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(71) Applicant: NGM BIOPHARMACEUTICALS, INC.

[US/US]; 333 Oyster Point Boulevard, South San Francisco, CA 94080 (US).

(72) Inventors: CHENG, Rujin; 333 Oyster Point Boulevard, South San Francisco, CA 94080 (US). FAN, Bin; 333 Oyster Point Boulevard, South San Francisco, CA 94080 (US). HANSEN, Simon; 333 Oyster Point Boulevard, South San Francisco, CA 94080 (US).

(74) Agent: LIANG, Bing et al.; Jones Day, 250 Vesey Street, New York, NY 10281-1047 (US).

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(54) Title: MULTIMERIZATION OF BINDING MOLECULES HAVING AN ANTIBODY CONSTANT REGION VARIANT

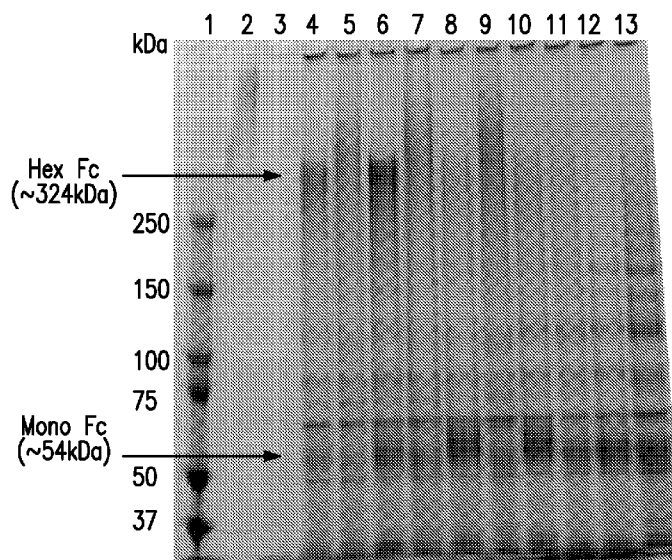


FIG. 1A

(57) Abstract: Molecules that are engineered to form an oligomer, wherein each of the molecules comprises an IgG C_H2 region with the position 253 by EU numbering substituted to be a cysteine and/or a human μ tailpiece.



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Multimerization of Binding Molecules Having an Antibody Constant Region Variant

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 63/180,969 filed April 28, 2021, the disclosure of which is incorporated by reference herein in its entirety.

SEQUENCE LISTING

[0002] This application incorporates by reference in its entirety a Sequence Listing submitted with this application as a text file entitled "13370-120-228_Sequence_Listing_ST25.txt", created on April 25, 2022, and is 26,426 bytes in size.

1. FIELD

[0003] Provided herein is a molecule comprising an engineered IgG C_{H2} region and oligomer complexes comprising same. Further provided herein are a pharmaceutical composition comprising the molecules or oligomers described herein, a host cell, nucleic acids, a vector that are related to the molecules described herein, and a method of producing and using such molecules and oligomers.

2. BACKGROUND

[0004] In the field of antibody therapeutics, design and generations of novel recombinant antibodies or derivatives have gained tremendous attention and made continuous progress. Specifically, novel designs are desirable for overcoming limitations of native antibodies, and for providing advantages such as improvement in antibody half-life, pharmacokinetics, stability, avidity, blood clearance, tissue or target cell penetration and retention, *etc.* The compositions and methods described herein satisfy this need and provide related advantages.

3. SUMMARY

[0005] In one aspect, provided herein is a molecule comprising an IgG C_{H2} region, wherein position 253 in the IgG C_{H2} region by EU numbering is substituted to be a cysteine.

[0006] In some embodiments, the molecule further comprises an IgG hinge region. In other embodiments, the molecule further comprises an IgG C_{H3} region. In yet other embodiments, the

molecule further comprises a human μ tailpiece. In still yet other embodiments, the molecule further comprises an IgG C_{H1} region.

[0007] In some specific embodiments, the human μ tailpiece comprises an amino acid sequence of SEQ ID NO: 1 or an amino acid sequence having at least 75%, 80%, 85%, or 90% identity to SEQ ID NO: 1. In other specific embodiments, the human μ tailpiece is conjugated to the C-terminus of the IgG C_{H2} region. In yet other specific embodiments, the human μ tailpiece is conjugated to the C-terminus of the IgG C_{H3} region.

[0008] In some embodiments, the IgG is a human IgG. In some specific embodiments, the human IgG is a human IgG1. In other specific embodiments, the human IgG is a human IgG2. In yet other specific embodiments, the human IgG is a human IgG3. In still yet other specific embodiments, the human IgG is a human IgG4.

[0009] In some embodiments, the molecule further comprises a binding domain that specifically binds to a target. In some specific embodiments, the binding domain is an antibody fragment. In other embodiments, the molecule is an antibody or antigen binding fragment thereof.

[0010] In one aspect, provided herein is an oligomer comprising two or more molecules as described herein. In another aspect, provided herein is an isolated nucleic acid encoding the molecule as described herein. In another aspect, provided herein is a vector comprising the nucleic acid as described herein.

[0011] In another aspect, provided herein is an oligomer, which comprises two or more molecules, and each molecule comprises an IgG C_{H2} region, wherein position 253 in the IgG C_{H2} region by EU numbering is substituted to be a cysteine.

