

PROVISIONAL PATENT APPLICATION
HETERODIMERIC ANTIBODY DESIGN AND CONSTRUCTS

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HETERODIMERIC ANTIBODY DESIGN AND CONSTRUCTS

BACKGROUND OF THE INVENTION

[0001] Field of Invention

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

[0003] Description of Related Art

[0004] Antibodies are a complex miracle of biological evolution. They are specific for antigens, and have unique biological properties dependent on their structure. They are key to disease defense in vivo, and have become, through human engineering, useful as diagnostic tools, components of chemical assays, and therapeutic agents. In their natural state, they have multiple functions in vivo, but can and do cause serious disease when they act in the wrong place or at the wrong time. Examples of such disease are rheumatoid arthritis, Type 1 Diabetes, Celiac disease, Lupus, Crohn's disease and many more.

[0005] Groups have attempted to harness the power of antibodies for decades, and innovations include those outlined in United States Patent No. 6129914 by Weiner et al., which discloses a bispecific monoclonal antibody; United States Patent Publication No. 20100143315 by Ralf-Holger Voss et al, which discloses an expression of a single component of an antibody; Patent Cooperation Treaty publication no. 2007115230 by Sergei Kotenko, which discloses chimeric polypeptides, and PCT Publication 2009089004 filed by Amgen, which discloses stabilized antibodies. Davis, Jonathan H. et al. (2010)Protein Engineering, Design & Selection 23(4): 195:202, Discloses asymmetric Fc fusion proteins, also in WO2007/110205. Arathoon et al. (2002) have described a method for making multispecific antibodies having heteromultimeric heavy chain components and common light chain components such as bispecific antibodies in US 7183076B2.

SUMMARY OF THE INVENTION

[0006] There is provided according to one aspect of the invention novel constructs comprising complexes of heterogeneous components designed to change the way the antibody behaves. Each of the CH3, CH2 and receptor binding region may be asymmetrically mutated to achieve calculated results in terms of dimerization, receptor binding, stability, orientation, and the like. An asymmetric immunoglobulin fc-like scaffold for engineering therapeutic agents with strongly modulated effector functions and multifunctional target recognition capabilities is provided.

- [0007] The provided scaffold has two heavy chains that are asymmetric in the various domains to accomplish selectivity between the various Fc receptors involved in modulating effector function, beyond those achievable with a natural homodimeric (symmetric) Fc molecule. These therapeutic novel molecules comprise complexes of heterogeneous components designed to alter the natural way antibodies behave.
- [0008] Thus there are provided components of therapeutic molecules that can effectively engage the natural immune system based effector modalities, and which are engineered to exhibit other effector mechanisms such as complement activation and FcRn based recycling.
- [0009] In accordance with another aspect of the invention, a heterodimeric Fc scaffold is provided.
- [0010] In accordance with one aspect of the invention, Fc variants with improved or altered receptor binding profiles are presented.
- [0011] In accordance to another aspect of the invention, a purified, stable heterodimeric Fc with asymmetric design of Fc-FcR interaction and selectivity is presented.
- [0012] Furthermore, this purified stable heterodimeric Fc may be bi-specific with respect to antigen specificity and optimization of Fc-FcRn interaction for improved pharmacokinetic properties such as half-life.
- [0013] In an aspect of the instant invention is an isolated heteromultimer comprising a first polypeptide and a second polypeptide which meet at an interface which has been engineered to promote heteromultimer formation, wherein at least one of said first and second polypeptide comprises a variant CH3 domain forming part of the interface, and wherein said variant CH3 domain comprises modifications relative to the wild-type CH3 domain such that a greater ratio of heteromultimer:homomultimer is formed as compared to a multimer with a non-engineered interface; wherein the amino acid modifications form favorable interactions between the first polypeptide and the second polypeptide; and wherein the interface comprises first and second faces, the first face having greater binding affinity to a receptor compared to the second face. In certain embodiments, the the favorable interactions are one or more interactions of electrostatics, hydrogen-bonding, surface complementarity, packing, and hydrophobic contact. In certain embodiments of the invention, the heteromultimer is a heterodimer. In some embodiments, one polypeptide comprises a variant CH3 domain forming part of the interface, while the other polypeptide comprises a wild-type CH3 domain. In some embodiments, in the absence of the modifications; the interface has no detectable binding to the receptor.
- [0014] In certain embodiments of the heteromultimer, first polypeptide comprises the amino acid modifications E357H and S364F; and the second polypeptide comprises the amino acid modifications L351Y and K370I. In some other embodiments, first polypeptide comprises the amino acid modifications E357W and S364F; and the second polypeptide comprises the amino acid modifications Y349A, L351Y and K370F. In some embodiments, first polypeptide comprises the amino acid modifications E357H and S364F; and the second polypeptide comprises the amino acid modifications L351Y and K370F. In certain embodiments, first polypeptide comprises the amino acid modifications E357L, T366A, K409F

and T411N; and the second polypeptide comprises the amino acid modifications L351Y and Y407A. In other embodiments, first polypeptide comprises the amino acid modifications L351Y, L368T, and Y407A; and the second polypeptide comprises the amino acid modifications E357L, T366A, K409Y, and T411N.

- [0015]** In certain embodiments of the heteromultimer, if one polypeptide comprises modifications of the amino acid 366, then the other polypeptide does not comprise a modification of the amino acid 407. In certain other embodiments, if one polypeptide comprises modifications of the amino acid 394, then the other polypeptide does not comprise a modification of the amino acid 405.
- [0016]** In certain embodiments of the heteromultimer, one polypeptide comprises the amino acid modifications, L351Y, F405A, and Y407V; and the second polypeptide comprises the amino acid modifications T366I, and T394W.
- [0017]** In an embodiment of the heteromultimer, one polypeptide comprises the amino acid modifications, L351Y, V397T, F405M, and Y407V; and a second polypeptide comprises the amino acid modifications T366I or T366L; and the modification T394W.
- [0018]** In an embodiment of the heteromultimer, one polypeptide comprises the amino acid modifications, L351Y, V397T, F405M, and Y407V; and the second polypeptide comprises the amino acid modifications T366M, and T394W.
- [0019]** In certain embodiments of the heteromultimer, one polypeptide comprises the amino acid modifications, R344H, L351Y, V397T, D401E, F405M, Y407V; and the second polypeptide comprises the amino acid modifications Q362R, T366I, and T394W.
- [0020]** In certain embodiments of the heteromultimer, one polypeptide comprises the amino acid modifications, R344H, L351Y, V397T, S400E, F405M, Y407V; and the second polypeptide comprises the amino acid modifications Q362R, T366I, and T394W.
- [0021]** In certain embodiments of the heteromultimer, one polypeptide comprises the amino acid modifications, Q347R, L351Y, V397T, F405M, and Y407V; and the second polypeptide comprises the amino acid modifications S354N, K360E, T366I, and T394W.
- [0022]** In certain embodiments of the heteromultimer, one polypeptide comprises the amino acid modifications, T350V, L351Y, V397T, S400E, F405M, and Y407V; and the second polypeptide comprises the amino acid modifications T350V, T366I, T394W, and T411R.
- [0023]** In certain embodiments of the heteromultimer, one polypeptide comprises the amino acid modifications, R344H, L351Y, V397T, S400E, F405M, and Y407V; and the second polypeptide comprises the amino acid modifications T366I, T394W, and T411R.
- [0024]** In certain embodiments of the heteromultimer, one polypeptide comprises the amino acid modifications, Q347R, T350V, L351Y, V397T, F405M, Y407V; and the second polypeptide comprises the amino acid modifications T350V, K360E, T366I, T394W, T411R.

- [0025] In certain embodiments of the heteromultimer, one polypeptide comprises at least three amino acid modifications selected from the modifications, R344H, L351Y, K370Q, G371D, L398D, F405M Y407V; and the second polypeptide comprises at least two modifications selected from the amino acid modifications Q362R, T366I, T394W, K409R, and T411Q.
- [0026] In certain embodiments of the heteromultimer, one polypeptide comprises at least three amino acid modifications selected from the modifications, R344H, L351Y, K370Q, G371D, S400E, and F405M Y407V; and the second polypeptide comprises at least two modifications selected from the amino acid modifications Q362R, T366I, N390R, T394W, K409R, T411Q.
- [0027] In certain embodiments of the heteromultimer, one polypeptide comprises at least three amino acid modifications selected from the modifications, R344H, L351Y, V397T, L398D, D399W, F405M and Y407V; and the second polypeptide comprises at least two modifications selected from the amino acid modifications Q362R, T366I, T394W, K409M.
- [0028] In an embodiment of the heteromultimer, one polypeptide comprises the amino acid modifications, L351Y, L368M, V397T, F405M, and Y407V; and the second polypeptide comprises the amino acid modifications T366L, and T394W.
- [0029] In certain embodiments of the heteromultimer of the invention, one or both first and second polypeptides further comprise a CH2 domain. In some embodiments, CH2 domain is a variant CH2 domain. In some embodiments, the variant CH2 domains comprise asymmetric amino acid substitutions in the first and/or second polypeptide chain. In some embodiments, the heteromultimer comprises asymmetric amino acid substitutions in the CH2 domain such that one chain of said heteromultimer selectively binds an Fc receptor.
- [0030] In certain embodiments, heteromultimer selectively binds an Fc receptor. In some embodiments, Fc receptor is a member of Fc γ receptor family. In some embodiments, the receptor is selected from Fc γ RI, Fc γ RIIa, Fc γ RIIb, Fc γ RIIc, Fc γ RIIIa and Fc γ RIIIb.
- [0031] In some embodiments, the heteromultimer binds selectively to Fc γ RIIIa. In some embodiments, the heteromultimer comprises asymmetric amino acid substitutions selected from S267D, K392D and K409D. In some embodiments, the heteromultimer binds selectively to Fc γ RIIa. In some embodiments, the heteromultimer comprises asymmetric amino acid substitutions selected from S239D, K326E, A330L and I332E. In some embodiments, the heteromultimer binds selectively to Fc γ RIIb. In some embodiments, the heteromultimer comprises asymmetric amino acid substitutions selected from S239D, D265S, E269K and I332E. In some embodiments, the heteromultimer binds selectively to Fc γ RIIIa and Fc γ RIIa. In some embodiments, the heteromultimer comprises asymmetric amino acid substitutions selected from S239D, D265S, and S298A. In some embodiments, the heteromultimer binds selectively to Fc γ RIIIa and Fc γ RIIb. In some embodiments, the heteromultimer comprises asymmetric amino acid substitutions selected from S239D, S298A, K326E, A330L and I332E. In some embodiments, the

- heteromultimer binds selectively to FcγRIIa and FcγRIIb. In some embodiments, the heteromultimer comprises asymmetric amino acid substitutions selected from S239D, D265S, S298A and I332E.
- [0032] In certain embodiments, the heteromultimer is a multispecific antibody. In exemplary embodiments, the heteromultimer is a bispecific antibody.
- [0033] In certain embodiments, parent polypeptide of the first and second polypeptide is a human IgG Fc region. In further embodiments, the human IgG Fc region is a human IgG1, IgG2, IgG3, or IgG4 Fc region. In some embodiments of the heteromultimer, the wherein the CH3 domain is from an immunoglobulin selected from the group consisting of IgG, IgA, IgD, IgE and IgM. In some embodiments, the IgG is of subtype selected from the group consisting of IgG1, IgG2a, IgG2b, IgG3 and IgG4.
- [0034] In some embodiments, the heteromultimer is a bispecific antibody. In some embodiments, the bispecific antibody is bispecific immunoadhesin or an antibody-immunoadhesin chimera (Ab/Ia).
- [0035] In certain embodiments of the heteromultimer, the first polypeptide binds a target antigen and the second polypeptide comprises a toxin moiety. In certain embodiments of the invention is a composition comprising the heteromultimer of claim 1 and a pharmaceutically acceptable carrier.
- [0036] In an embodiment, is a host cell comprising nucleic acid encoding one or more of the heteromultimers described herein. In an embodiment, the host cell is a mammalian cell. In certain embodiments, the nucleic acid encoding the first polypeptide and the nucleic acid encoding the second polypeptide are present in a single vector. In certain embodiments, nucleic acid encoding the first polypeptide and the nucleic acid encoding the second polypeptide are present in separate vectors.
- [0037] In certain exemplary embodiments, the heteromultimer described herein, comprises at least one therapeutic antibody. In some embodiments, the therapeutic antibody binds a cancer target antigen. In an embodiment, the therapeutic antibody may be one of is selected from the group consisting of abagovomab, adalimumab, alemtuzumab, aurograb, bapineuzumab, basiliximab, belimumab, bevacizumab, briakinumab, canakinumab, catumaxomab, certolizumab pegol, cetuximab, daclizumab, denosumab, efalizumab, galiximab, gemtuzumab ozogamicin, golimumab, ibritumomab tiuxetan, infliximab, ipilimumab, lumiliximab, mepolizumab, motavizumab, muromonab, mycograb, natalizumab, nimotuzumab, ocrelizumab, ofatumumab, omalizumab, palivizumab, panitumumab, pertuzumab, ranibizumab, reslizumab, rituximab, teplizumab, tocilizumab/atlizumab, tositumomab, trastuzumab, ProxiniumTM, RencarexTM, ustekinumab, zalutumumab, and any other antibodies. In some embodiments, the target antigen is selected from the group consisting of a-chain (CD25) of IL-2R, Amyloid beta, anti-EpCAM x anti-CD3, BLYS (or BAFF), CD11a, 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 receptor, Integrin alpha-4/beta-1, Mucin 16 / CA-125, RANKL, TNF alpha, VEGF-A, and other therapeutically advantageous targets.

