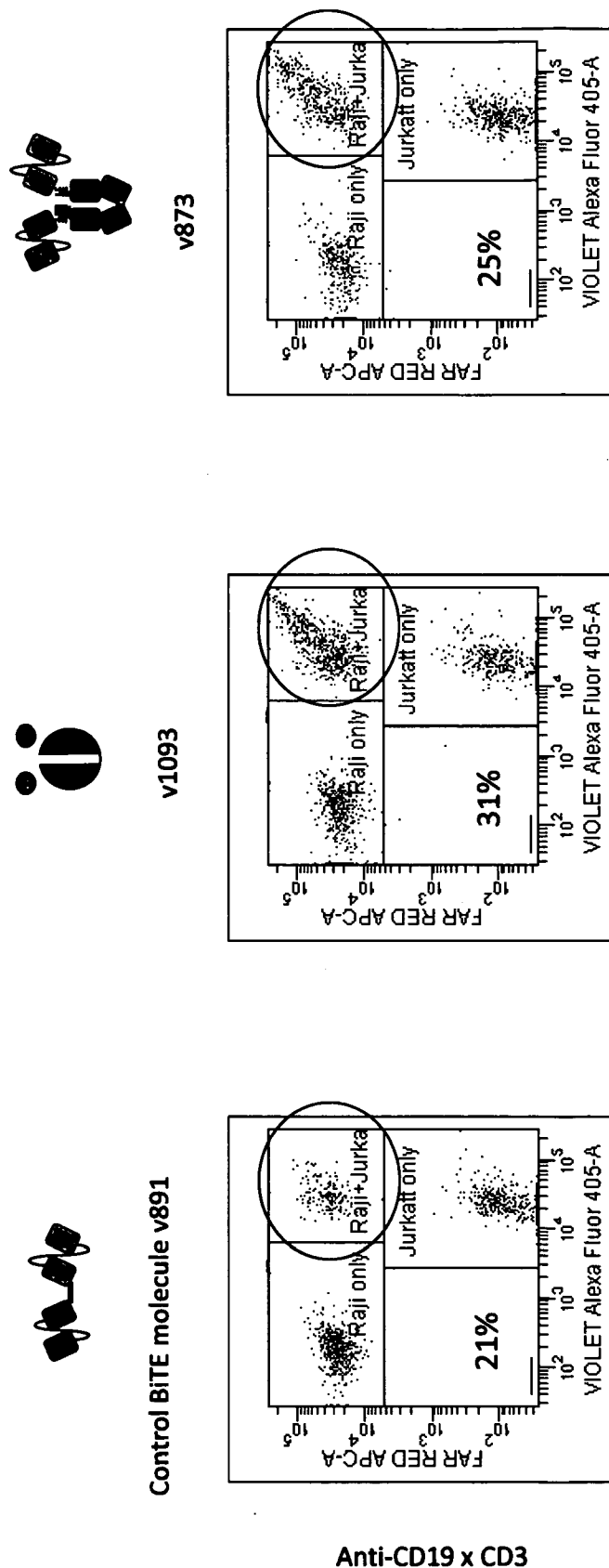


Figure 30B