[0012] In some embodiments, the molecule further comprises an IgG hinge region. In other embodiments, the molecule further comprises an IgG C_{H3} region. In yet other embodiments, the molecule further comprises a human μ tailpiece. In still yet other embodiments, the molecule further comprises an IgG C_{H1} region.

[0013] In some specific embodiments, the human μ tailpiece comprises an amino acid sequence of SEQ ID NO: 1 or an amino acid sequence having at least 75%, 80%, 85%, or 90% identity to SEQ ID NO: 1. In other specific embodiments, the human μ tailpiece is conjugated to

the C-terminus of the IgG C_{H2} region. In yet other specific embodiments, the human μ tailpiece is conjugated to the C-terminus of the IgG C_{H3} region.

[0014] In some embodiments, the IgG is a human IgG. In some specific embodiments, the human IgG is a human IgG1. In other specific embodiments, the human IgG is a human IgG2. In yet other specific embodiments, the human IgG is a human IgG3. In still yet other specific embodiments, the human IgG is a human IgG4.

[0015] In some embodiments, the molecule further comprises a binding domain that specifically binds to a target. In some specific embodiments, the binding domain is an antibody fragment. In other embodiments, the molecule is an antibody or antigen binding fragment thereof.

[0016] In some embodiments, the oligomer is a pentamer. In other embodiments, the oligomer is a hexamer.

[0017] In some embodiments, the oligomer is homomeric and the two or more molecules bind to the same target. In other embodiments, the oligomer is heteromeric. In some specific embodiments, the two or more molecules bind to two or more different targets.

[0018] In another aspect, provided herein is a pharmaceutical composition, which comprises the molecule as described herein, the oligomer as described herein, the isolated nucleic acid as described herein, or the vector as described herein, and a pharmaceutically acceptable excipient. In another aspect, provided herein is a method for treating a disease or disorder in a subject comprising administering to the subject the pharmaceutical composition as described herein.

[0019] In yet another aspect, provided herein is a method of making an oligomer comprising two or more molecules each comprising an IgG C_{H2} region, and the method comprises introducing into each molecule cysteine amino acid substitution at position 253 by EU numbering in the IgG C_{H2} region.

[0020] In still yet another aspect, provided herein is a method of producing oligomerized molecules, which comprises (i) introducing the vector of claim 40 to a host cell; (ii) cultivating the host cell under suitable conditions for the production of the oligomerized molecules; and (iii) purifying the oligomerized molecules.

4. BRIEF DESCRIPTION OF THE DRAWINGS

[0021] FIGS. 1A-1B: analysis of transient expression of human IgG1 and variants thereof.

FIG. 1A: Analysis of transient expression of different human IgG1 (huIgG1) Fc- μ tp and huIgG1 Fc- α tp variants and controls by SDS-PAGE. Lane 1 depicts protein ladder; lane 2 depicts IgM positive control (250 mg/L); lane 3 depicts Expi293 expression system without transfection; lane 6 depicts huIgG1 Fc with I253C mutation followed by an immunoglobulin μ chain tail piece (μ tp) (SEQ ID NO: 3); lane 7 depicts huIgG1 Fc with I253C mutation followed by an immunoglobulin α chain tail piece (α tp); lane 8 depicts huIgG1 Fc with Q438C mutation followed by a μ tp (SEQ ID NO: 5); lane 9 depicts huIgG1 Fc with Q438C mutation followed by an α tp; lane 10 depicts huIgG1 Fc with Y436C mutation followed by a μ tp (SEQ ID NO: 4); lane 11 depicts huIgG1 Fc with Y436C mutation followed by an α tp; lane 12 depicts huIgG1 Fc followed by a μ tp; lane 13 depicts huIgG1 Fc followed by an α tp. **FIG. 1B:** Analysis of transient expression of different human IgG1 (huIgG1) Fc- μ tp variants and controls by SDS-PAGE. Lane 1 depicts protein ladder; lane 2 depicts purified IgG1 positive control (100 mg/L); lane 3 depicts purified IgG1 positive control (200 mg/L); lane 4 depicts conditioned medium from Expi293 expression system without transfection; lane 5 depicts huIgG1 Fc with H310C mutation followed by an immunoglobulin μ chain tail piece (μ tp) [huIgG1 Fc (H310C)- μ tp]; lane 6 depicts huIgG1 Fc with L251C, I253G and S254C mutations followed by an immunoglobulin μ chain tail piece (μ tp) [huIgG1 Fc (L251C, I253G, and S254C)- μ tp]; lane 7 depicts huIgG1 Fc with S254C and N434C mutations followed by an immunoglobulin μ chain tail piece (μ tp) [huIgG1 Fc (S254C and N434C)- μ tp]; lane 8 depicts huIgG1 Fc with L251C and S254C mutations followed by an immunoglobulin μ chain tail piece (μ tp) [huIgG1 Fc (L251C and S254C)- μ tp]. **FIG 1C:** Analysis of transient expression of different human IgG1 (huIgG1) Fc- μ tp variants and controls by SDS-PAGE. Lane 1 depicts protein ladder; lane 2 depicts purified IgG1 positive control (100 mg/L); lane 3 depicts purified IgG1 positive control (200 mg/L); lane 4 depicts conditioned medium from Expi293 expression system without transfection; lane 5 depicts huIgG1 Fc with N286C mutation followed by an immunoglobulin μ chain tail piece (μ tp) [huIgG1 Fc (N286C)- μ tp].