- [0038] In some embodiments is a method of treating cancer in a patient having a cancer characterized by a cancer antigen, said method comprising administering to said patient a therapeutically effective amount of a heteromultimer described herein. In certain embodiments, the patient is a human patient. In some other embodiments is a method of treating immune disorders in a patient having an immune disorder characterized by an immune antigen, said method comprising administering to said patient a therapeutically effective amount of a heteromultimer described herein.
- [0039] In certain embodiments is a method of designing multi-functional therapeutics comprising heteromultimer described herein. In some embodiments is method of designing bi-functional therapeutics comprising heteromultimer. In some embodiments is a method for the design of asymmetric mutations in the CH2 domain of an heterodimeric Fc derived with mutations in the CH3 domain. In some embodiments is a method to design selectivity for the different Fc gamma receptors based on the mutations in the asymmetric Fc. In certain embodiments is a method to design mutations that bias binding of the Fc gamma receptors to one face of the Fc molecule. In certain embodiments is a method to design polarity drivers that bias the Fc γ receptors to interact with only one face of the asymmetric Fc scaffold of the heteromultimer described herein.
- [0040] In some embodiments is a polypeptide comprising mutations in the CH2 domain of the asymmetric Fc that lead to preferential Fc gamma receptor selectivity profiles. In some embodiments mutations in the CH3 domain lead to preferential formation of heterodimeric Fc. In certain embodiments is a method to a method to design bispecific therapeutic entities based on the asymmetric Fc described herein. In certain embodiments is a method to design multi-specific therapeutic entities based on the asymmetric Fc described herein.
- [0041] 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

- [0042] In drawings which illustrate embodiments of the invention,
- [0043] Figure 1 is a graphical 3-D structure of a wild type antibody showing the CH3 (top), CH2 (middle) and receptor regions. The dotted line rectangle on the left hand side is expanded to the right hand side showing two regions, Region 1 and Region 2, of the target area of CH3;
- [0044] Figure 2 is a graphical 3-D representation of showing the wild type residue at position 368;
- [0045] Figure 3 is a graphical 3-D representation of Region 1 showing mutated position 368;
- [0046] Figure 4 is a graphical 3-D representation of additional mutations in Region 2;

- [0047] Figure 5 is a table of in silico calculations for clash score, interface area difference, packing different, electrostatic energy difference and overall “affinity score” for the first three variants AZ1, AZ2 and AZ3;
- [0048] Figure 6 shows a graphical 3-D image showing variants AZ2 and AZ3 which are “built onto” variant AZ1;
- [0049] Figure 7 show graphical 3-D representations of AZ2 and AZ3 variants;
- [0050] Figure 8 shows a table as in Figure 5 but for AZ1, AZ2 and AZ3 heterodimers, and homodimers. Affinity score is not relevant for homodimers so there is no score showing for that aspect for the homodimers;
- [0051] Figure 9 is a graphical representation of a 3-D representation of wild type (left) and mutated AZ4 (right);
- [0052] Figure 10 is a table as Figure 5 showing in silico calculation s for ZZ4 heterodimer and homodimers;
- [0053] Figure 11 is a graphical representation of CH3 variants AZ5 (left) and AZ6 (right);
- [0054] Figure 12 is a table as described for Figure 5 showing in silico data for AZ4, AZ5 and AZ6;
- [0055] Figure 13 is a graphical 3-D representation of an antibody on the left, with a drawing of the possibilities of binding characteristics at the receptor region using a heterodimeric approach;
- [0056] Figure 14 is a schematic representation of the IgG molecule;
- [0057] Figure 15 shows multiple sequence alignment of Fc γ receptors. Genebank/Uniprot Sequence ID’s: Fc γ RIIA (sp P12318), Fc γ RIIB (sp P31994), Fc γ RIIC (gi 126116592), Fc γ RIIIA (sp P08637), Fc γ RIIIB (sp O75015);
- [0058] Figure 16 is a schematic of the crystal structure of Fc-Fc γ RIIb Complex [PDB ID: 1T83, Radaev & Sun]. A 1:1 complex of the Fc and Fc γ receptor is observed with an asymmetric contact between the two chains of Fc and the Fc γ R;
- [0059] Figure 17 shows a schematic of alternative multifunctional molecules based on the asymmetric Fc scaffold: Asymmetric Fc Scaffold and Asymmetric Fc- Monomeric IgG Arm;
- [0060] Figure 18 shows a schematic of alternative multifunctional molecules based on the asymmetric Fc scaffold: Asymmetric Fc-Monospecific IgG arms and Asymmetric Fc – Bispecific IgG Arms (Common Light Chain);
- [0061] Figure 19 shows an illustration of alternative multifunctional molecules based on the asymmetric Fc scaffold. Asymmetric Fc- Bispecific IgG Arms and a functional molecule such as toxin;
- [0062] Figure 20 illustrates alternative multifunctional molecules based on the asymmetric Fc scaffold: Asymmetric Fc- Single scFv arm and Asymmetric Fc- bispecific scFv Arms;
- [0063] Figure 21 illustrations of alternative multifunctional molecules based on the assymetric Fc scaffold: Asymmetric Fc- Trispecific scFv Arms and Asymmetric Fc-tetraspecific scFv arms.

[0064] Figure 22 displays asymmetric design of mutations on one face of the Fc for better Fc γ R selectivity introduces a productive side for Fc γ R interactions and a non-productive face with wild type like interactions. Mutations on the non-productive face of the Fc can be introduced to block interactions with FcR and bias polarity of the Fc so as to interact on the productive face only.

DETAILED DESCRIPTION

- [0065]** In an aspect of the instant invention is an isolated heteromultimer comprising a first polypeptide and a second polypeptide which meet at an interface which has been engineered to promote heteromultimer formation, wherein at least one of said first and second polypeptide comprises a variant CH3 domain forming part of the interface, and wherein said variant CH3 domain comprises modifications relative to the wild-type CH3 domain such that a greater ratio of heteromultimer:homomultimer is formed as compared to a multimer with a non-engineered interface; wherein the amino acid modifications form favorable interactions between the first polypeptide and the second polypeptide; and wherein the interface comprises first and second faces, the first face having greater binding affinity to a receptor compared to the second face. In certain embodiments, the the favorable interactions are one or more interactions of electrostatics, hydrogen-bonding, surface complementarity, packing, and hydrophobic contact. In certain embodiments of the invention, the heteromultimer is a heterodimer. In some embodiments, one polypeptide comprises a variant CH3 domain forming part of the interface, while the other polypeptide comprises a wild-type CH3 domain. In some embodiments, in the absence of the modifications; the interface has no detectable binding to the receptor.
- [0066]** In certain embodiments of the heteromultimer, first polypeptide comprises the amino acid modifications E357H and S364F; and the second polypeptide comprises the amino acid modifications L351Y and K370I. In some other embodiments, first polypeptide comprises the amino acid modifications E357W and S364F; and the second polypeptide comprises the amino acid modifications Y349A, L351Y and K370F. In some embodiments, first polypeptide comprises the amino acid modifications E357H and S364F; and the second polypeptide comprises the amino acid modifications L351Y and K370F. In certain embodiments, first polypeptide comprises the amino acid modifications E357L, T366A, K409F and T411N; and the second polypeptide comprises the amino acid modifications L351Y and Y407A. In other embodiments, first polypeptide comprises the amino acid modifications L351Y, L368T, and Y407A; and the second polypeptide comprises the amino acid modifications E357L, T366A, K409Y, and T411N.
- [0067]** In certain embodiments of the heteromultimer, if one polypeptide comprises modifications of the amino acid 366, then the other polypeptide does not comprise a modification of the amino acid 407. In certain other embodiments, if one polypeptide comprises modifications of the amino acid 394, then the other polypeptide does not comprise a modification of the amino acid 405.
- [0068]** In certain embodiments of the heteromultimer of the invention, one or both first and second polypeptides further comprise a CH2 domain. In some embodiments, CH2 domain is a variant CH2 domain. In some embodiments, the variant CH2 domains comprise asymmetric amino acid substitutions in the first and/or second polypeptide chain. In some embodiments, the heteromultimer comprises

asymmetric amino acid substitutions in the CH2 domain such that one chain of said heteromultimer selectively binds an Fc receptor.

- [0069] In certain embodiments, heteromultimer selectively binds an Fc receptor. In some embodiments, Fc receptor is a member of Fc γ receptor family. In some embodiments, the receptor is selected from Fc γ RI, Fc γ RIIa, Fc γ RIIb, Fc γ RIIc, Fc γ RIIIa and Fc γ RIIIb.
- [0070] In some embodiments, the heteromultimer binds selectively to Fc γ RIIIa. In some embodiments, the heteromultimer comprises asymmetric amino acid substitutions selected from S267D, K392D and K409D. In some embodiments, the heteromultimer binds selectively to Fc γ RIIa. In some embodiments, the heteromultimer comprises asymmetric amino acid substitutions selected from S239D, K326E, A330L and I332E. In some embodiments, the heteromultimer binds selectively to Fc γ RIIb. In some embodiments, the heteromultimer comprises asymmetric amino acid substitutions selected from S239D, D265S, E269K and I332E. In some embodiments, the heteromultimer binds selectively to Fc γ RIIIa and Fc γ RIIa. In some embodiments, the heteromultimer comprises asymmetric amino acid substitutions selected from S239D, D265S, and S298A. In some embodiments, the heteromultimer binds selectively to Fc γ RIIIa and Fc γ RIIb. In some embodiments, the heteromultimer comprises asymmetric amino acid substitutions selected from S239D, S298A, K326E, A330L and I332E. In some embodiments, the heteromultimer binds selectively to Fc γ RIIa and Fc γ RIIb. In some embodiments, the heteromultimer comprises asymmetric amino acid substitutions selected from S239D, D265S, S298A and I332E.
- [0071] In certain embodiments, the heteromultimer is a multispecific antibody. In exemplary embodiments, the heteromultimer is a bispecific antibody.
- [0072] In certain embodiments, parent polypeptide of the first and second polypeptide is a human IgG Fc region. In further embodiments, the human IgG Fc region is a human IgG1, IgG2, IgG3, or IgG4 Fc region. In some embodiments of the heteromultimer, the wherein the CH3 domain is from an immunoglobulin selected from the group consisting of IgG, IgA, IgD, IgE and IgM. In some embodiments, the IgG is of subtype selected from the group consisting of IgG1, IgG2a, IgG2b, IgG3 and IgG4.
- [0073] In some embodiments, the heteromultimer is a bispecific antibody. In some embodiments, the bispecific antibody is bispecific immunoadhesin or an antibody-immunoadhesin chimera (Ab/Ia).
- [0074] In certain embodiments of the heteromultimer, the first polypeptide binds a target antigen and the second polypeptide comprises a toxin moiety. In certain embodiments of the invention is a composition comprising the heteromultimer of claim 1 and a pharmaceutically acceptable carrier.
- [0075] In an embodiment, is a host cell comprising nucleic acid encoding one or more of the heteromultimers described herein. In an embodiment, the host cell is a mammalian cell. In certain embodiments, the nucleic acid encoding the first polypeptide and the nucleic acid encoding the second polypeptide are

present in a single vector. In certain embodiments, nucleic acid encoding the first polypeptide and the nucleic acid encoding the second polypeptide are present in separate vectors.

- [0076] In certain exemplary embodiments, the heteromultimer described herein, comprises at least one therapeutic antibody. In some embodiments, the therapeutic antibody binds a cancer target antigen. In an embodiment, the therapeutic antibody may be one of is selected from the group consisting of abagovomab, adalimumab, alemtuzumab, aurograb, bapineuzumab, basiliximab, belimumab, bevacizumab, briakinumab, canakinumab, catumaxomab, certolizumab pegol, cetuximab, daclizumab, denosumab, efalizumab, galiximab, gemtuzumab ozogamicin, golimumab, ibritumomab tiuxetan, infliximab, ipilimumab, lumiliximab, mepolizumab, motavizumab, muromonab, mycograb, natalizumab, nimotuzumab, ocrelizumab, ofatumumab, omalizumab, palivizumab, panitumumab, pertuzumab, ranibizumab, reslizumab, rituximab, teplizumab, tocilizumab/atlizumab, tositumomab, trastuzumab, ProxiniumTM, RencarexTM, ustekinumab, zalutumumab, and any other antibodies. In some embodiments, the target antigen is selected from the group consisting of a-chain (CD25) of IL-2R, Amyloid beta, anti-EpCAM x anti-CD3 , BLYS (or BAFF), CD1 1 a , 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 receptor, Integrin alpha-4/beta-1, Mucin 16 / CA-125, RANKL, TNF alpha, VEGF-A, and other therapeutically advantageous targets.
- [0077] In some embodiments is a method of treating cancer in a patient having a cancer characterized by a cancer antigen, said method comprising administering to said patient a therapeutically effective amount of a heteromultimer described herein. In certain embodiments, the patient is a human patient. In some other embodiments is a method of treating immune disorders in a patient having an immune disorder characterized by an immune antigen, said method comprising administering to said patient a therapeutically effective amount of a heteromultimer described herein.
- [0078] In certain embodiments is a method of designing multi-functional therapeutics comprising heteromultimer described herein. In some embodiments is method of designing bi-functional therapeutics comprising heteromultimer. In some embodiments is a method for the design of asymmetric mutations in the CH2 domain of an heterodimeric Fc derived with mutations in the CH3 domain. In some embodiments is a method to design selectivity for the different Fc gamma receptors based on the mutations in the asymmetric Fc. In certain embodiments is a method to design mutations that bias binding of the Fc gamma receptors to one face of the Fc molecule. In certain embodiments is a method to design polarity drivers that bias the Fc γ receptors to interact with only one face of the asymmetric Fc scaffold of the heteromultimer described herein.
- [0079] In some embodiments is a polypeptide comprising mutations in the CH2 domain of the asymmetric Fc that lead to preferential Fc gamma receptor selectivity profiles. In some embodiments mutations in the CH3 domain lead to preferential formation of heterodimeric Fc. In certain embodiments is a method to

a method to design bispecific therapeutic entities based on the asymmetric Fc described herein. In certain embodiments is a method to design multi-specific therapeutic entities based on the asymmetric Fc described herein.

[0080] Monoclonal antibodies (mAb) such as IgG are symmetric molecules composed of two equivalent heavy and two light polypeptide chains (Figure 14), each comprising multiple immunoglobulin (Ig) structural domains. The IgG class of mAb's exists in one of four isoforms, IgG1, IgG2, IgG3, or IgG4. The heavy chain is composed of four (VH, CH1, CH2 and CH3) and the light chain of two (VL and CL) Ig domains, respectively. The VH and CH1 domains from each of the heavy chains combine with the VL and CL domains of light chain to form the two Fab ("fragment antigen binding") arms of the mAb. The CH3 and CH2 domains of the two heavy chains interact via protein-protein contacts across the CH3 domains and glycosylation in the CH2 domains to form the homodimeric Fc ("fragment crystallizable") region. The linker region between CH1 and CH2 domains of the antibody constitutes the hinge region of the antibody molecule. Apart from connecting the Fab and Fc regions of the mAb, the hinge also maintains disulphide links across the two heavy chains and holds them together. The number of amino acids and disulphide links in the hinge region is notably different among the four isotypes of IgG. The glycosylation pattern in IgG molecules can be significantly diverse, about 30 different carbohydrate moieties have been observed in IgG molecules [Arnold J.N.; Wormald M.R.; Sim R.B.; Rudd P.M. and Dwek R.A. (2007) Annual Reviews of Immunology 25, 21-50].

[0081] The symmetric nature of the mAb structure results in both Fab arms having their antigen binding capability affinity matured to recognize the same epitope. At the other end, the Fc portion of the antibody molecule is involved in interactions with various receptor molecules on the immune or "effector" cells, and some of these interactions are responsible for mediating effector functions such as antibody dependent cellular cytotoxicity (ADCC), antibody dependent cellular phagocytosis (ADCP) and complement activation. Generally, the effector function involves immune responses leading to pathogen or toxin neutralization and elimination, complement activation, and phagocytic response from the humoral immune system. The Fcγ receptor (FcγR) molecules on the effector cells contact the Fc of the activated IgG antibody involved in integral antibody-antigen immune complex to mediate and regulate the effector response. Optimizing the interaction of mAb based protein therapeutic agents to these Fcγ receptors can lead to improvements in the efficacy of these drug candidates.

[0082] In humans there are three known classes of FcγR's with further polymorphic types within each class. The Fc in the IgG1 molecule is known to bind FcγRI (CD64) at nanomolar concentrations while FcγRII (CD32) and FcγRIII (CD16) binding occurs at weaker micromolar concentrations [Bruhns P.; Iannascoli B.; England P.; Mancardi D.A.; Fernandez N.; Jorieux S. and Daron M. (2009) Blood 113: 3716-25].

The high affinity FcγRI receptors can bind IgG in monomeric forms while the low affinity FcγRII and FcγRIII receptors can only bind antigen-antibody immune complexes or IgG aggregates as a result of avidity effects. The different IgG forms have varying affinities for the different FcγR's; in particular, the IgG1 and IgG3 exhibit stronger activity. The Fcγ receptors are the extracellular domains of trans-membrane proteins and possess cytoplasmic domains that are involved in regulating signaling pathways within the cell. When clustered on the immune cell surface on association with the antibody mediated immune complexes, depending on the nature of signaling units linked to the FcγR's on the cytoplasmic end of these cell surface receptors, these molecules regulate the effector response [Nimmerjahn F. and Ravetch J.V. (2008) Nature Immu Rev 8(1):34-47].