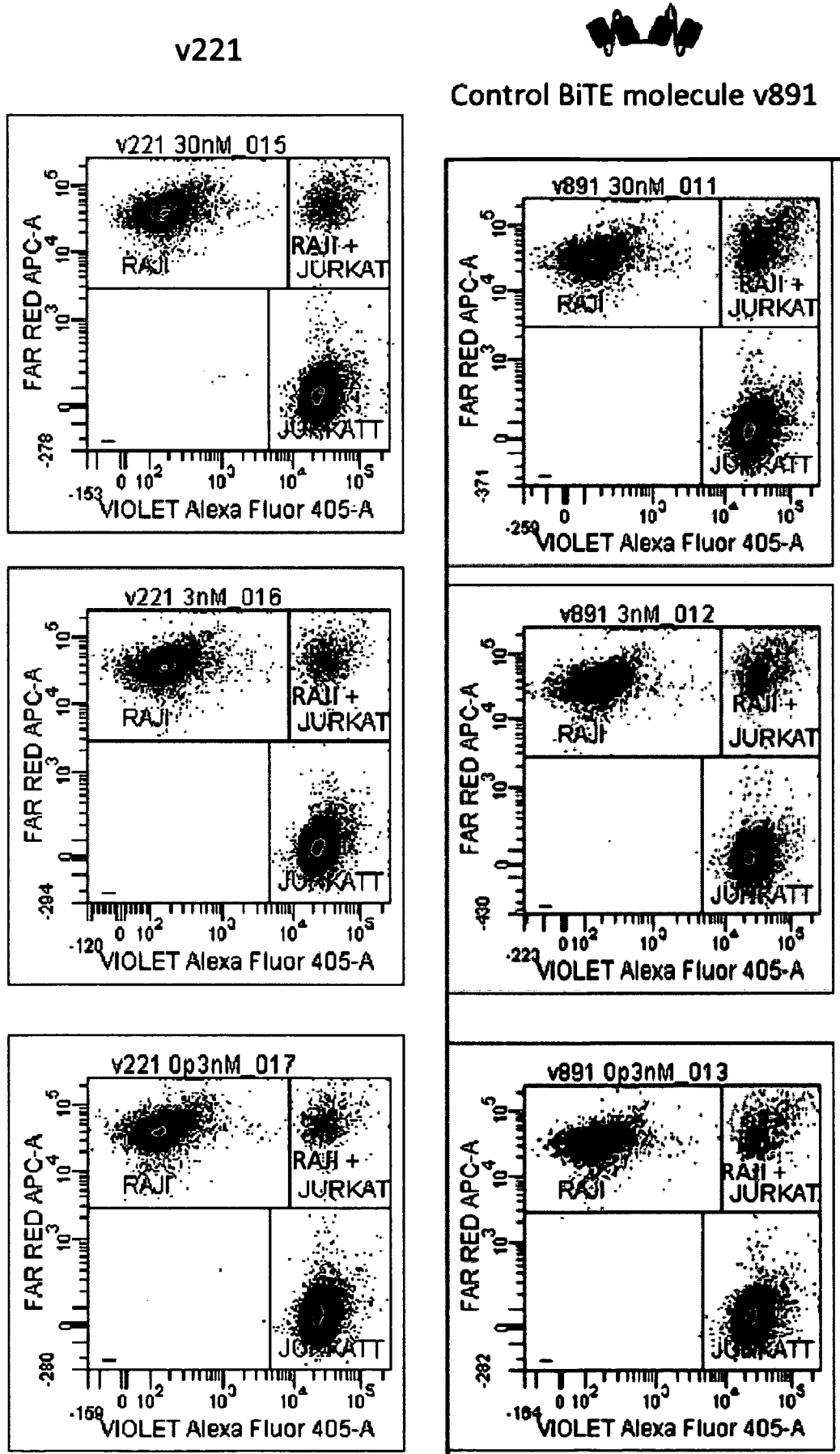


Figure 30 B cont'd