[0022] FIGS. 2A-2C: purification of huIgG1 Fc with I253C followed by a μ tp (huIgG1 Fc (I253C)- μ tp). **FIG. 2A:** purification with MabSelectTM protein A resin. **FIG. 2B:** purification

with CaptureSelect™ FcXL Affinity Matrix. **FIG. 2C**: purification with anion exchange chromatography.

[0023] FIGS. 3A-3D: characterization of huIgG1 Fc (I253C)-μtp. **FIG. 3A**: intact mass analysis of purified huIgG1 Fc (I253C)-μtp; **FIG. 3B**: HPLC-SEC analysis of huIgG1 Fc (I253C)-μtp overlaid with BioRad gel filtration protein standard; **FIG. 3C**: HPLC-SEC analysis of huIgG1 Fc (I253C)-μtp: quantification of purity by integration. **FIG. 3D**: Thermal stability analysis of huIgG1 Fc (I253C)-μtp by nano-format of Differential Scanning Fluorimetry (nano DSF).

[0024] FIG. 4: expression of IgG hexamers by SDS-PAGE. Lane 1 depicts purified human IgG1 positive control (100 mg/L); lane 2 depicts purified human IgG1 hexamer positive control (100 mg/L); lane 3 depicts conditioned medium from Expi293 expression system without transfection; lane 4 depicts anti-beta blotho huIgG1 hexamer; lane 5 depicts anti-GDNF Family Receptor Alpha Like (GFRAL) huIgG1 hexamer; lane 6 depicts anti-vascular endothelial growth factor (VEGF) huIgG1 hexamer.

5. DETAILED DESCRIPTION

[0025] The present disclosure is based in part on the surprising finding that certain amino acid substitution at an antibody constant region (*i.e.*, substitution to cysteine at position 253 by EU numbering in IgG C_H2 region) can increase the formation of oligomers such as hexamers as demonstrated in Section 6 below. The number of inter-molecule bonds among congregate of molecules determines the level of the complexity of the corresponding oligomers that can possibly be formed. If a molecule only contains one inter-molecule binding site, only dimers can be formed, since the only inter-molecule binding site of each molecule is occupied after forming bonds with the other component of the dimer, and no other binding sites are available for forming oligomers with more complex structures. In contrast, if a molecule contains more than one inter-molecule binding sites, for this given molecule, more than one bond can be formed with more than one binding partner. Therefore, oligomers with more complex structures may be formed among congregate of such molecules. In addition, the nature and location of the amino

acid residues within a molecule that can form inter-molecule bonds significantly impact the efficiency for formation of oligomers.

[0026] In one aspect, provided herein are molecules engineered to form oligomers. Also provided herein is an oligomer formed by the disclosed molecules. Also provided herein is a pharmaceutical composition comprising the molecules and the oligomers provided herein. Also provided herein is a kit comprising the compositions provided herein. Additionally provided herein are nucleic acid molecules, vectors and host cells that express the disclosed molecules. Further provided herein are methods of generating the molecules and oligomers provided herein. In yet further aspect, provided herein are methods for using the present molecules and oligomers.

5.1 Definitions

[0027] Techniques and procedures described or referenced herein include those that are generally well understood and/or commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in Sambrook et al., *Molecular Cloning: A Laboratory Manual* (3d ed. 2001); *Current Protocols in Molecular Biology* (Ausubel et al. eds., 2003); *Therapeutic Monoclonal Antibodies: From Bench to Clinic* (An ed. 2009); *Monoclonal Antibodies: Methods and Protocols* (Albitar ed. 2010); and *Antibody Engineering* Vols 1 and 2 (Kontermann and Dübeler eds., 2d ed. 2010). Unless otherwise defined herein, technical and scientific terms used in the present description have the meanings that are commonly understood by those of ordinary skill in the art. For purposes of interpreting this specification, the following description of terms will apply and whenever appropriate, terms used in the singular will also include the plural and vice versa. In the event that any description of a term set forth conflicts with any document incorporated herein by reference, the description of the term set forth below shall control.

[0028] As used herein, the term “disulfide bonds” or “disulfide bonding” refers to a covalent link between the sulfur atoms of the thiol groups (–SH) in certain residues of a molecule. The bond is formed upon oxidation of the two thiols, thus linking the two residues and their respective main body of the molecules by the covalent disulfide bond. Specifically when the molecule is referenced to a polypeptide or its derivative, disulfide bonds are typically in a configuration of six atoms, $C\alpha - C\beta - S\gamma - S'\gamma - C'\beta - C'\alpha$, linking two amino acid residues.

[0029] As used herein, the term “EU numbering” refers to a widely used numbering scheme for the constant domain (including portions of the C_H1, hinge, and the Fc). *See* Kabat, E.A. (1991) Sequences of Proteins of Immunological Interest: Tabulation and Analysis of Amino Acid and Nucleic Acid Sequences of Precursors, V-Regions, C-Regions, J-Chain, T-Cell Receptors for Antigen T-Cell Surface Antigens, [Beta]2-Microglobulins, Major Histocompatibility Antigens, Thy-1, Complement, C-Reactive Protein, Thymopoietin, Integrins, PostGamma Globulin, [Alpha]2-Macroglobulins, and Other Related Proteins, 5th edn, National Institutes of Health.