[0083] At the chromosomal level, three genes encode the FcγRI (FcγRIA, FcγRIB, FcγRIC) and FcγRII (FcγRIIA, FcγRIIB, FcγRIIC) and two genes encode the FcγRIII (FcγRIIIA, FcγRIIIB). Among the IgG binding human Fcγ receptors, the FcγRIA, FcγRIC and FcγRIIIA types have been shown to be membrane associated with a common γ-chain signal adaptor protein which contains a cytoplasmic immunoreceptor tyrosine based activation motif (ITAM) that leads to the activation of effector function. The FcγRIIA and FcγRIIC also comprise a cytoplasmic ITAM, but without the common γ-chain signal adaptor protein. At the same time, the FcγRIIB is linked to an immunoreceptor tyrosine-based inhibitory motif (ITIM). Activation of FcγRIIB resulting in ITIM phosphorylation results in inhibition of the activating signaling cascade. The FcγRIIIB, while lacking either of the tyrosine based immuno-modulatory cytoplasmic tails, has a GPI (glycosyl-phosphatidyl-inositol) anchor and has been shown to contribute to activation of some granulocytes in the presence of FcγRIIA.

[0084] Table 1: Fcγ Receptor Characteristics

Receptor	Alleles	Signaling Motif	Function	IgG Binding Affinity
FcγRI (CD64)		ITAM	Activating	IgG1 ≈ IgG3 > IgG4
FcγRIIa (CD32a)	131(H/R)	ITAM	Activating	IgG1 > IgG3 > IgG2 > IgG4
FcγRIIb (CD32b)	232(I/T)	ITIM	Inhibitory	IgG3 ≈ IgG1 ≈ IgG4 > IgG2
FcγRIIc (CD32c)	57(Q/Truncation)	ITAM	Activating	IgG3 ≈ IgG1 ≈ IgG4 > IgG2

FcγRIIIa (CD16a)	158(V/F)	ITAM	Activating	IgG3 > IgG1 > IgG4 > IgG2
FcγRIIIb (CD16b)	NA1/2 SH/78(A/D)	GPI	Activating	IgG3 > IgG1

ITAM: Immuno-receptor Tyrosine based Activation Motif; ITIM: Immuno-receptor Tyrosine based Inhibition Motif; GPI Glycophosphoinositol

[0085] While the functional role of ITAM and ITIM motifs and the associated receptor molecules are known, the nature and mechanisms of the modulation of signaling in combination is not completely understood, especially when combined with the activity of a host of other immune cell surface receptors and adaptor molecules (e.g. BCR's, CD22, CD45 etc) involved in signal transduction. In this context, the design of Fc-like molecules that can interact with these Fcγ receptors with exquisite selectivity profiles is a valuable scaffold in any attempt to de-convolute and modulate the effect of such receptor molecules with subtle regulatory activities.

[0086] In the context of designing antibody molecules that can differentiate the FcγR's, the effort is complicated by the fact that the extracellular Fc binding sections of the FcγRII and FcγRIII receptor types exhibit high sequence similarity, which can be attributed at least in part to ancestral segmental duplication. The two major types of FcγRII receptors, A and B, have 69% sequence identity while the FcγRIIA and FcγRIIB exhibit about 44% sequence identity. The FcγRIIB and FcγRIIC differ by only 2 residues in the extracellular region, although they are significantly different in the intracellular region, notable being the presence of ITIM and ITAM motifs respectively. As a result it can be anticipated that therapeutic antibody molecules required to bind one receptor would also potentially bind to other receptor classes, possibly resulting in unintended therapeutic effects.

[0087] Complicating matters further, each of the receptor class presents multiple single nucleotide polymorphisms (SNPs) and copy number variations (CNVs). The resulting receptor diversity differentially impact their affinity to IgG's and its mechanism of action. These genetic variations could affect the affinity of particular IgG subclasses for the Fcγ receptors, alter the downstream effector events or impact mechanisms that alter the levels of receptor expression resulting in functionally relevant phenotypes, non-functional or functionally unknown receptor variants (Bournazos S.; Woof J.M.; Hart S.P. and Dransfield I. (2009) *Clinical and Experimental Immunology* 157(2):244-54). They potentially lead to complex effects, altering the balance between activating and inhibitory receptor signaling, resulting in the creation of disease susceptible phenotypes.

[0088] Some of these allelic variations is listed in Table 2. Notably, the R131 variant in FcγRIIa is a high responder with IgG1 while the alternate H131 variants show more efficient interactions with IgG2 and IgG3. In the case of FcγRIIIa, donors homozygous for V at position 158 exhibit increased NK cell activity in comparison to homozygous F/F158 individuals due to higher affinity of the former allotype for human IgG1, IgG3 and IgG4. The allelic variants NA1 and NA2 of FcγRIIb is the result of a four amino acid substitution which in turn leads to differences in the glycosylation of the receptor. The NA1 allele presents enhanced binding and phagocytosis of the immune complex by neutrophils. The FcγRIIB has two known allelic variants, 232I and 232T. The 232T variant is known to be strongly impaired in its negative regulatory activity. The frequencies of FcγR polymorphisms and its associations to differential responsiveness to infections or predisposition to disease conditions such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), vasculitis, immune-mediated thrombocytic purpura (ITP), myasthenia gravis, multiple sclerosis (MS), and immuno neuropathies (Guillian-Barre syndrome (GBS)) have been reported.

[0089] Copy number variation in the locus of FcγR genes, in particular for FcγRIIB, FcγRIIc and FcγRIIIA has been demonstrated, and further correlation of these differences to cell surface expression of these receptors have been noted. In contrast FcγRIIa and FcγRIIb do not show gene copy number variation. Low copy number of FcγRIIb has in fact been associated with glomerulonephritis in the autoimmune disease systemic lupus erythematosus (SLE) [Aitman TJ et al. (2006) Nature 16;439(7078):851-5]. This is particularly interesting given the fact that a non-signaling GPI module anchors the FcγRIIb receptor. It can be hypothesized that the presence of these FcγRIIb receptors could potentially act as competitive inhibitors of Fc interactions with other signaling FcγR's. The effect of copy number variation in FcγRIIc is also especially interesting. A C/T SNP at position 202 in FcγRIIc converts a glutamine residue to a stop codon preventing the generation of a functional protein. The functional open reading frame of FcγRIIc is expressed in 9% of healthy individuals (white population) and there is a significant overrepresentation (19%) of the allele in the ITP population implying a predisposition of these phenotypes for ITP [Breunis WB et al. (2008) Blood 111(3):1029-38]. It has been demonstrated that in individuals expressing functional FcγRIIc on NK cells, the ADCC achieved is mediated by these receptors to a greater extent than the FcγRIIIa. Such complexities associated with these polymorphisms and genetic variations highlights the need for personalized treatment strategies requiring high tailored therapeutics.

[0090] The various effector cells differ in the presentation of these Fc γ receptors as well as in their humoral and tissue distribution, thus contributing to variations in their mechanism of activation and action [Table 2]. Tuning the selectivity of therapeutic antibodies towards the recognition of specific Fc γ R types and modulating the impact of certain classes of effector cells, leads to optimization of the effector mechanism for particular disease conditions. This is meant to selectively activate or inhibit specific effector modalities, depending on the disease condition being treated.

[0091] Table 2: Cellular distribution of Fc γ R's.

	Fc γ RI (CD64)	Fc γ RIIa (CD32a)	Fc γ RIIb (CD32b)	Fc γ RIIc (CD32c)	Fc γ RIIIa (CD16a)	Fc γ RIIIb (CD16b)	Distribution
Lymphoid							
B cell			✓				Blood
Plasma cell			✓				Tissue
NK cell				✓	✓		Blood
Myeloid							
Monocyte	✓	✓	✓		✓		Blood
Dendritic cell	✓	✓	✓		✓		Tissue
Platelet		✓					Blood
Macrophage	✓	✓	✓		✓		Tissue
Neutrophil	✓		✓			✓	Blood
Eosinophil	✓					✓	Blood
Basophil			✓				Blood
Mast cell			✓			✓	Tissue

[0092] In addition, Fc γ R's are also expressed by follicular dendritic cells, endothelial cells, microglial cells, osteoclasts and mesangial cells. Currently, the functional significance of Fc γ R expression on these other cells is not known.

[0093] The high affinity Fc γ RI is composed of three C-type immunoglobulin superfamily (IgSF) domains while the low affinity Fc γ RII and Fc γ RIII are constituted of two C-type IgSF domains each. The structure of Fc γ RIIa, Fc γ RIIb, Fc γ RIIIa and Fc γ RIIIb receptor proteins has been solved by

crystallography. The two IgSF domains in these structures are bent by 50-55 degrees relative to each other and are connected by a hinge.

- [0094] The publicly available structure of an Fc-Fc γ R co-complex is that of the Fc-Fc γ RIIIb system and the Fc γ R geometry in the complex is maintained very close to that observed in the apo state of the protein [Sondermann P.; Huber R.; Oosthuizen V. and Jacob U. (2000) *Nature* 406, 267-273. ; Radaev S.; Motyaka S.; Fridman W.; Sautes-Fridman C. and Sun P.D. (2001) *J Biol Chem* 276, 16469-16477; Sondermann P. et al. *Biochem Soc Trans.* 2002 Aug;30(4):481-6; Sondermann P, Oosthuizen V. *Immunol Lett.* 2002 Jun 3;82(1-2):51-6; Radaev S, Sun P. *Mol Immunol.* 2002 May;38(14):1073-83.][Figure 15b]. The strong sequence and structural similarity between the receptors forms the basis of comparative models of the Fc bound to the other receptors. On the other hand, the sequence and structural similarity between these receptor molecules also makes the design of Fc with the exquisite selectivity between the receptors and their diverse isotypes challenging.
- [0095] Prior to the structural evaluation of Fc-Fc γ R complex based on crystallography, there were questions if the 2-fold axis of symmetry in the Fc molecule means two potential binding sites and an effective 2:1 stoichiometry for the Fc-Fc γ R association. Nuclear magnetic resonance (NMR) based structural studies of Fc - Fc γ R interactions indicate that binding an Fc to one Fc γ R on one face of the molecule induces a conformational change that precludes the binding of a second Fc γ R molecule to the Fc of the same antibody molecule [Kato K. et al (2000) *J Mol Biol.* 295(2):213-24]. The geometry of the available co-crystal complex of the Fc-Fc γ RIIIb confirms the association of the Fc γ R to Fc in an asymmetric orientation with a 1:1 stoichiometry. As shown in Figure 16, the Fc γ R binds to a cleft on one end of the horseshoe-shaped Fc molecule, and is in contact with the CH2 domains from both the chains.
- [0096] Alanine scanning mutagenesis [Shields RL et al. (2001) *JBC* 276(9): 6591-604] provides insight on the residues of the Fc interfacing with the diverse receptor types and hence involved in the Fc-Fc γ R interaction and recognition. Traditionally, optimization of the therapeutic antibodies has been focused around mutations that exhibit increased binding to the activating receptors Fc γ RIII [US6737056] or decreased affinity to Fc γ RIIb [US2009/0010920A1]. In all these alternate variants, mutations are introduced concurrently in both the chains.
- [0097] MAb molecules often exhibit their therapeutic activity by inducing spatial localization of the target and effector immune cells. A natural antibody mediates this by interacting with the target using its Fab domains and the effector cell using Fc domain. They are able to juxtaposition the immune complex vis-

à-vis the effector cell such that the cell mediated response can be induced. Avidity effects required for FcγR signaling, originating in the formation of immune complexes involving the targeting of a single target by multiple antibody molecules, is another example of significance of spatio-temporal organization in immune action.

[0098] There is also a spatio-temporal aspect to the cell signaling that is induced as part of the effector activity of mAb molecules. Cell signaling such as those based on FcγR molecule activation involves localization of the relevant receptor molecules within a region of membrane domain referred to as lipid rafts. Lipid rafts are enriched with glycosphingolipid and cholesterol and several classes of upstream signal transducers including the Src family kinases. Upon cell stimulation various signaling molecules, adaptor proteins and the signaling kinases as well as phosphatases are recruited. Molecular assembly at lipid rafts is important for signal transduction.

[0099] A non-natural design strategy, combining different antigen specificities and increased avidity to provide better binding properties is the basis of bispecific therapeutic design. Bispecific antibodies or other forms of bispecific or multifunctional protein therapeutics are designed to mediate interactions between the target and a variety of effector cells [Müller & Kontermann (2010) *BioDrugs* 24(2):89-98]. Multispecific therapeutic molecules are engineered to redirect the Helper T-cells or other immune effector cells against specific target cells.

[00100] Recently, Catumaxomab (Removab®), a trifunctional antibody with one arm targeting the CD3 trigger molecule on T-cells, the second arm targeting a tumor associated EPCAM antigen, and the Fc portion of the molecule interacting with Fcγ receptors, has been approved by the European Agency for the Evaluation of Medicinal Products for the intraperitoneal treatment of malignant ascites in patients with EPCAM positive carcinomas.

[00101] The production of a bispecific antibody molecule with the two Fab arms selected to bind two different targets using traditional hybridoma techniques is challenging [Segal DM et al. (2001) *J Immunol Methods*. **248**, 1-6.]. The intrinsic tendency of Fc portion of the antibody molecule to dimerize leads to the formation of complex mixtures of up to 10 different IgG molecules consisting of various combinations of heavy and light chains. The previously mentioned trifunctional antibody, Catumaxomab, is a rat/mouse quadroma derived bispecific mAb and the purification of this molecule is achieved on the basis of a pH dependent elution in protein A column based chromatographic separation [Lindhofer H. et al. (1995) *J Immunol* **155**, 219-225].

[00102] Previous efforts to design complementary mutations in the CH3 domain to selectively form a heterodimeric Fc vis-à-vis a homodimer have involved mutations that introduce “knobs” and “holes” on the two complementary Fc chains. This leads to selective heterodimerization of “knob into holes” in comparison to homodimerization involving “knobs with knobs” or “holes with holes” [US005731168A, US007183076B2, Ridgway JB, Presta LG, Carter P. *Protein Eng* 1996 Jul; 9(7): 617-21; Atwell S, Ridgway JB, Wells JA, Carter P. *J Mol Biol* 1997 Jul 4; 270(1): 26-35.] Gunsekaran and coworkers [Gunasekaran K. et al. (2010) *J Biol Chem.* **285**, 19637-46] have recently employed a complementary electrostatic design strategy to achieve the selective heterodimerization goal. Davis and coworkers [Davis, JH. et al. (2010) *Prot Eng Des Sel* ;23(4):195-202] have designed CH3 domains using a strand exchange engineered domain (SEED), which comprises of alternating segments of human IgA and IgG CH3 sequences, and these preferentially associate in the form of heterodimers.

[00103] In another embodiment, the invention relates to a method for identifying Fc variant polypeptides *in silico* based on calculated binding affinities to FcγRIIa, FcγRIIb and/or FcγRIIIa. In another embodiment, the method further comprises calculating *in silico* electrostatics, solvation, packing, packing density, hydrogen binding, and entropic effects of said Fc variant polypeptides. In yet another embodiment, the method of the current invention further includes constructing the said Fc variant polypeptides and expressing said polypeptides in the context of a therapeutic antibody and further expressing said antibody in mammalian cells. In still another embodiment the method of the current invention comprises constructing the Fc variant polypeptides identified *in silico* by site directed mutagenesis, PCR based mutagenesis, cassette mutagenesis or *de novo* synthesis.