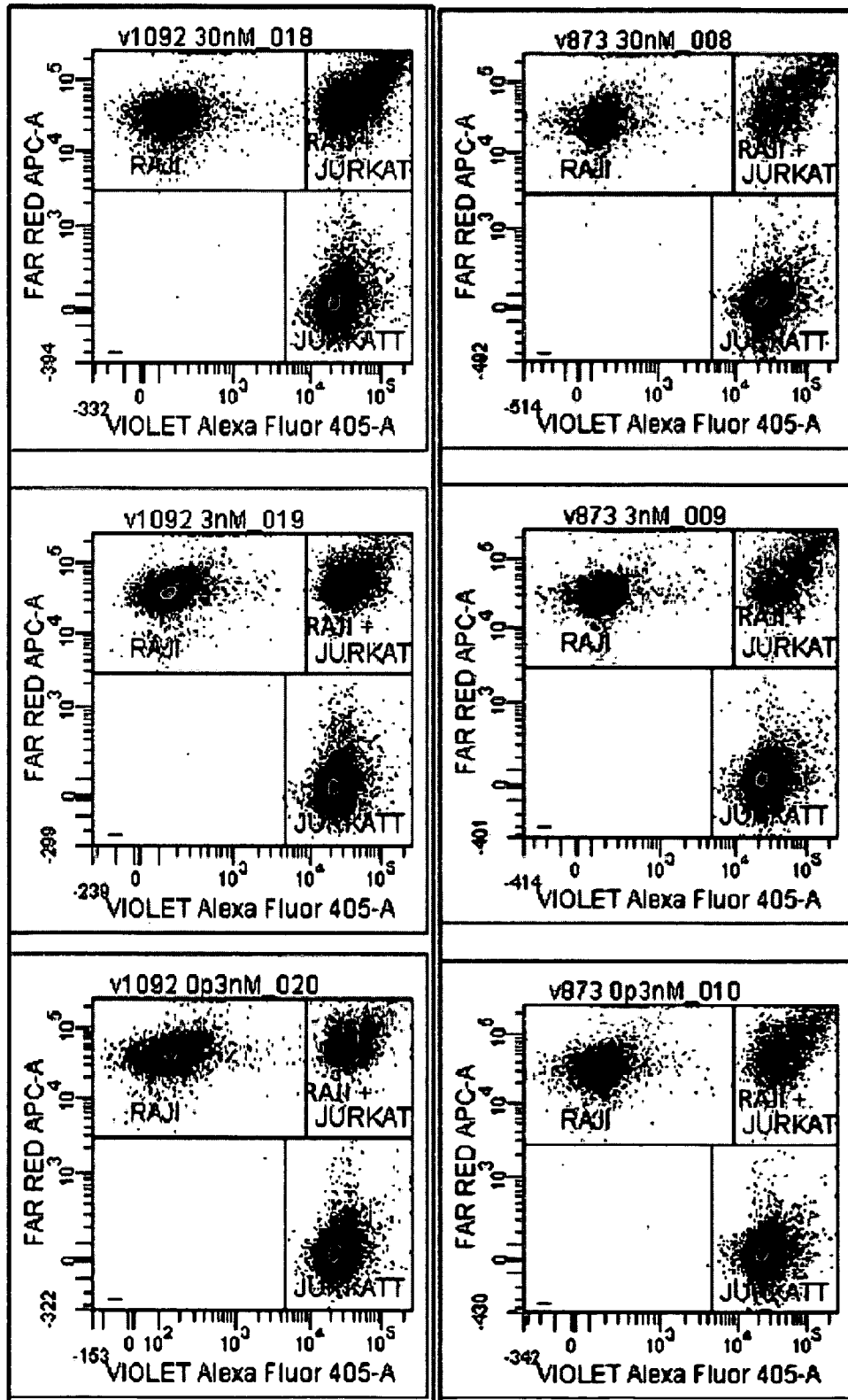


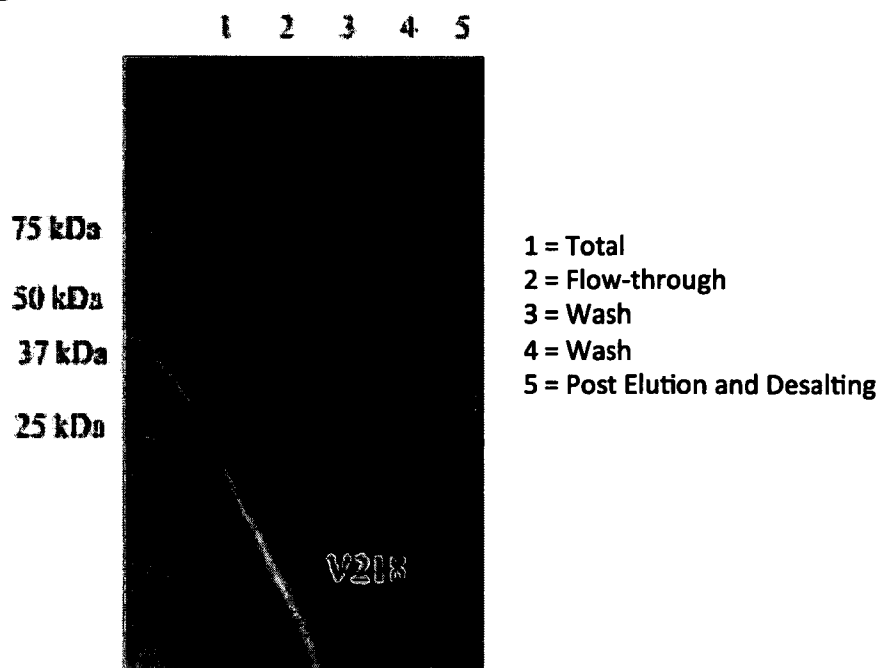