[0030] As used herein, the term “oligomer,” “oligomerized molecule,” or “multimerized molecule” used herein refer to a structure of a complex that consists of several similar or identical repeating units. The oligomer can be dimer, triplet, tetramer, pentamer, hexamer, heptamer, octamer, nonamer, decamer, undecamer, twelve-mer, thirteen-mer, fourteen-mer, fifteen-mer, sixteen-mer, seventeen-mer, eighteen-mer, nineteen-mer, twenty-mer, *etc.* which correspond to a complex that consists of two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, *etc.* similar or identical repeating units.

[0031] The term “antibody,” “immunoglobulin,” or “Ig” is used interchangeably herein, and is used in the broadest sense and specifically covers, for example, monoclonal antibodies (including agonist, antagonist, neutralizing antibodies, full length or intact monoclonal antibodies), antibody compositions with polyepitopic or monoepitopic specificity, polyclonal or monovalent antibodies, multivalent antibodies, multispecific antibodies (*e.g.*, bispecific antibodies so long as they exhibit the desired biological activity), formed from at least two intact antibodies, single chain antibodies, and fragments thereof (*e.g.*, domain antibodies) as described below. The term also includes all antibody variants including those carrying various mutations (*e.g.*, in constant regions or Fc region) and other modifications (*e.g.*, with additional peptide sequence to the C-terminus or N-terminus). An antibody can be human, humanized, chimeric and/or affinity matured, as well as an antibody from other species, for example, mouse, rabbit, llama, *etc.* The term “antibody” is intended to include a polypeptide product of B cells within the immunoglobulin class of polypeptides that is able to bind to a specific molecular antigen and is composed of two identical pairs of polypeptide chains, wherein each pair has one heavy chain

(about 50-70 kDa) and one light chain (about 25 kDa), each amino-terminal portion of each chain includes a variable region of about 100 to about 130 or more amino acids, and each carboxy-terminal portion of each chain includes a constant region. *See, e.g., Antibody Engineering* (Borrebaeck ed., 2d ed. 1995); and Kuby, *Immunology* (3d ed. 1997). Antibodies also include, but are not limited to, synthetic antibodies, recombinantly produced antibodies, single domain antibodies including from Camelidae species (*e.g.*, llama or alpaca) or their humanized variants, intrabodies, anti-idiotypic (anti-Id) antibodies, and functional fragments (*e.g.*, antigen-binding fragments) of any of the above, which refers to a portion of an antibody heavy or light chain polypeptide that retains some or all of the binding activity of the antibody from which the fragment was derived. Non-limiting examples of functional fragments (*e.g.*, antigen-binding fragments) include single-chain Fvs (scFv) (*e.g.*, including monospecific, bispecific, *etc.*), Fab fragments, F(ab') fragments, F(ab)₂ fragments, F(ab')₂ fragments, disulfide-linked Fvs (dsFv), Fd fragments, Fv fragments, diabody, triabody, tetrabody, and minibody. In particular, antibodies provided herein include immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, for example, antigen-binding domains or molecules that contain an antigen-binding site that binds to an antigen (*e.g.*, one or more CDRs of an antibody). Such antibody fragments can be found in, for example, Harlow and Lane, *Antibodies: A Laboratory Manual* (1989); *Mol. Biology and Biotechnology: A Comprehensive Desk Reference* (Myers ed., 1995); Huston *et al.*, 1993, *Cell Biophysics* 22:189-224; Plückthun and Skerra, 1989, *Meth. Enzymol.* 178:497-515; and Day, *Advanced Immunochemistry* (2d ed. 1990). The antibodies provided herein can be of any class (*e.g.*, IgG, IgE, IgM, IgD, and IgA) or any subclass (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2) of immunoglobulin molecule. Antibodies may be agonistic antibodies or antagonistic antibodies. Antibodies may be neither agonistic nor antagonistic.

[0032] An “antigen” is a structure to which an antibody can selectively bind. A target antigen may be a polypeptide, carbohydrate, nucleic acid, lipid, hapten, or other naturally occurring or synthetic compound. In some embodiments, the target antigen is a polypeptide. In certain embodiments, an antigen is associated with a cell, for example, is present on or in a cell.

[0033] The terms “binds” or “binding” refer to an interaction between molecules including, for example, to form a complex. Interactions can be, for example, non-covalent interactions

including hydrogen bonds, ionic bonds, hydrophobic interactions, and/or van der Waals interactions. A complex can also include the binding of two or more molecules held together by covalent or non-covalent bonds, interactions, or forces. The strength of the total non-covalent interactions between a single antigen-binding site on an antibody and a single epitope of a target molecule, such as an antigen, is the affinity of the antibody or functional fragment for that epitope. The ratio of dissociation rate (k_{off}) to association rate (k_{on}) of a binding molecule (*e.g.*, an antibody) to a monovalent antigen ($k_{\text{off}}/k_{\text{on}}$) is the dissociation constant K_D , which is inversely related to affinity. The lower the K_D value, the higher the affinity of the antibody. The value of K_D varies for different complexes of antibody and antigen and depends on both k_{on} and k_{off} . The dissociation constant K_D for an antibody provided herein can be determined using any method provided herein or any other method well known to those skilled in the art. The affinity at one binding site does not always reflect the true strength of the interaction between an antibody and an antigen. When complex antigens containing multiple, repeating antigenic determinants, such as a polyvalent antigen, come in contact with antibodies containing multiple binding sites, the interaction of antibody with antigen at one site will increase the probability of a reaction at a second site. The strength of such multiple interactions between a multivalent antibody and antigen is called the avidity.