[00104] Factors taken into account in the design of the synthetic Fc scaffold include *in silico* calculations for steric repulsion, change in buried interface area, relative contact density, relative solvation and electrostatic effect. All these matrices were used to arrive at an affinity score.

[00105] This application describes a molecular design for achieving exquisite FcγR selectivity profiles via the design of an asymmetric scaffold built on a heterodimeric Fc. This scaffold allows for asymmetric mutations in the CH2 domain to achieve a variety of novel selectivity profiles. Further, the scaffold has inherent features for the engineering of multifunctional (bi, tri, tetra or penta functional) therapeutic molecules.

[00106] The asymmetric scaffold can be optimized for pH dependent binding properties to the neonatal Fc receptor (FcRn) to enable better recycling of the molecule and enhance its half life and related pharmacokinetic properties.

[00107] The asymmetric scaffold can be optimized for binding to the functionally relevant FcγRI receptor allotypes. FcγRI is a prominent marker on macrophages that are involved in chronic inflammatory disorders such as Rheumatoid Arthritis, Atopic Dermatitis, Psoriasis and a number of pulmonary diseases.

[00108] The asymmetric scaffold can be optimized for protein A binding. Protein A binding is often employed for separation and purification of antibody molecules. Mutations can be introduced in the asymmetric scaffold to avoid aggregation of the therapeutic during storage.

CH3 Variants for asymmetric scaffold dimerization

[00109] We employ a structure and computation guided approach to our protein engineering goals. We employ a combination of positive and negative design strategies to form our heterodimeric Fc variants. A positive design strategy introduces mutations that stabilize the dimer but may not be very successful in preferentially forming a heterodimer. A negative design strategy aims to introduce mutations in the CH3 domain of Fc that preferentially form heterodimers in comparison to homodimers.

[00110] Like all specific protein-protein complexes, a number of features can be altered at the protein interaction surface to achieve dimer formation. These include changes in hydrophobics, electrostatics, hydrogen bonds and surface complementarity between the two partner proteins. Our computational approach involves simulating and scoring these functionally relevant properties. Molecular association is a complex process with a number of compensatory effects in play and scoring mechanism is meant to assist in evaluating these factors in models of the wild type and mutant structures.

[00111] The CH3-CH3 interface between the two chains A and B has a central core around which there is a C2 axis of symmetry. Our optimization is aimed at introducing compensatory mutations in the two regions flanking the central core.

[00112] Table 3: CH3 domain mutations for the creation of asymmetric Fc scaffold.

	Variant	Chains	Fc Mutations						
Wild-Type		A	-	-	-	-	-	-	-
		B	-	-	-	-	-	-	-
Zymeworks Asymmetric™ Variants	AZ1	A	L368D	K370Q	-	-	-	-	-
		B	E357R	L368R	-	-	-	-	-
	AZ2	A	L351I	L368D	K370Q	-	-	-	-
		B	E357R	L368R	-	-	-	-	-
	AZ3	A	L351D	L368D	K370Q	-	-	-	-
		B	E357R	L368R	-	-	-	-	-
	AZ4	A	L368D	K370E	-	-	-	-	-
		B	E357R	L368R	-	-	-	-	-
	AZ5	A	L368D	K370E	-	-	-	-	-
		B	E357K	L368R	-	-	-	-	-
	AZ6	A	V397S	F405A	Y407V	-	-	-	-
		B	K392V	T394W	-	-	-	-	-
	AZ7	A	L351R	V397S	F405A	Y407V	-	-	-
		B	K392V	T394W	-	-	-	-	-
	AZ8	A	L351Y	V397S	F405A	Y407V	-	-	-
		B	K392V	T394W	-	-	-	-	-
	AZ9	A	V397S	F405A	Y407V	-	-	-	-
		B	L368R	K392V	T394W	-	-	-	-
	AZ10	A	V397T	F405I	-	-	-	-	-
		B	K392V	T394H	-	-	-	-	-
	AZ11	A	E357W	S364F	-	-	-	-	-
		B	Y349A	L351Y	K370I	-	-	-	-
	AZ12	A	E357H	S364F	-	-	-	-	-
		B	L351Y	K370I	-	-	-	-	-
	AZ13	A	E357W	S364F	-	-	-	-	-
		B	Y349A	L351Y	K370F	-	-	-	-
	AZ14	A	E357H	S364F	-	-	-	-	-
		B	L351Y	K370F	-	-	-	-	-
	AZ15	A	E357L	T366A	K409F	T411N	-	-	-
		B	L351Y	Y407A	-	-	-	-	-
	AZ16	A	L351Y	L368T	Y407A	-	-	-	-
		B	E357L	T366A	K409Y	T411N	-	-	-

AZ17	A	L351Y	F405A	Y407V	-	-	-	-
	B	T366I	T394W	-	-	-	-	-
AZ18	A	L351Y	V397T	F405M	Y407V	-	-	-
	B	T366I	T394W	-	-	-	-	-
AZ19	A	L351Y	V397T	F405M	Y407V	-	-	-
	B	T366L	T394W	-	-	-	-	-
AZ20	A	L351Y	V397T	F405M	Y407V	-	-	-
	B	T366M	T394W	-	-	-	-	-
AZ21	A	L351Y	L368M	V397T	F405I	Y407V	-	-
	B	T366L	T394W	-	-	-	-	-
AZ22	A	L351Y	L368M	V397T	F405I	Y407V	-	-
	B	T366M	T394W	-	-	-	-	-
AZ23	A	L351Y	V397T	F405M	Y407V	-	-	-
	B	L351I	T366I	T394W	-	-	-	-
AZ24	A	L351Y	V397T	L398D	F405M	Y407V	-	-
	B	S354E	T366I	T394W	-	-	-	-
AZ25	A	L351Y	V397T	L398D	S400E	F405M	Y407V	-
	B	T366I	N390R	T394W	-	-	-	-
AZ26	A	R344H	L351Y	V397T	S400E	F405M	Y407V	-
	B	Q362R	T366I	T394W	-	-	-	-
AZ27	A	R344H	L351Y	V397T	D401E	F405M	Y407V	-
	B	Q362R	T366I	T394W	-	-	-	-
AZ28	A	Q347R	L351Y	V397T	F405M	Y407V	-	-
	B	S354E	K360E	T366I	T394W	-	-	-
AZ29	A	Q347R	L351Y	V397T	F405M	Y407V	-	-
	B	S354N	K360E	T366I	T394W	-	-	-
AZ30	A	T350V	L351Y	V397T	S400E	F405M	Y407V	-
	B	T350V	T366I	T394W	T411R	-	-	-
AZ31	A	R344H	L351Y	V397T	L398D	F405M	Y407V	-
	B	T366I	T394W	T411R	-	-	-	-
AZ32	A	Q347R	T350V	L351Y	V397T	F405M	Y407V	-
	B	T350V	K360E	T366I	T394W	T411R	-	-
AZ33	A	L351Y	F405A	Y407V	-	-	-	-
	B	T366I	K392M	T394W	-	-	-	-
AZ34	A	L351Y	S400E	F405A	Y407V	-	-	-
	B	T366I	N390R	K392M	T394W	-	-	-

AZ35	A	L351Y	K370Q	G371D	F405M	Y407V	-	-
	B	Q362R	T366I	T394W	K409R	T411Q	-	-
AZ36	A	L351Y	K370Q	G371D	F405S	Y407V	-	-
	B	Q362R	T366I	T394W	K409R	T411Q	-	-
AZ37	A	R344H	L351Y	K370Q	G371D	L398D	F405M	Y407V
	B	Q362R	T366I	T394W	K409R	T411Q	-	-
AZ38	A	R344H	L351Y	K370Q	G371D	S400E	F405M	Y407V
	B	Q362R	T366I	N390R	T394W	K409R	T411Q	-
AZ39	A	L351Y	K370Q	G371D	F405M	Y407V	-	-
	B	T366I	T394W	T411R	-	-	-	-
AZ40	A	L351Y	K370Q	G371D	F405M	Y407V	-	-
	B	T366I	T394W	K409M	T411R	-	-	-
AZ41	A	R344H	L351Y	K370Q	G371D	L398D	F405M	Y407V
	B	T366I	T394W	K409M	T411R	-	-	-
AZ42	A	R344H	L351Y	K370Q	G371D	S400E	F405M	Y407V
	B	T366I	N390R	T394W	K409M	T411R	-	-
AZ43	A	L351Y	K370T	G371D	F405I	Y407V	-	-
	B	E357Q	S364R	T394W	-	-	-	-
AZ44	A	L351Y	K370T	G371D	F405M	Y407V	-	-
	B	E357Q	S364R	T394W	K409I	-	-	-
AZ45	A	R344H	L351Y	K370T	G371D	S400E	F405M	Y407V
	B	E357Q	S364R	T366I	N390R	T394W	K409I	-
AZ46	A	R344H	L351Y	K370T	G371D	F405M	Y407V	-
	B	E357Q	S364R	T366I	T394W	K409I	T411R	-
AZ47	A	L351Y	K370A	G371S	D399R	F405S	Y407V	-
	B	E357Q	Q362R	T364Y	T366I	T394W	K409S	-
AZ48	A	L351Y	V397S	D399W	F405M	Y407V	-	-
	B	Q362R	T366I	T394W	K409M	-	-	-
AZ49	A	L351Y	V397S	D399Y	F405M	Y407V	-	-
	B	Q362R	T366I	T394W	K409I	-	-	-
AZ50	A	R344H	L351Y	V397T	L398D	D399W	F405M	Y407V
	B	Q362R	T366I	T394W	K409M	-	-	-
AZ51	A	R344H	L351Y	V397T	D399W	S400E	F405M	Y407V
	B	Q362R	T366I	T394W	K409M	-	-	-
AZ52	A	L368V	K370F	F405I	Y407V	-	-	-
	B	E357Q	S364Y	T366I	T394W	-	-	-

AZ53	A	L368V	K370Y	F405I	Y407V	-	-	-
	B	E357Q	S364Y	T394W	-	-	-	-
AZ54	A	R344H	L368V	K370Y	F405M	Y407V	-	-
	B	E357Q	Q362R	S364Y	T394W	-	-	-
AZ55	A	L368V	K370Y	S400E	F405M	Y407V	-	-
	B	E357Q	S364Y	N390R	T394W	-	-	-
AZ56	A	L368V	K370Y	L398D	F405M	Y407V	-	-
	B	E357Q	S364Y	T394W	T411R	-	-	-
AZ57	A	R344H	L351Y	K370Y	F405M	Y407V	-	-
	B	E357Q	Q362R	T364T	T366I	T394W	-	-
AZ58	A	L368V	V397T	F405M	Y407V	-	-	-
	B	T366Y	T394W	-	-	-	-	-
AZ59	A	L368V	K370Q	V397T	F405M	Y407V	-	-
	B	T366Y	T394W	-	-	-	-	-
AZ60	A	R344H	L368V	V397T	S400E	F405M	Y407V	-
	B	Q362R	T366Y	T394W	-	-	-	-
AZ61	A	L368V	V397T	S400E	F405M	Y407V	-	-
	B	T366Y	N390R	T394W	-	-	-	-
AZ62	A	L368V	V397T	L398D	F405M	Y407V	-	-
	B	T366Y	T394W	T411R	-	-	-	-

CH2 variations for fcγR selectivity

[00113] The Fc-FcγR protein-protein interaction in this complex indicates that the two chains in the Fc molecule interact with two distinct sites on the FcγR molecule. Although there is symmetry in the two heavy chains in the natural Fc molecules, the local FcγR environment around residues on one chain is different from the FcγR residues surrounding the same residue position on the opposite Fc chain. The two symmetry related positions interact with different selection of FcγR residues.

[00114] Given the asymmetry in the association of Fc to FcγR, concurrent mutations in chain A and B of the Fc molecule do not impact the interactions with FcγR in a symmetric manner. When introducing mutations to optimize interactions on one chain of the Fc with its local FcγR environment, in a homodimeric Fc structure, the corresponding mutation in the second chain may be favorable, unfavorable or non-contributing to the required FcγR binding and selectivity profile.

[00115] Using a structure and computation guided approach, asymmetric mutations are engineered in the two chains of the Fc to overcome these limitations of traditional Fc engineering strategies. One can achieve better binding selectivity between the receptors if the two chains of Fc are optimized independently for enhanced binding to their corresponding face of the receptor molecule.

[00116] For instance, mutations at a particular position on one chain of the Fc can be designed to enhance selectivity to a particular residue, a positive design effort, while the same residue position can be mutated to unfavorably interact with its local environment in an alternate Fc γ receptor type, hence achieving better selectivity between the two receptors.

[00117] The asymmetric design of mutations in the CH2 domain are tailored to recognize the Fc γ R on one face of the Fc molecule. This constitutes the productive face of the asymmetric Fc scaffold while the opposite face presents wild type like interaction propensity without the designed selectivity profile and can be considered a non-productive face. A negative design strategy can be employed to introduce mutations on the non-productive face to block Fc γ R interactions to this side of the asymmetric Fc scaffold, thereby forcing the desired interaction tendencies to the Fc γ receptors.

[00118] Table 4: Selectivity Profiles of Fc for different Fc γ Receptors

Variant Selectivity	Receptor Binding		
	Fc γ RIIIa F/V	Fc γ RIIIa H/R	Fc γ RIIIb F/Y
	↑ / -	×	×
	×	↑ / -	×
	×	×	↑ / -
	↑ / -	↑ / -	×
	↑ / -	×	↑ / -
	×	↑ / -	↑ / -

[00119] (↑/-) indicates a variant which exhibits an increased or wild type like binding to the particular receptor type. (×) indicates no binding to the receptor type.

[00120] Asymmetric Variants

1	S267D_K392D_K409D
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2	S239D_K326E_A330L_I332E
3	S239D_D265S_E269K_I332E
4	S239D_D265S_S298A
5	S239D_S298A_K326E_A330L_I332E
6	S239D_D265S_S298A_I332E

Design of bispecific or other multifunctional targeting molecules

[00121] The asymmetric scaffold provides the framework for engineering bispecific and other multifunctional targeting therapeutic agents. Various embodiments of multifunctional targeting molecules can be designed on the basis of this asymmetric scaffold as shown in Figure 20.

[00122] Bispecific or multifunctional targeting therapeutic agents can be designed for a number of clinical applications.

[00123] Antigen binding features can be built on the scaffold either using the normal IgG like Fab arm involving the two domains in heavy chain and the VL and CL domains in the light chain.

[00124] Alternately, single chain fragment variable (scFv) which have the variable light and heavy chains linked together has been used for antigen binding.

[00125] Bispecific molecules that interact with two different receptor molecules can be useful in the activation of signaling processes that require the co-stimulation of two or more receptor molecules concurrently.

[00126] The scaffold can be employed to generate immunotoxins, immune based molecules capable of delivering toxin molecules to tumors or other cell types of interest. One arm of the asymmetric scaffold is connected to a Fab or scFv capable of recognizing a target cell surface receptor while the second chain can be linked to the toxin molecule.

[00127] Fab, scFv's or their derivatives that recognize a number of know targets can be employed.

[00128] A number of known toxins can be linked to the scaffold to achieve multifunctional protein.

Manufacture of the heterodimer

[00129] Literature provides many methods for preparing antibodies. The detection of heterodimers is done by co-expressing heavy chains with different fusion tags ((His)₆ or RFP, for example) and detection of the heterodimer formation by SDS-PAGE. Quantification is performed by Western blotting techniques.

[00130] Functionality was assayed through conformation of wild type activity of heterodimeric trastuzumab with CH3 variants of the invention as well as previously published polypeptides.