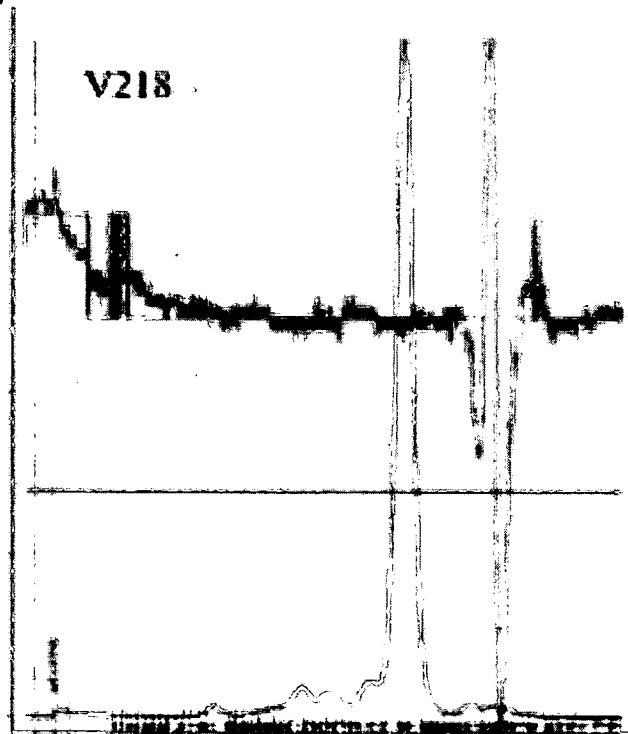
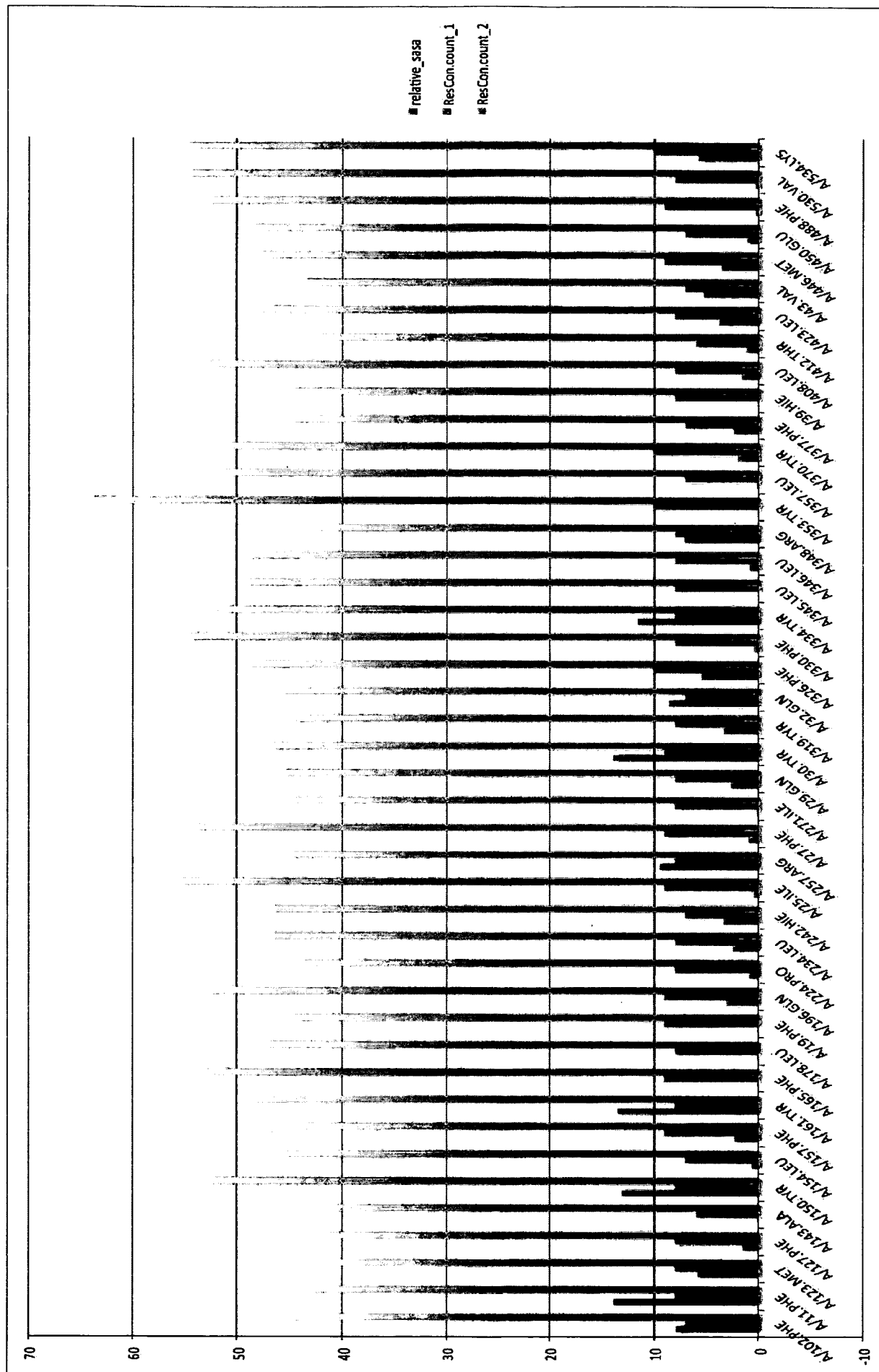