[0034] In connection with the binding molecules described herein terms such as “bind to,” “that specifically bind to,” and analogous terms are also used interchangeably herein and refer to binding molecules of antigen binding domains that specifically bind to an antigen, such as a polypeptide. A binding molecule or antigen binding domain that binds to or specifically binds to an antigen can be identified, for example, by immunoassays, Octet[®], Biacore[®], or other techniques known to those of skill in the art. In some embodiments, a binding molecule or antigen binding domain binds to or specifically binds to an antigen when it binds to an antigen with higher affinity than to any cross-reactive antigen as determined using experimental techniques, such as radioimmunoassay (RIA) and enzyme linked immunosorbent assay (ELISA). Typically, a specific or selective reaction will be at least twice background signal or noise and may be more than 10 times background. See, *e.g.*, Fundamental Immunology 332-36 (Paul ed., 2d ed. 1989) for a discussion regarding binding specificity. In certain embodiments, the extent of binding of a binding molecule or antigen binding domain to a “non-target” protein is less than

about 10% of the binding of the binding molecule or antigen binding domain to its particular target antigen, for example, as determined by fluorescence activated cell sorting (FACS) analysis or RIA. A binding molecule or antigen binding domain that binds to an antigen includes one that is capable of binding the antigen with sufficient affinity such that the binding molecule is useful, for example, as a therapeutic and/or diagnostic agent in targeting the antigen. In certain embodiments, a binding molecule or antigen binding domain that binds to an antigen has a dissociation constant (K_D) of less than or equal to 1 μ M, 800 nM, 600 nM, 550 nM, 500 nM, 300 nM, 250 nM, 100 nM, 50 nM, 10 nM, 5 nM, 4 nM, 3 nM, 2 nM, 1 nM, 0.9 nM, 0.8 nM, 0.7 nM, 0.6 nM, 0.5 nM, 0.4 nM, 0.3 nM, 0.2 nM, or 0.1 nM. In certain embodiments, a binding molecule or antigen binding domain binds to an epitope of an antigen that is conserved among the antigen from different species.

[0035] In certain embodiments, the antibodies or antigen binding fragments of the molecules described herein can comprise “chimeric” sequences in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (*see* U.S. Pat. No. 4,816,567; and Morrison et al., 1984, Proc. Natl. Acad. Sci. USA 81:6851-55).

[0036] In certain embodiments, the antibodies or antigen binding fragments of the molecules described herein can comprise portions of “humanized” forms of nonhuman (*e.g.*, murine) antibodies that are chimeric antibodies that include human immunoglobulins (*e.g.*, recipient antibody) in which the native CDR residues are replaced by residues from the corresponding CDR of a nonhuman species (*e.g.*, donor antibody) such as mouse, rat, rabbit, or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, one or more FR region residues of the human immunoglobulin are replaced by corresponding nonhuman residues. Furthermore, humanized antibodies can comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. A humanized antibody heavy or light chain can comprise substantially all of at least one or more variable regions, in which all or substantially all of the CDRs correspond

to those of a nonhuman immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. In certain embodiments, the humanized antibody will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, *see*, Jones et al., 1986, Nature 321:522-25; Riechmann et al., 1988, Nature 332:323-29; Presta, 1992, Curr. Op. Struct. Biol. 2:593-96; Carter et al., 1992, Proc. Natl. Acad. Sci. USA 89:4285-89; U.S. Pat. Nos: 6,800,738; 6,719,971; 6,639,055; 6,407,213; and 6,054,297.

[0037] In certain embodiments, the antibodies or antigen binding fragments of the molecules described herein can comprise portions of a “fully human antibody” or “human antibody,” wherein the terms are used interchangeably herein and refer to an antibody that comprises a human variable region and, for example, a human constant region. In specific embodiments, the terms refer to an antibody that comprises a variable region and constant region of human origin. “Fully human” antibodies, in certain embodiments, can also encompass antibodies which bind polypeptides and are encoded by nucleic acid sequences which are naturally occurring somatic variants of human germline immunoglobulin nucleic acid sequence. The term “fully human antibody” includes antibodies having variable and constant regions corresponding to human germline immunoglobulin sequences as described by Kabat et al. (*See* Kabat et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). A “human antibody” is one that possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries (Hoogenboom and Winter, 1991, J. Mol. Biol. 227:381; Marks et al., 1991, J. Mol. Biol. 222:581) and yeast display libraries (Chao et al., 2006, Nature Protocols 1: 755-68). Also available for the preparation of human monoclonal antibodies are methods described in Cole et al., Monoclonal Antibodies and Cancer Therapy 77 (1985); Boerner et al., 1991, J. Immunol. 147(1):86-95; and van Dijk and van de Winkel, 2001, Curr. Opin. Pharmacol. 5: 368-74. Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic

challenge, but whose endogenous loci have been disabled, *e.g.*, mice (*see, e.g.*, Jakobovits, 1995, Curr. Opin. Biotechnol. 6(5):561-66; Brüggemann and Taussing, 1997, Curr. Opin. Biotechnol. 8(4):455-58; and U.S. Pat. Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). *See also*, for example, Li et al., 2006, Proc. Natl. Acad. Sci. USA 103:3557-62 regarding human antibodies generated via a human B-cell hybridoma technology.