[00131] For the in vitro experiments, peripheral blood mononuclear cells (PBMCs) purified from human blood donors were used (lymphocytes, monocytes, macrophages). Surface Her-2 expressing SKBR-3 cells were used as targets. The viability of the SKBR-3 cells was measured 24 hours after the antibodies of the invention were added.

EXAMPLE 1:

Investigation of Fc-FcYRIIb engagement and inhibition.

[00132] Table 5: Fc Variants synthesized and tested:

Identifier	Mutated residues - separated by chain (chain A/chain B)	Characteristics
AZ1	L368D_K370Q/E357R_L368R	positive design salt bridge variant
AZ2	L368D_K370Q_L351I/E357R_L368R	positive design salt bridge variant
AZ3	L368D_K370Q_L351D/E357R_L368R	positive design salt bridge variant
AZ4	L368D_K370E/E357R_L368R	positive design salt bridge variant
AZ5	L368D_K370E/E357K_L368R	positive design salt bridge variant
AZ6	V397S_F405A_Y407V/K392V_T394W	negative design variant Trp
AZ7	L351R_V397S_F405A_Y407V/K392V_T394W	negative design variant Trp
AZ8	L351Y_V397S_F405A_Y407V/K392V_T394W	negative design variant Trp
AZ9	V397S_F405A_Y407V/L368R_K392V_T394W	negative design variant Trp
AZ10	V397T_F405I/K392V_T394H	negative design variant His

[00133] In design, first the CH3 region was optimized using hotspot determination, asymmetry analyses, cavity analyses, affinity mutations and heterodimer drivers, dynamics characterization for individual sites and variants, and stability and unfolding analysis.

[00134] While specific embodiments of the invention have been described and illustrated, such embodiments should be considered illustrative of the invention only and not as limiting the invention as construed in accordance with the accompanying claims.

[00135] Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

CLAIMS

What is claimed is:

1. An isolated heteromultimer comprising a first polypeptide and a second polypeptide which meet at an interface which has been engineered to promote heteromultimer formation, wherein at least one of said first polypeptide and second polypeptide comprises a variant CH3 domain forming part of the interface, and wherein said variant CH3 domain comprises modifications relative to the wild-type CH3 domain such that a greater ratio of heteromultimer:homomultimer is formed as compared to a multimer with a non-engineered interface; wherein the amino acid modifications form favorable interactions between the first polypeptide and the second polypeptide; and wherein the heteromultimer comprises first and second faces, the first face having greater binding affinity to a receptor compared to the second face.
2. The heteromultimer of claim 1, wherein the favorable interactions are one or more interactions of electrostatics, hydrogen-bonding, surface complementarity, packing, and hydrophobic contact.
3. The heteromultimer of claim 1, wherein said heteromultimer is a heterodimer
4. The heteromultimer of claim 1, wherein one polypeptide comprises a variant CH3 domain forming part of the interface, while the other polypeptide comprises a wild-type CH3 domain.
5. The heteromultimer of claim 1 wherein in the absence of the modifications; the interface has no detectable binding to the receptor.
6. The heteromultimer of claims 1, wherein first polypeptide or second polypeptide comprises amino acid modifications selected from L368D and K370Q.
7. The heteromultimer of claims 1, wherein first polypeptide or second polypeptide comprises amino acid modifications selected from E357R and L368R.
8. The heteromultimer of claims 1- wherein first polypeptide comprises the amino acid modifications L368D and K370Q; and the second polypeptide comprises the amino acid modifications E357R and L368R.
9. The heteromultimer of claim 8 wherein first polypeptide or second polypeptide further comprises the amino acid modification L351I.
10. The heteromultimer of claims 1 wherein first polypeptide comprises the amino acid modifications L351I, L368D and K370Q; and the second polypeptide comprises the amino acid modifications E357R and L368R.
11. The heteromultimer of claims 1 wherein first polypeptide comprises the amino acid modifications L351D, L368D and K370Q; and the second polypeptide comprises the amino acid modifications E357R and L368R.

12. The heteromultimer of claims 1 wherein first polypeptide comprises the amino acid modifications L368D and K370E; and the second polypeptide comprises the amino acid modifications E357R and L368R.
13. The heteromultimer of claims 1 wherein first polypeptide comprises the amino acid modifications E357W and S364F; and the second polypeptide comprises the amino acid modifications Y349A, L351Y and K370I.
14. The heteromultimer of claims 1 wherein first polypeptide comprises the amino acid modifications E357H and S364F; and the second polypeptide comprises the amino acid modifications L351Y and K370I.
15. The heteromultimer of claims 1 wherein first polypeptide comprises the amino acid modifications E357W and S364F; and the second polypeptide comprises the amino acid modifications Y349A, L351Y and K370F.
16. The heteromultimer of claims 1 wherein first polypeptide comprises the amino acid modifications E357H and S364F; and the second polypeptide comprises the amino acid modifications L351Y and K370F.
17. The heteromultimer of claims 1 wherein first polypeptide comprises the amino acid modifications E357L, T366A, K409F and T411N; and the second polypeptide comprises the amino acid modifications L351Y and Y407A.
18. The heteromultimer of claims 1 wherein first polypeptide comprises the amino acid modifications L351Y, L368T, and Y407A; and the second polypeptide comprises the amino acid modifications E357L, T366A, K409Y, and T411N.
19. The heteromultimer of claims 1, wherein if one polypeptide comprises modifications of the amino acid 366, then the other polypeptide does not comprise a modification of the amino acid 407.
20. The heteromultimer of claims 1, wherein if one polypeptide comprises modifications of the amino acid 394, then the other polypeptide does not comprise a modification of the amino acid 405.
21. The heteromultimer of claim 1, wherein one or both first and second polypeptides further comprise a CH2 domain.
22. The heteromultimer of claim 21, wherein said CH2 domain is a variant CH2 domain.
23. The heteromultimer of claim 21, wherein said variant CH2 domains comprise asymmetric amino acid substitutions in the first and/or second polypeptide chain.
24. The heteromultimer of claim 23, wherein said heteromultimer selectively binds an Fc receptor.
25. The heteromultimer of claim 24, wherein said Fc receptor is a member of Fc γ receptor family.
26. The heteromultimer of claim 25, wherein said Fc γ receptor is selected from Fc γ RI, Fc γ RIIa, Fc γ RIIb, Fc γ RIIc, Fc γ RIIIa and Fc γ RIIIb.

27. The heteromultimer of claim 25, comprising asymmetric amino acid substitutions in the CH2 domain such that one chain of said heteromultimer selectively binds an Fc receptor.
28. The heteromultimer of claim 27, wherein said Fc receptor is an Fcγ receptor.
29. The heteromultimer of claim 28, wherein said heteromultimer binds selectively to FcγRIIIa.
30. The heteromultimer of claim 29, comprising asymmetric amino acid substitutions selected from S267D, K392D and K409D.
31. The heteromultimer of claim 28, wherein said heteromultimer binds selectively to FcγRIIa.
32. The heteromultimer of claim 31, comprising asymmetric amino acid substitutions selected from S239D, K326E, A330L and I332E.
33. The heteromultimer of claim 28, wherein said heteromultimer binds selectively to FcγRIIb.
34. The heteromultimer of claim 33, comprising asymmetric amino acid substitutions selected from S239D, D265S, E269K and I332E.
35. The heteromultimer of claim 28, wherein said heteromultimer binds selectively to FcγRIIIa and FcγRIIa.
36. The heteromultimer of claim 35, comprising asymmetric amino acid substitutions selected from S239D, D265S, and S298A.
37. The heteromultimer of claim 28, wherein said heteromultimer binds selectively to FcγRIIIa and FcγRIIb.
38. The heteromultimer of claim 37, comprising asymmetric amino acid substitutions selected from S239D, S298A, K326E, A330L and I332E.
39. The heteromultimer of claim 28, wherein said heteromultimer binds selectively to FcγRIIa and FcγRIIb.
40. The heteromultimer of claim 39, comprising asymmetric amino acid substitutions selected from S239D, D265S, S298A and I332E.
41. The heteromultimer of claims 1-40, wherein said heteromultimer is a bispecific antibody.
42. The heteromultimer of claims 1-40, wherein said heteromultimer is a multispecific antibody.
43. The heteromultimer of claim 1, wherein the parent polypeptide of the first and second polypeptide is a human IgG Fc region.
44. The heteromultimer of claim 43, wherein the human IgG Fc region is a human IgG1, IgG2, IgG3, or IgG4 Fc region.
45. The heteromultimer of claim 1, wherein the CH3 domain is from an immunoglobulin selected from the group consisting of IgG, IgA, IgD, IgE and IgM.
46. The heteromultimer of claim 45, wherein the immunoglobulin is IgG.

47. The heteromultimer of claim 46, wherein the IgG is of subtype selected from the group consisting of IgG1, IgG2a, IgG2b, IgG3 and IgG4.
48. The heteromultimer of claim 47, wherein the IgG is a human IgG.
49. The heteromultimer of claim 1, wherein the heteromultimer is a bispecific antibody.
50. The heteromultimer of claim 49, wherein the heteromultimer is a bispecific immunoadhesin.
51. The heteromultimer of claim 50, wherein the heteromultimer is an antibody-immunoadhesin chimera (Ab/Ia).
52. The heteromultimer of claim 1, wherein the first polypeptide binds a target antigen and the second polypeptide comprises a toxin moiety.
53. A composition comprising the heteromultimer of claim 1 and a pharmaceutically acceptable carrier.
54. A host cell comprising nucleic acid encoding the heteromultimer of claim 50.
55. The host cell of claim 51, wherein the nucleic acid encoding the first polypeptide and the nucleic acid encoding the second polypeptide are present in a single vector.
56. The host cell of claim 51, wherein the nucleic acid encoding the first polypeptide and the nucleic acid encoding the second polypeptide are present in separate vectors.
57. A method of making a heteromultimer comprising culturing the host cell of claim 51 so that the nucleic acid is expressed and recovering the heteromultimer from the cell culture.
58. The method of claim 57, wherein the host cell is a mammalian cell.
59. The method of claim 58, wherein the heteromultimer is recovered from the cell culture media.
60. The heteromultimer of claim 1, wherein the heteromultimer comprises at least one therapeutic antibody.
61. The heterodimer of claim 60, wherein said therapeutic antibody binds a cancer target antigen.
62. The heteromultimer of claim 61, wherein said therapeutic antibody is selected from the group consisting of abagovomab, adalimumab, alemtuzumab, aurograb, bapineuzumab, basiliximab, belimumab, bevacizumab, briakinumab, canakinumab, catumaxomab, certolizumab pegol, cetuximab, daclizumab, denosumab, efalizumab, galiximab, gemtuzumab ozogamicin, golimumab, ibritumomab tiuxetan, infliximab, ipilimumab, lumiliximab, mepolizumab, motavizumab, muromonab, mycograb, natalizumab, nimotuzumab, ocrelizumab, ofatumumab, omalizumab, palivizumab, panitumumab, pertuzumab, ranibizumab, reslizumab, rituximab, teplizumab, tocilizumab/atlizumab, tositumomab, trastuzumab, ProxiniumTM, RencarexTM, ustekinumab, zalutumumab, and any other antibodies.
63. The heteromultimer of claim 57, wherein said target antigen is selected from the group consisting of a-chain (CD25) of IL-2R, Amyloid beta, anti-EpCAM x anti-CD3, BLyS (or BAFF), CD11a, 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 receptor,

Integrin alpha-4/beta-1, Mucin 16 / CA-125, RANKL, TNF alpha, VEGF-A, and other therapeutically advantageous targets.

64. A method of treating cancer in a patient having a cancer characterized by a cancer antigen, said method comprising administering to said patient a therapeutically effective amount of a heteromultimer of claim 57.

65. The method of claim 64, wherein said patient is human.

66. A method of treating immune disorders in a patient having an immune disorder characterized by an immune antigen, said method comprising administering to said patient a therapeutically effective amount of a heteromultimer of claim 60.

67. A method of designing multi-functional therapeutics comprising heteromultimer of claim 1.

68. A method of designing bi-functional therapeutics comprising heteromultimer of claim 1.

69. A method for the design of asymmetric mutations in the CH2 domain of an heterodimeric Fc derived with mutations in the CH3 domain.

70. A method to design selectivity for the different Fc gamma receptors based on the mutations in the asymmetric Fc of claim 69.

71. A method to design mutations that bias binding of the Fc gamma receptors to one face of the Fc molecule.

72. A polypeptide comprising mutations in the CH2 domain of the asymmetric Fc of claim 69 that lead to preferential Fc gamma receptor selectivity profiles.

73. A method to design polarity drivers that bias the Fc γ receptors to interact with only one face of the asymmetric Fc scaffold of claim 66.

74. Heteromultimer of claim 1 comprising mutations in the CH3 domain that lead to preferential formation of heterodimeric Fc.

75. A method to design bispecific therapeutic entities based on the asymmetric Fc of claim 69.

76. A method to design multifunctional therapeutic entities based on the asymmetric Fc of claim 69.

HETERODIMERIC ANTIBODY DESIGN AND CONSTRUCTS

ABSTRACT

[00136] The provided scaffolds have heavy chains that are asymmetric in the various domains to accomplish selectivity between the various Fc receptors involved in modulating effector function, beyond those achievable with a natural homodimeric (symmetric) Fc molecule. These therapeutic novel molecules comprise complexes of heterogeneous components designed to alter the natural way antibodies behave.