Figure 31A**Figure 31B**

Figure 32



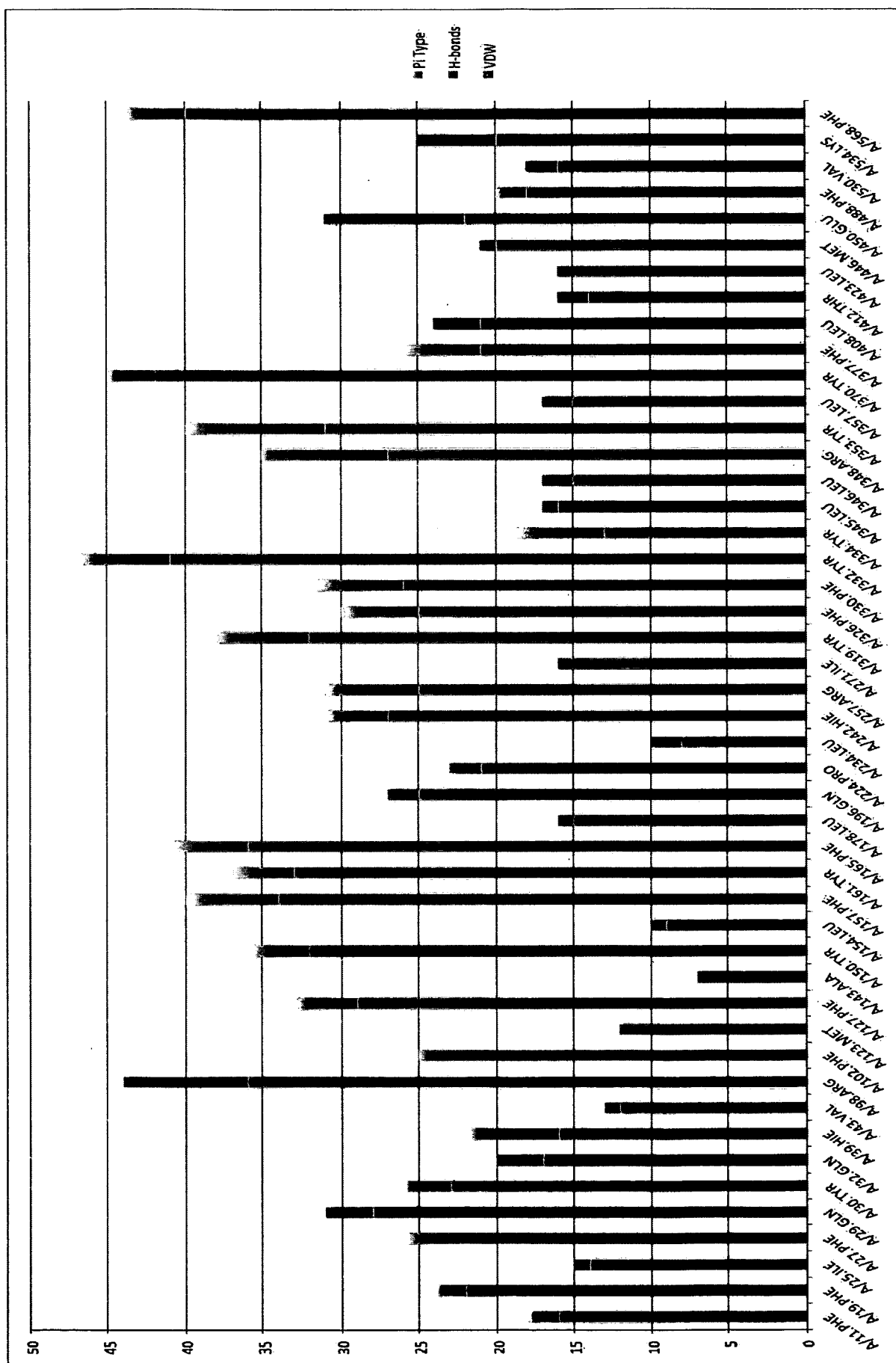


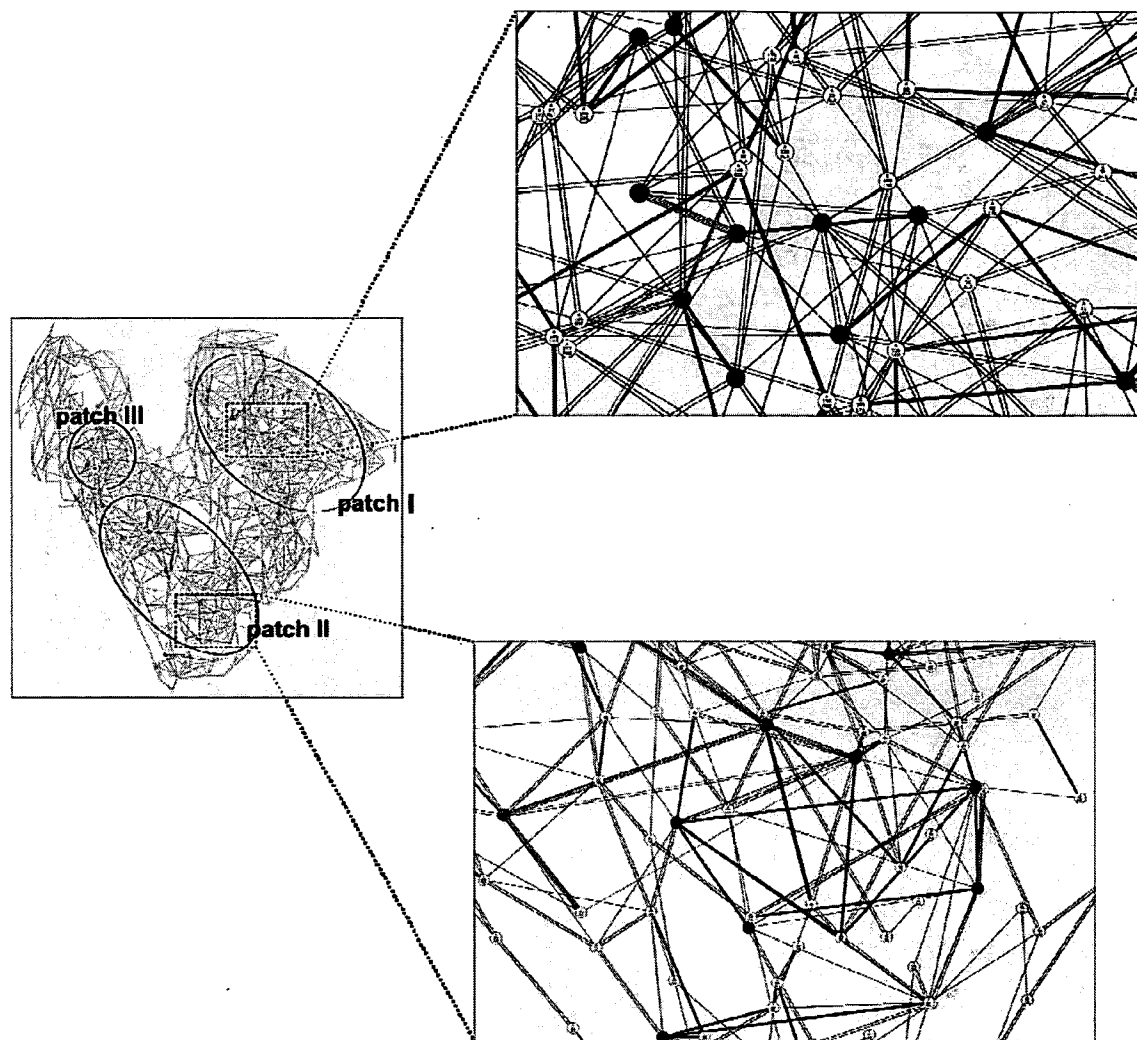