[0038] A typical 4-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains. In the case of IgGs, the 4-chain unit is generally about 150,000 daltons. Each L chain is linked to an H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. Each H and L chain also has regularly spaced intrachain disulfide bridges. Each H chain has at the N-terminus, a variable domain (VH) followed by three constant domains (CH) for each of the α and γ chains and four CH domains for μ and ϵ isotypes. Each L chain has at the N-terminus, a variable domain (VL) followed by a constant domain (CL) at its other end. The VL is aligned with the VH, and the CL is aligned with the first constant domain of the heavy chain (CH1). Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains. The pairing of a VH and VL together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, *see*, for example, Basic and Clinical Immunology 71 (Stites *et al.* eds., 8th ed. 1994); and Immunobiology (Janeway *et al.* eds., 5th ed. 2001).

[0039] The term “Fab” or “Fab region” refers to an antibody region that binds to antigens. A conventional IgG usually comprises two Fab regions, each residing on one of the two arms of the Y-shaped IgG structure. Each Fab region is typically composed of one variable region and one constant region of each of the heavy and the light chain. More specifically, the variable region and the constant region of the heavy chain in a Fab region are VH and CH1 regions, and the variable region and the constant region of the light chain in a Fab region are VL and CL regions. The VH, CH1, VL, and CL in a Fab region can be arranged in various ways to confer an antigen binding capability according to the present disclosure. For example, VH and CH1 regions can be on one polypeptide, and VL and CL regions can be on a separate polypeptide, similarly to a Fab region of a conventional IgG. Alternatively, VH, CH1, VL and CL regions can all be on the same polypeptide and oriented in different orders.

[0040] The term “variable region,” “variable domain,” “V region,” or “V domain” refers to a portion of the light or heavy chains of an antibody that is generally located at the amino-terminal of the light or heavy chain and has a length of about 120 to 130 amino acids in the heavy chain and about 100 to 110 amino acids in the light chain, and are used in the binding and specificity of each particular antibody for its particular antigen. The variable region of the heavy chain may be referred to as “VH.” The variable region of the light chain may be referred to as “VL.” The term “variable” refers to the fact that certain segments of the variable regions differ extensively in sequence among antibodies. The V region mediates antigen binding and defines specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the 110-amino acid span of the variable regions. Instead, the V regions consist of less variable (*e.g.*, relatively invariant) stretches called framework regions (FRs) of about 15-30 amino acids separated by shorter regions of greater variability (*e.g.*, extreme variability) called “hypervariable regions” that are each about 9-12 amino acids long. The variable regions of heavy and light chains each comprise four FRs, largely adopting a β sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases form part of, the β sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (*see, e.g.*, Kabat *et al.*, Sequences of Proteins of Immunological Interest (5th ed. 1991)). The constant regions are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC). The variable regions differ extensively in sequence between different antibodies. In specific embodiments, the variable region is a human variable region.

[0041] The term “heavy chain” when used in reference to an antibody refers to a polypeptide chain of about 50-70 kDa, wherein the amino-terminal portion includes a variable region of about 120 to 130 or more amino acids, and a carboxy-terminal portion includes a constant region. The constant region can be one of five distinct types, (*e.g.*, isotypes) referred to as alpha (α), delta (δ), epsilon (ϵ), gamma (γ), and mu (μ), based on the amino acid sequence of the heavy chain constant region. The distinct heavy chains differ in size: α , δ , and γ contain approximately 450 amino acids, while μ and ϵ contain approximately 550 amino acids. When combined with a

light chain, these distinct types of heavy chains give rise to five well known classes (*e.g.*, isotypes) of antibodies, IgA, IgD, IgE, IgG, and IgM, respectively, including four subclasses of IgG, namely IgG1, IgG2, IgG3, and IgG4.

[0042] The term “light chain” when used in reference to an antibody refers to a polypeptide chain of about 25 kDa, wherein the amino-terminal portion includes a variable region of about 100 to about 110 or more amino acids, and a carboxy-terminal portion includes a constant region. The approximate length of a light chain is 211 to 217 amino acids. There are two distinct types, referred to as kappa (κ) or lambda (λ) based on the amino acid sequence of the constant domains.

[0043] As used herein, the terms “hypervariable region,” “HVR,” “Complementarity Determining Region,” and “CDR” are used interchangeably. A “CDR” refers to one of three hypervariable regions (H1, H2 or H3) within the non-framework region of the immunoglobulin (Ig or antibody) VH β -sheet framework, or one of three hypervariable regions (L1, L2 or L3) within the non-framework region of the antibody VL β -sheet framework. Accordingly, CDRs are variable region sequences interspersed within the framework region sequences. CDR regions are well known to those skilled in the art and have been defined by well-known numbering systems.