FIG. 1

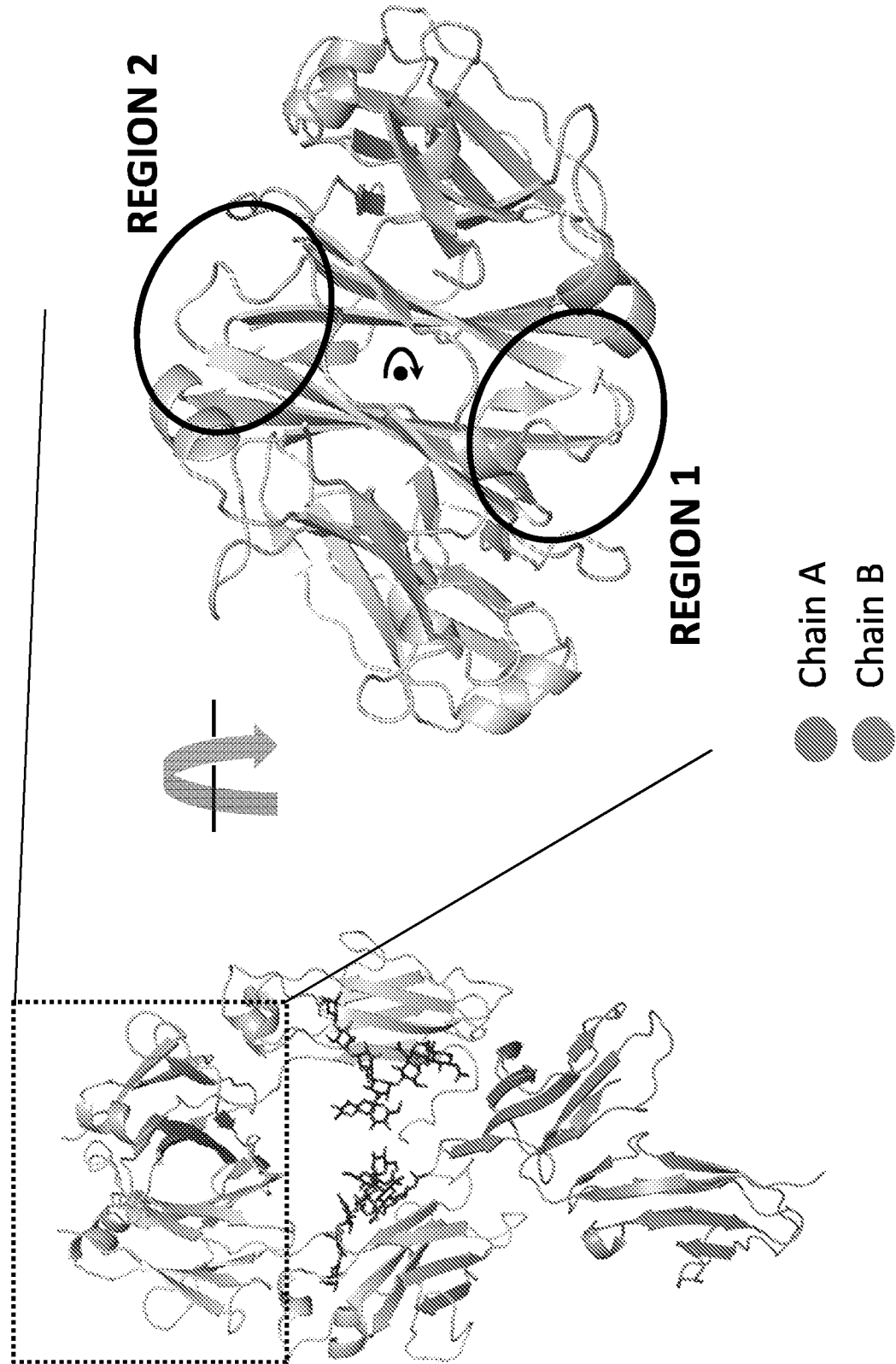
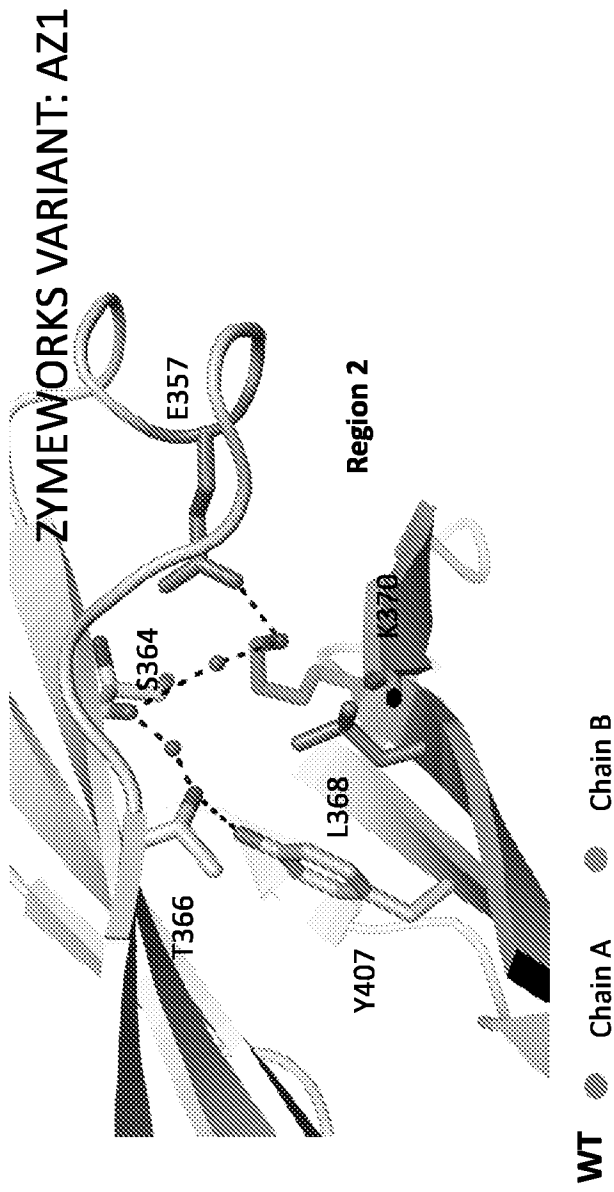
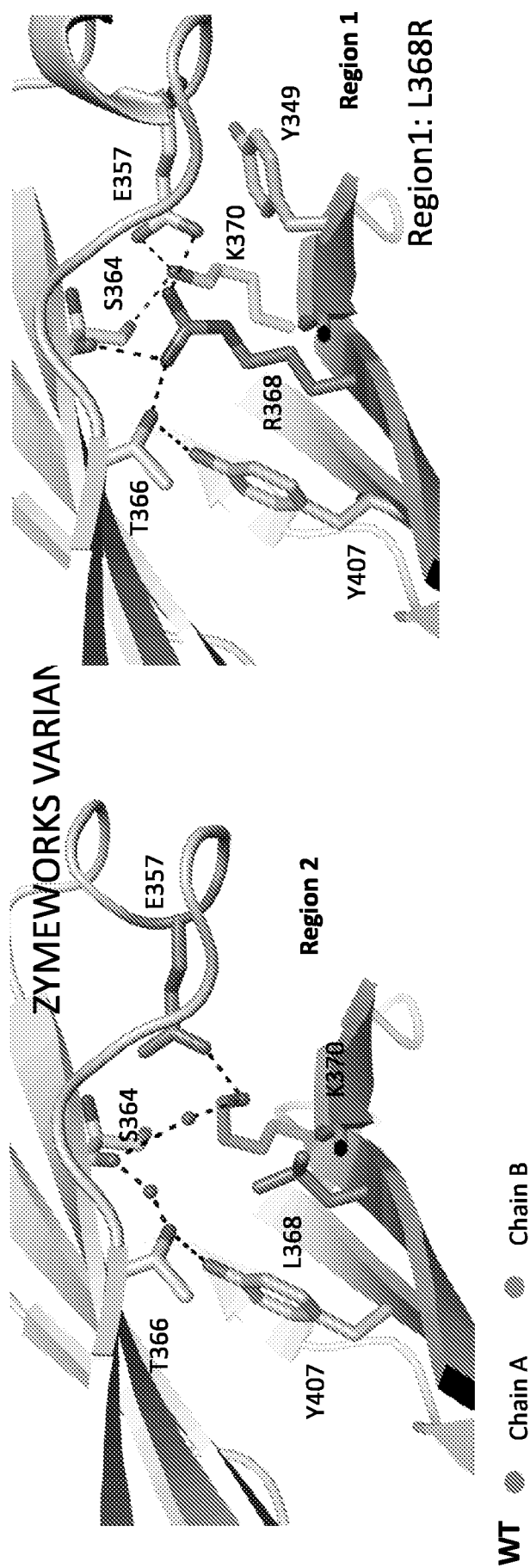


FIG. 2



- L368 faces a critical natural cavity

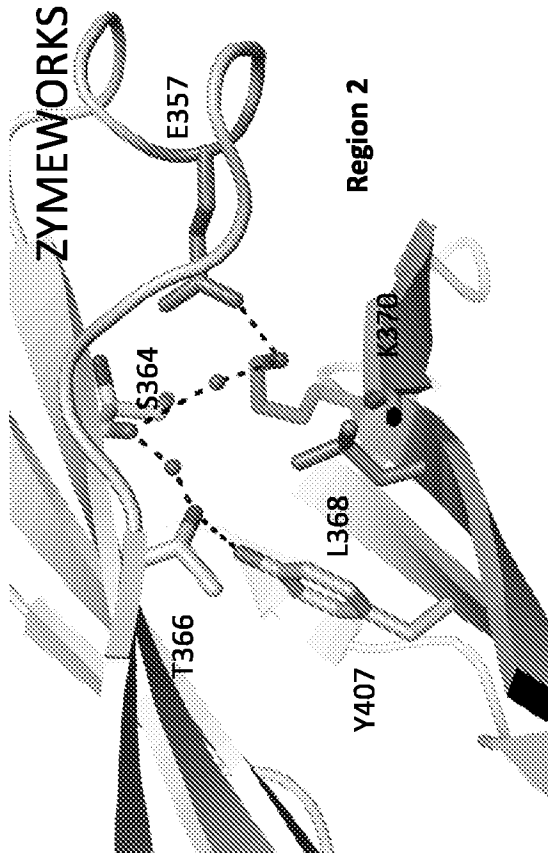
FIG. 3



- L368 faces a critical natural cavity
- L368R increases stability by improving packing in Region 1

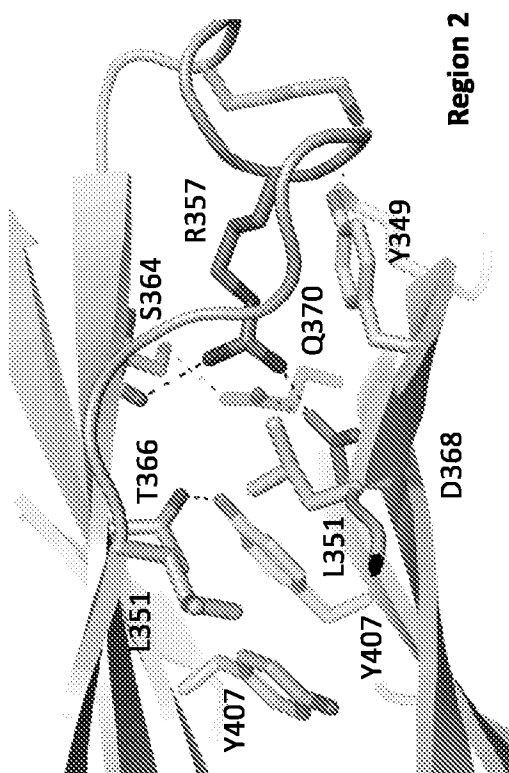
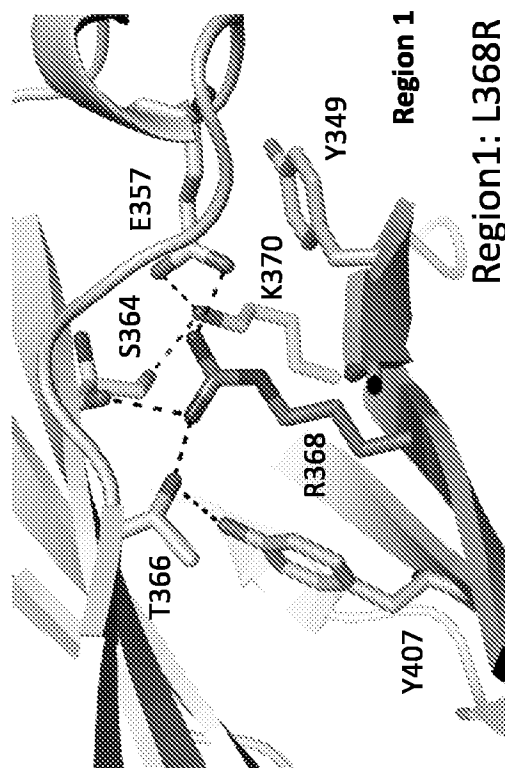
FIG. 4

ZYMEWORKS VARIANT



WT ● Chain A ● Chain B

- L368 faces a critical natural cavity
- L368R increases stability by improving packing in Region 1
- Additional mutations create favorable electrostatic interactions

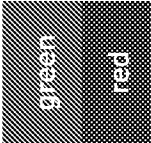


Region 2: E357R / L368D_K370Q

FIG. 5

VARIANTS: AZ1, AZ2, AZ3

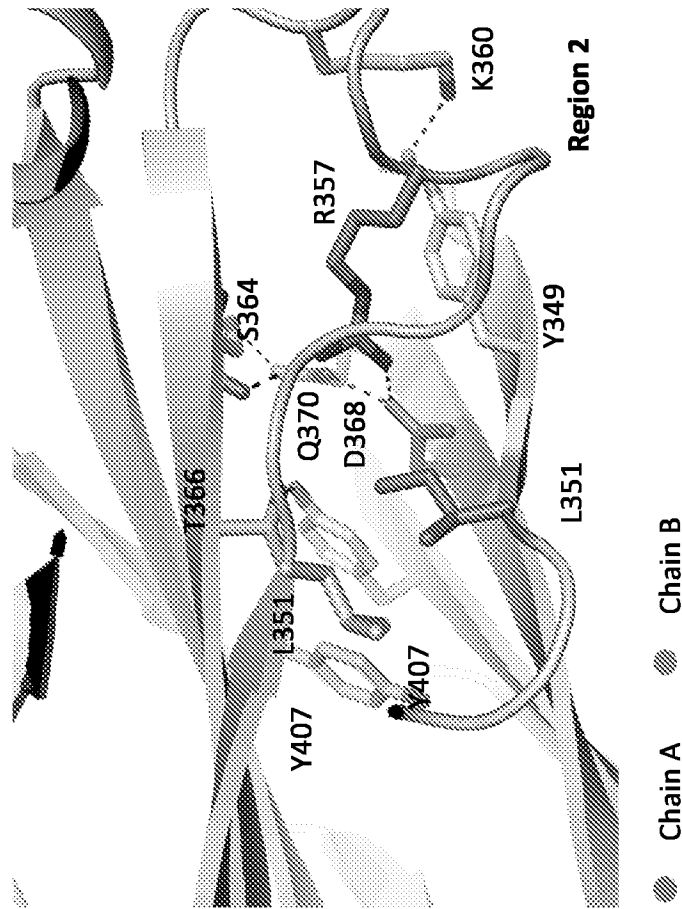
	clash score (kcal/mol) - wt +	Δ interface area ($\text{\AA}^2$) - wt +	Δ packing (sd) - wt +	Δ electrostatic energy (kcal/mol) - wt +	affinity score - wt +
Heterodimer	Amgen	-26.2	-46.0	12.3	2.1
	AZ1	-14.6	9.9	-12.3	-0.4
Homodimer	Amgen (chain A)	-33.6	-0.5	94.4	N/A
	Amgen (chain B)	-11.5	-0.1	69.5	N/A
	AZ1 (chain A)	-36.6	-0.2	87.3	N/A
	AZ1 (chain B)	-21.3	-0.1	30.76	N/A



Favorable
Unfavorable

FIG. 6

L368R_E357R / L368D_K370Q_ZA151I HEWORKS VARIANT: AZ2, AZ3

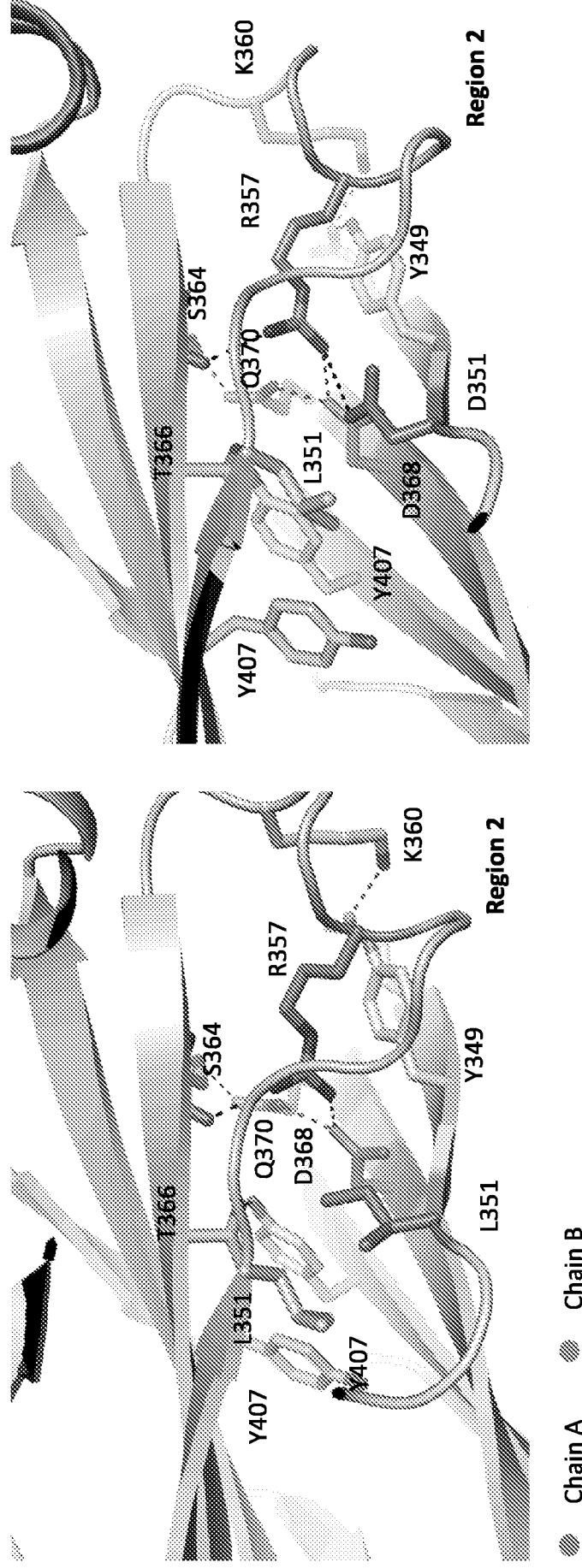


- Variants AZ 2 and 3 build on AZ 1
- L351I (AZ2) is *negatively* designed and favors heterodimer formations by improving packing