Figure 34

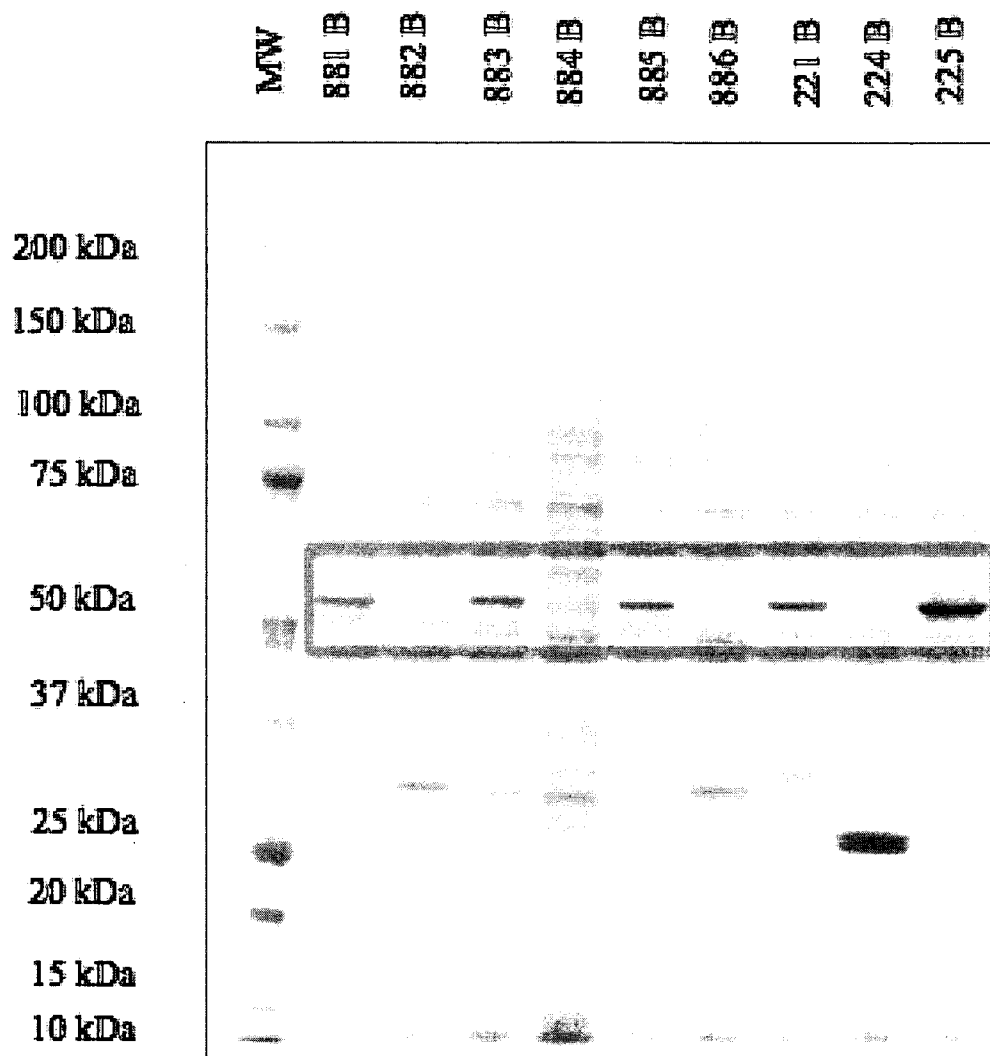
Figure 35