[0044] The term “constant region” or “constant domain” refers to a carboxy terminal portion of the light and heavy chain which is not directly involved in binding of the antibody to antigen but exhibits various effector function, such as interaction with the Fc receptor. The term refers to the portion of an immunoglobulin molecule having a more conserved amino acid sequence relative to the other portion of the immunoglobulin, the variable region, which contains the antigen binding site. The constant region may contain the CH1, CH2, and CH3 regions of the heavy chain and the CL region of the light chain. As used herein, the terms “IgG CH1,” “IgG CH2,” and “IgG CH3” refer to the commonly defined first, second and third domains, respectively, in the constant region of a heavy chain of IgG. A heavy chain from an IgG usually contains three constant domains IgG CH1, CH2, CH3, and a spacer hinge region between the CH1 and CH2 domains.

[0045] The term “Fc region” herein is used to define a C-terminal region of an immunoglobulin heavy chain, including, for example, native sequence Fc regions, recombinant

Fc regions, and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is often defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody.

Accordingly, a composition of intact antibodies may comprise antibody populations with all K447 residues removed, antibody populations with no K447 residues removed, and antibody populations having a mixture of antibodies with and without the K447 residue. A “functional Fc region” possesses an “effector function” of a native sequence Fc region. Exemplary “effector functions” include C1q binding; CDC; Fc receptor binding; ADCC; phagocytosis; downregulation of cell surface receptors (*e.g.*, B cell receptor), *etc.* Such effector functions generally require the Fc region to be combined with a binding region or binding domain (*e.g.*, an antibody variable region or domain) and can be assessed using various assays known to those skilled in the art. A “variant Fc region” comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification (*e.g.*, substituting, addition, or deletion). In certain embodiments, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, for example, from about one to about ten amino acid substitutions, or from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of a parent polypeptide. The variant Fc region herein can possess at least about 80% homology with a native sequence Fc region and/or with an Fc region of a parent polypeptide, or at least about 90% homology therewith, for example, at least about 95% homology therewith.

[0046] The term “framework” or “FR” refers to those variable region residues flanking the CDRs. FR residues are present, for example, in chimeric, humanized, human, domain antibodies (*e.g.*, single domain antibodies), diabodies, linear antibodies, and bispecific antibodies. FR residues are those variable domain residues other than the hypervariable region residues or CDR residues.

[0047] As used herein, an “epitope” is a term in the art and refers to a localized region of an antigen to which a binding molecule (*e.g.*, an antibody comprising a single domain antibody

sequence) can specifically bind. An epitope can be a linear epitope or a conformational, non-linear, or discontinuous epitope. In the case of a polypeptide antigen, for example, an epitope can be contiguous amino acids of the polypeptide (a “linear” epitope) or an epitope can comprise amino acids from two or more non-contiguous regions of the polypeptide (a “conformational,” “non-linear” or “discontinuous” epitope). It will be appreciated by one of skill in the art that, in general, a linear epitope may or may not be dependent on secondary, tertiary, or quaternary structure. For example, in some embodiments, a binding molecule binds to a group of amino acids regardless of whether they are folded in a natural three dimensional protein structure. In other embodiments, a binding molecule requires amino acid residues making up the epitope to exhibit a particular conformation (*e.g.*, bend, twist, turn or fold) in order to recognize and bind the epitope.

[0048] The term “targeting region” or “binding domain” is used herein in broadest sense and refers to a region of a molecule that can specifically bind to a target (or an antigen). The targeting region can be the antigen-binding fragment without a C_H1 region, or any reformatted antigen-binding fragment such as single-chain variable fragment (scFv), any polypeptide that is non-immunoglobulin but forms specific binding to a target via enzyme-substrate, receptor-ligand or other protein-protein interactions. The targeting region can also be a sequence of nucleic acids that forms specific binding to the target via reverse complementary binding. The targeting region can also be an entity that can form any covalent or non-covalent connection with a target. In some embodiments, the targeting region or binding domain is derived from an antibody such as antigen binding domain or fragment of antibody. The terms “antigen-binding fragment,” “antigen-binding domain,” “antigen-binding region,” and similar terms when used in the context of an antibody refer to that portion of the antibody, which comprises the amino acid residues that interact with an antigen and confer on the binding agent its specificity and affinity for the antigen (*e.g.*, the CDRs). “Antigen binding fragment” as used herein include “antibody fragment,” which comprise a portion of an intact antibody, such as the antigen binding or variable region of the intact antibody. Examples of antibody fragments are described above, and include, without limitation, Fab, Fab’, F(ab’)2, and Fv fragments; diabodies and di-diabodies; single-chain antibody molecules; dual variable domain antibodies; single variable domain antibodies (sdAbs); and multispecific antibodies formed from antibody fragments.

[0049] As used herein, the terms “polypeptide” and “peptide” and “protein” are used interchangeably herein and refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid, including but not limited to, unnatural amino acids, as well as other modifications known in the art. It is understood that, because the polypeptides of this disclosure may be based upon antibodies or other members of the immunoglobulin superfamily, in certain embodiments, a “polypeptide” can occur as a single chain or as two or more associated chains.