FIG. 7

L368R_E357R / L368D_K370Q_L351I

AZ2, AZ3 L368R_E357R / L368D_K370Q_L351D

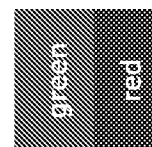


- Variants AZ 2 and 3 build on AZ 1
- L351I (AZ2) is *negatively* designed and favors heterodimer formations by improving packing
- L351D (AZ3) is *positively* designed and favors heterodimers formation by improving electrostatic interactions

FIG. 8

VARIANTS: AZ1, AZ2, AZ3

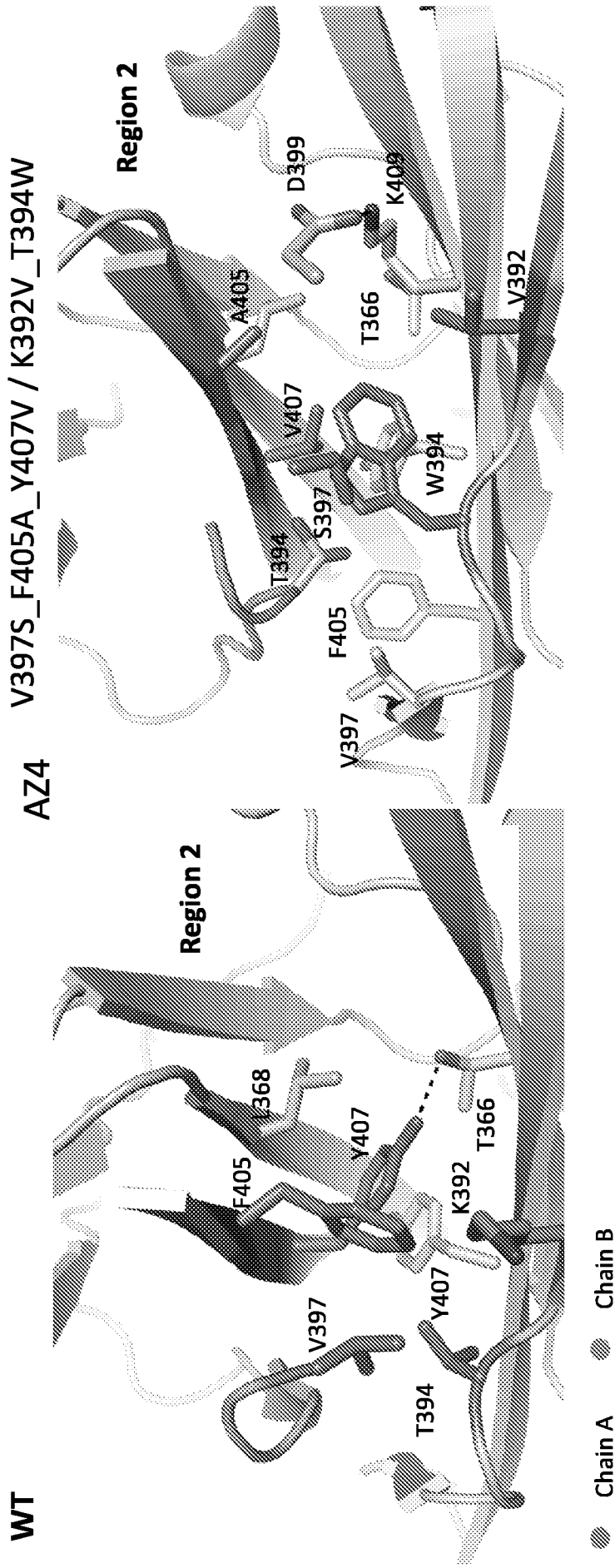
	clash score (kcal/mol) - wt +	Δ interface area ($\text{\AA}^2$) - wt +	Δ packing (sd) - wt +	Δ electrostatic energy (kcal/mol) - wt +	affinity score - wt +
Heterodimer	Amgen	-46.0	-0.41	12.3	2.1
	AZ1	9.9	0.09	-12.3	-0.4
	AZ2	0.7	0.01	-17.2	-0.4
	AZ3	-16.1	0.07	-6.4	-0.4
Homodimer	Amgen (chain A)	9.3	-0.5	94.4	N/A
	Amgen (chain B)	-77.9	-0.1	69.5	N/A
	AZ1 (chain A)	23.1	-0.2	87.3	N/A
	AZ1 (chain B)	-50.8	-0.1	30.76	N/A
	AZ2 (chain B)	-123.5	-0.3	52.2	N/A
	AZ3 (chain B)	-80.2	-0.4	60.6	N/A



Favorable

Unfavorable

FIG. 9



- T394W is negatively designed at the interface core to drive heterodimer formation
- Surrounding mutations are designed to optimize packing and rescue stability

FIG. 10

AZ4, AZ5, AZ6

	clash score (kcal/mol) - wt +	Δ interface area ($\text{\AA}^2$) - wt +	Δ packing (sd) - wt +	Δ electrostatic energy (kcal/mol) - wt +	affinity score - wt +
Heterodimer					
	Genentech	8.4	-0.1	8.1	0.7
	AZ4	-31.1	0.2	3.03	-0.7
Homodimer	Genentech (chain A)	-4.7	N/A	15.6	N/A
	Genentech (chain B)	-71.7	-0.3	2.3	N/A
	AZ4 (chain A)	553.6	N/A	32.1	N/A
	AZ4 (chain B)	5.6	-0.5	6.87	N/A

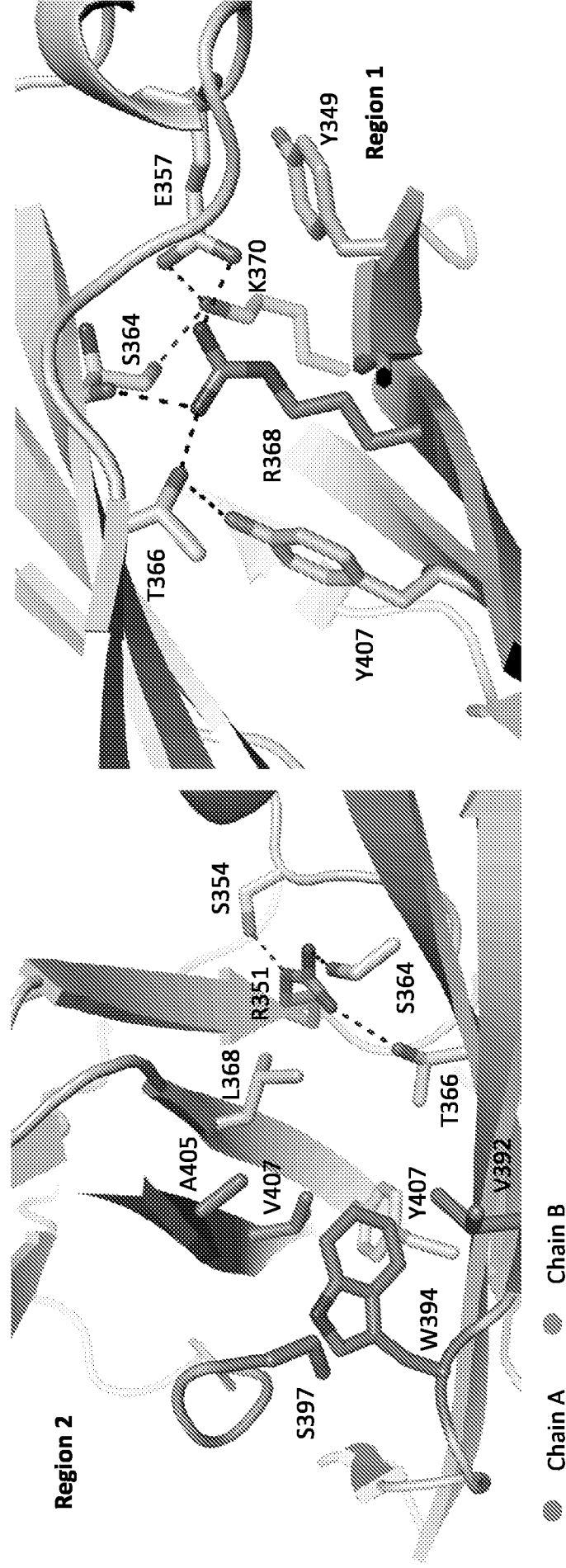
green

red

Favorable
Unfavorable

FIG. 11

L351R_V397S_F405A_Y407V / K392V_T394W AZ5, ~~AZ6~~ X397S_F405A_Y407V / K392V_T394W_L368R

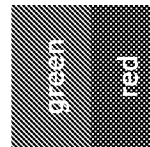


- The possible destabilizing effect of negative design (AZ4) is rescued by adding:
 - L351R in AZ5 to improve electrostatic interactions in Region 2
 - L368R in AZ6 to improve packing and electrostatic interactions in Region 1

FIG. 12

AZ4, AZ5, AZ6

	clash score (kcal/mol) - wt +	Δ interface area ($\text{\AA}^2$) - wt +	Δ packing (sd) - wt +	Δ electrostatic energy (kcal/mol) - wt +	affinity score - wt +
Heterodimer					
	Genentech	8.4	-0.1	8.1	0.7
	AZ4	-31.1	0.2	3.03	-0.7
	AZ5	-25.1	0.2	-5.5	-0.8
Homodimer	AZ6	-32.5	0.0	-11.7	-0.3
	Genentech (chain A)	-4.7	N/A	15.6	N/A
	Genentech (chain B)	-71.7	-0.3	2.3	N/A
	AZ4 (chain A)	5.1	N/A	32.1	N/A
	AZ4 (chain B)	-115.5	-0.5	6.87	N/A
	AZ5 (chain A)	-82.3	N/A	35.5	N/A
	AZ6 (chain B)	-100.9	-0.3	-16.1	N/A



Favorable
Unfavorable

FIG. 13

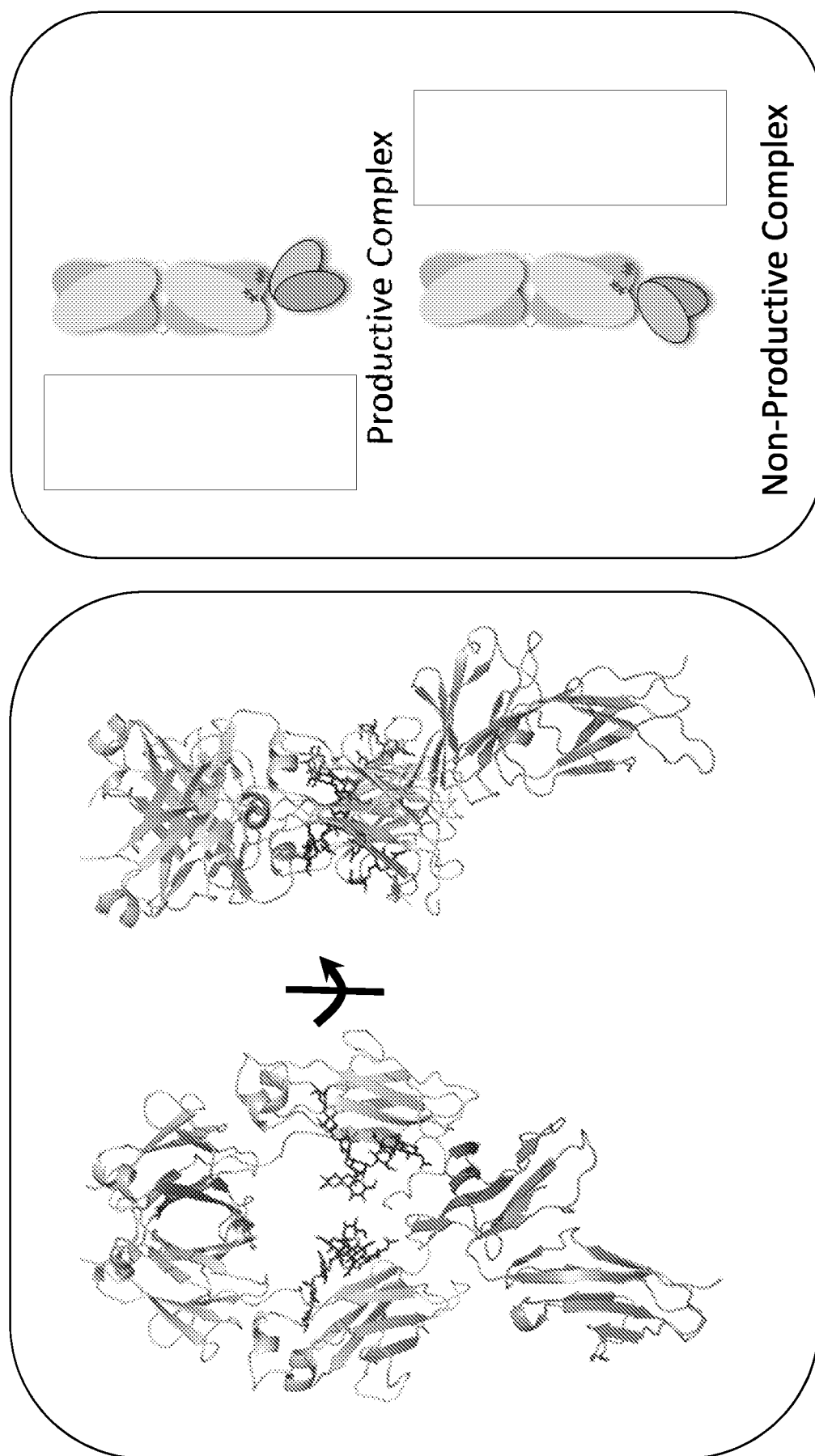
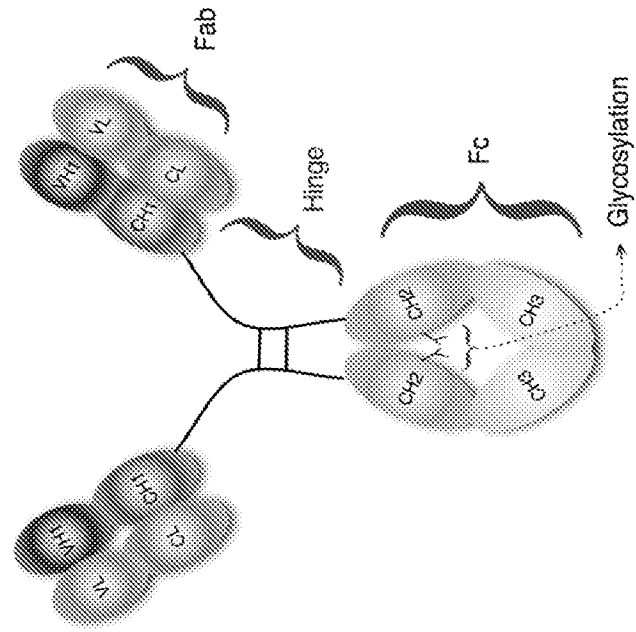