Figure 36 A

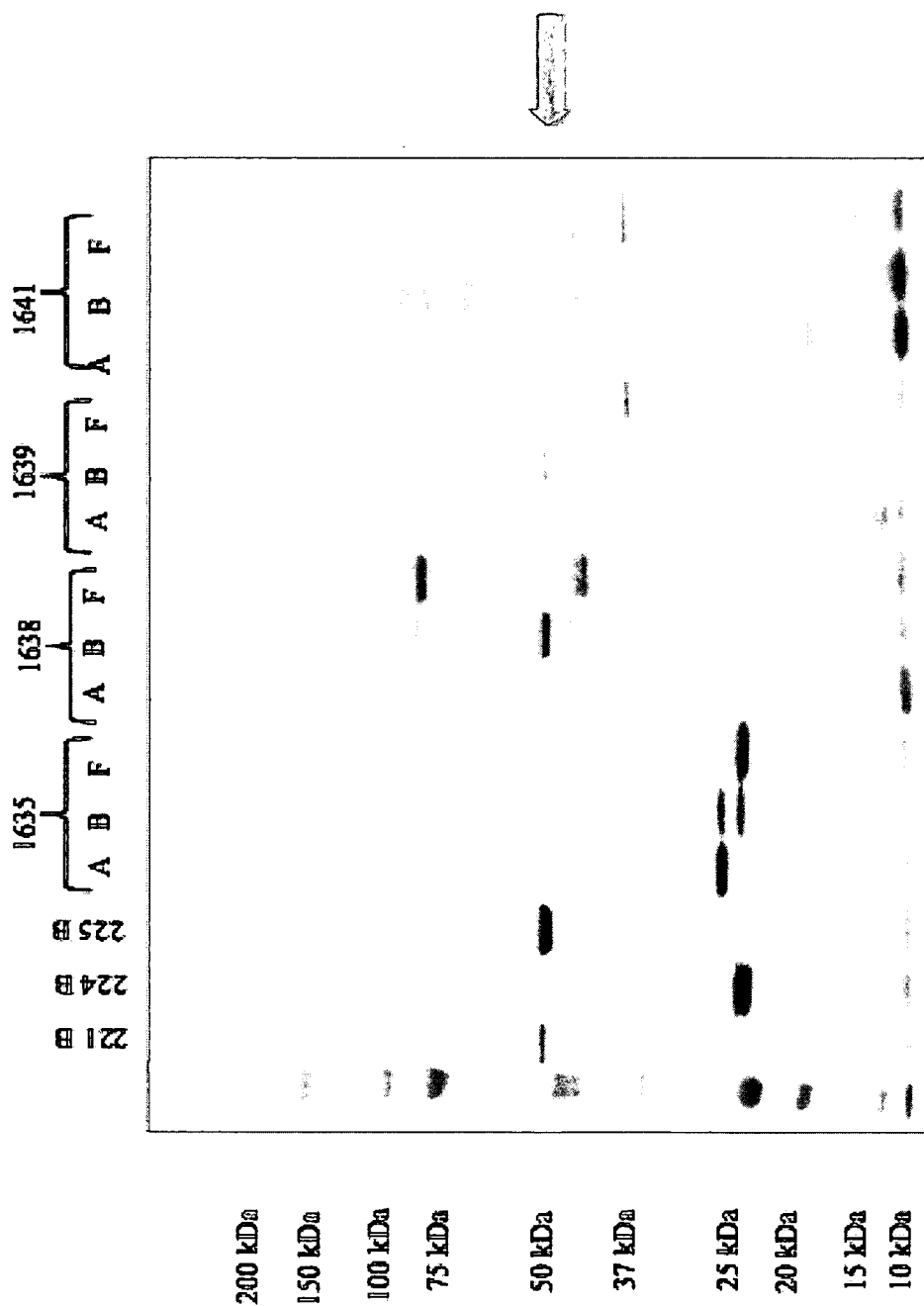


Figure 36 B