Fig. 14



Fim. 15

[illegible]

Fig. 16

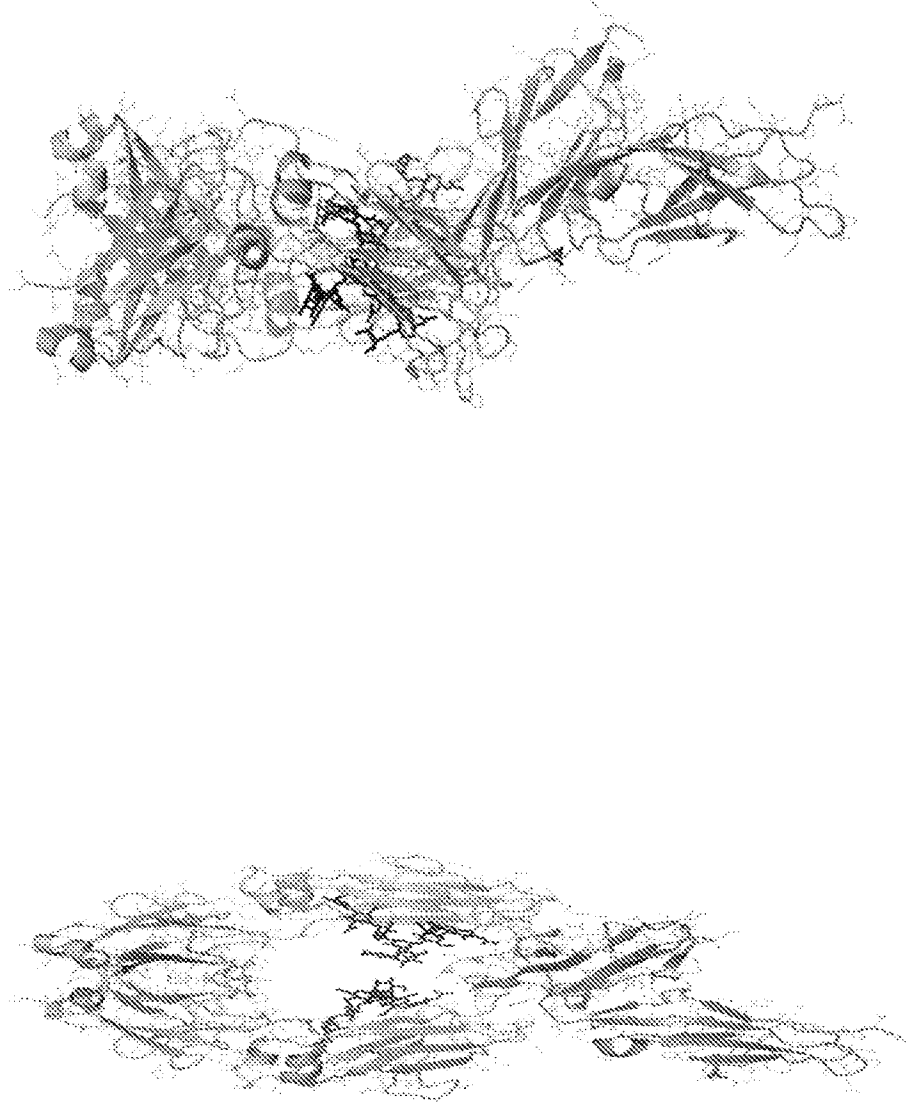


Fig. 17

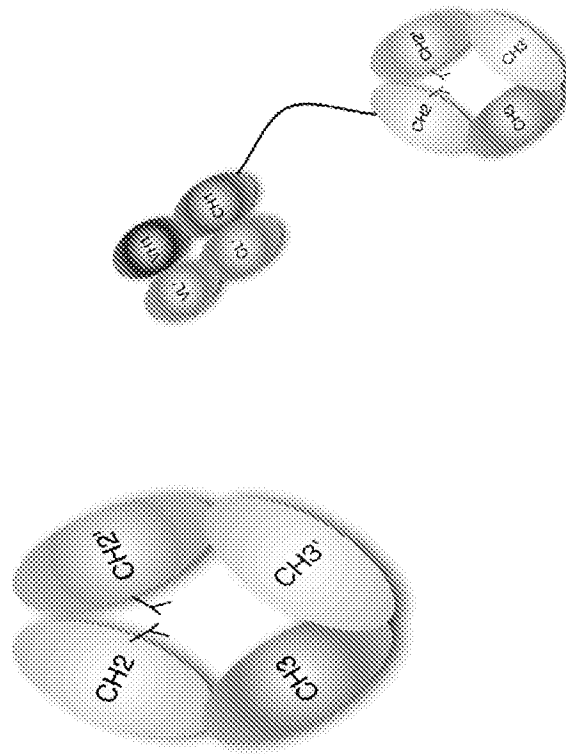


Fig. 18

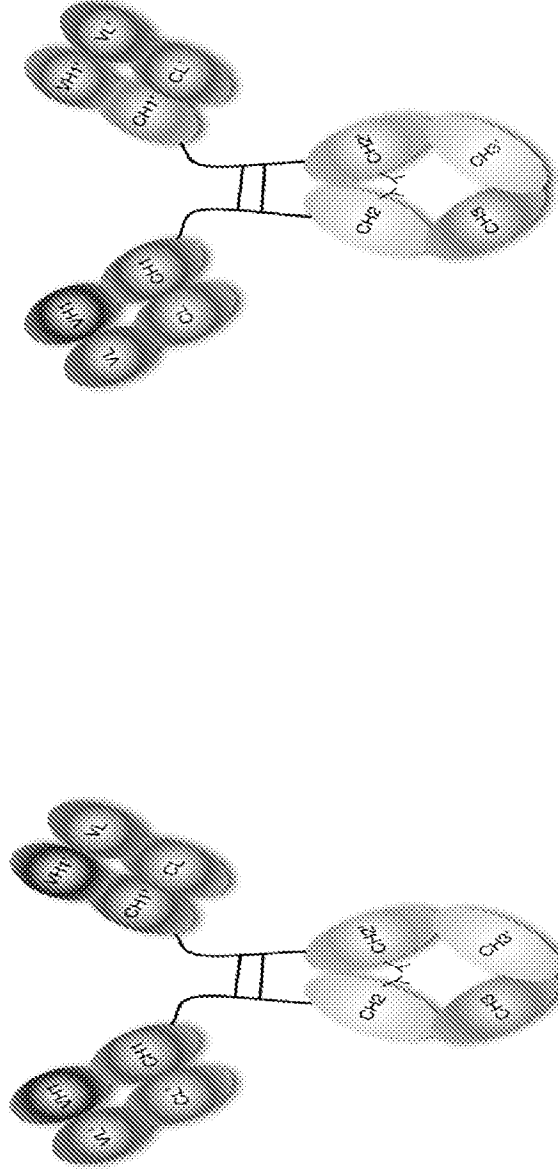


Fig. 19

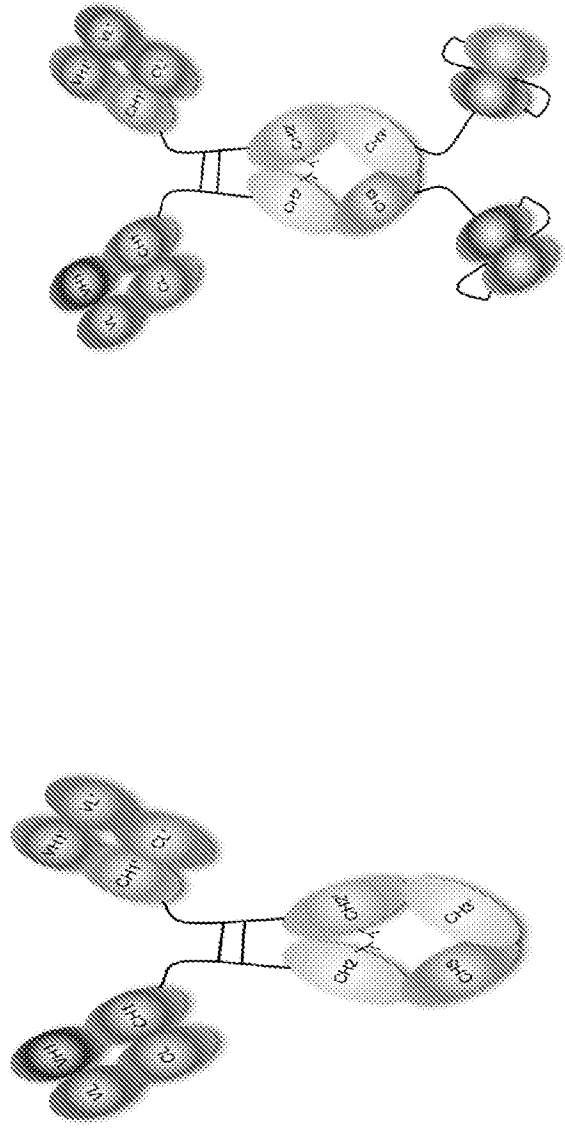


Fig. 20

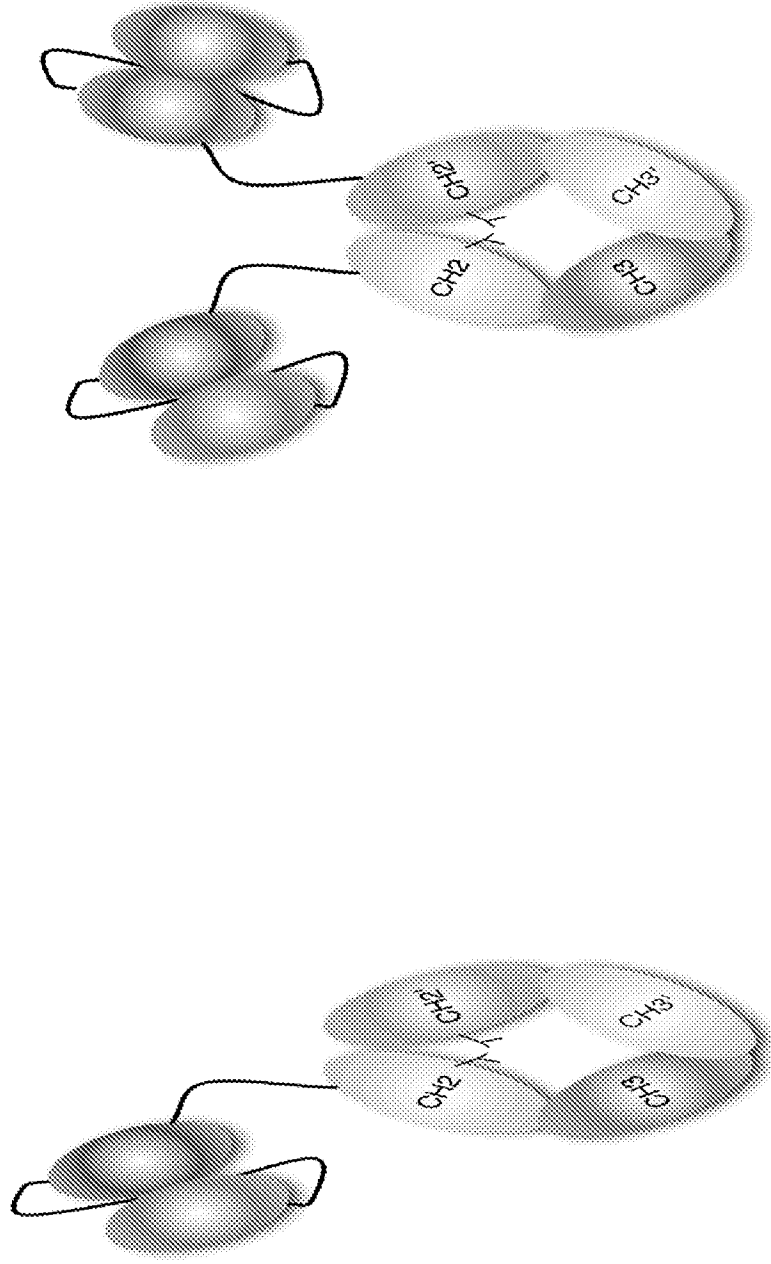


Fig. 21

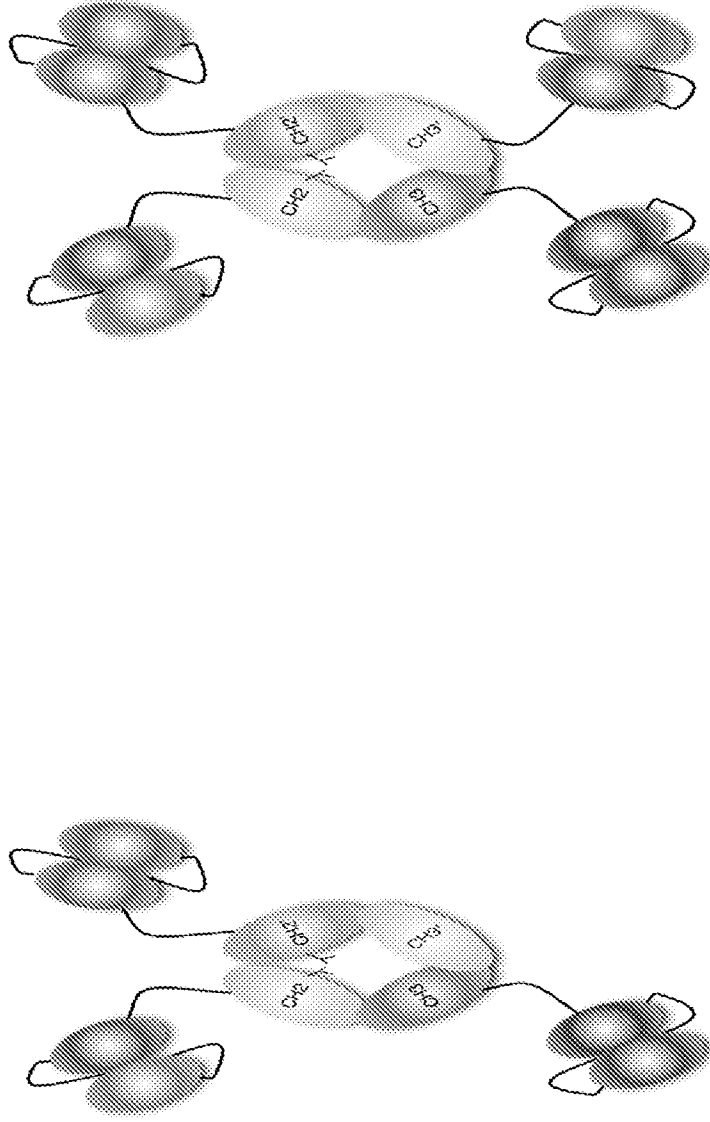


Figure 22