[0050] “Polynucleotide” or “nucleic acid,” as used interchangeably herein, refers to polymers of nucleotides of any length and includes DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase or by a synthetic reaction. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. “Oligonucleotide,” as used herein, refers to short, generally single-stranded, synthetic polynucleotides that are generally, but not necessarily, fewer than about 200 nucleotides in length. The terms “oligonucleotide” and “polynucleotide” are not mutually exclusive. The description above for polynucleotides is equally and fully applicable to oligonucleotides. A cell that produces a binding molecule of the present disclosure may include a parent hybridoma cell, as well as bacterial and eukaryotic host cells into which nucleic acids encoding the antibodies have been introduced. Unless specified otherwise, the left-hand end of any single-stranded polynucleotide sequence disclosed herein is the 5' end; the left-hand direction of double-stranded polynucleotide sequences is referred to as the 5' direction. The direction of 5' to 3' addition of nascent RNA transcripts is referred to as the transcription direction; sequence regions on the DNA strand having the same sequence as the RNA transcript that are 5' to the 5' end of the RNA transcript are referred to as “upstream sequences”; sequence regions on the DNA strand having the same sequence as the RNA transcript that are 3' to the 3' end of the RNA transcript are referred to as “downstream sequences.”

[0051] An “isolated nucleic acid” is a nucleic acid, for example, an RNA, DNA, or a mixed nucleic acids, which is substantially separated from other genome DNA sequences as well as proteins or complexes such as ribosomes and polymerases, which naturally accompany a native sequence. An “isolated” nucleic acid molecule is one which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid molecule. Moreover, an “isolated” nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. In a specific embodiment, one or more nucleic acid molecules encoding a single domain antibody or an antibody as described herein are isolated or purified. The term embraces nucleic acid sequences that have been removed from their naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogues or analogues biologically synthesized by heterologous systems. A substantially pure molecule may include isolated forms of the molecule.

[0052] Unless otherwise specified, a “nucleotide sequence encoding an amino acid sequence” includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. The phrase nucleotide sequence that encodes a protein or an RNA may also include introns to the extent that the nucleotide sequence encoding the protein may in some version contain an intron(s).

[0053] As used herein, the term “vector” refers to a substance that is used to carry or include a nucleic acid sequence, including for example, a nucleic acid sequence encoding an antibody or antigen binding fragment as described herein, in order to introduce a nucleic acid sequence into a host cell. Vectors applicable for use include, for example, expression vectors, plasmids, phage vectors, viral vectors, episomes, and artificial chromosomes, which can include selection sequences or markers operable for stable integration into a host cell’s chromosome. Additionally, the vectors can include one or more selectable marker genes and appropriate expression control sequences. Selectable marker genes that can be included, for example, provide resistance to antibiotics or toxins, complement auxotrophic deficiencies, or supply critical nutrients not in the culture media. Expression control sequences can include constitutive and inducible promoters, transcription enhancers, transcription terminators, and the like, which

are well known in the art. When two or more nucleic acid molecules are to be co-expressed (*e.g.*, both an antibody heavy and light chain or an antibody VH and VL), both nucleic acid molecules can be inserted, for example, into a single expression vector or in separate expression vectors. For single vector expression, the encoding nucleic acids can be operationally linked to one common expression control sequence or linked to different expression control sequences, such as one inducible promoter and one constitutive promoter. The introduction of nucleic acid molecules into a host cell can be confirmed using methods well known in the art. Such methods include, for example, nucleic acid analysis such as Northern blots or polymerase chain reaction (PCR) amplification of mRNA, immunoblotting for expression of gene products, or other suitable analytical methods to test the expression of an introduced nucleic acid sequence or its corresponding gene product. It is understood by those skilled in the art that the nucleic acid molecules are expressed in a sufficient amount to produce a desired product and it is further understood that expression levels can be optimized to obtain sufficient expression using methods well known in the art.

[0054] As used herein, the term “host cell” refers to a particular subject cell that may be transfected with a nucleic acid molecule and the progeny or potential progeny of such a cell. Progeny of such a cell may not be identical to the parent cell transfected with the nucleic acid molecule due to mutations or environmental influences that may occur in succeeding generations or integration of the nucleic acid molecule into the host cell genome.

[0055] As used herein, the term “identity” refers to a relationship between the sequences of two or more polypeptide molecules or two or more nucleic acid molecules, as determined by aligning and comparing the sequences. “Percent (%) amino acid sequence identity” with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, or MEGALIGN (DNASar, Inc.) software. Those skilled in the art can determine appropriate parameters for

aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0056] The term “specificity” refers to selective recognition of an antigen binding molecule (such as an antibody) for a particular epitope of an antigen. Natural antibodies, for example, are monospecific. The term "multispecific" as used herein denotes that an antigen binding protein has two or more antigen-binding sites of which at least two bind different antigens. "Bispecific" as used herein denotes that an antigen binding molecule has two different antigen-binding specificities. The term "monospecific" used herein denotes an antigen binding molecule that has one or more binding sites each of which bind the same antigen.

[0057] The term “valent” as used herein denotes the presence of a specified number of binding sites in an antigen binding molecule. A natural antibody for example or a full length antibody has two binding sites and is bivalent. As such, the terms "trivalent", "tetravalent", "pentavalent" and "hexavalent" denote the presence of two binding site, three binding sites, four binding sites, five binding sites, and six binding sites, respectively, in an antigen binding molecule.

[0058] As used herein, the term “pharmaceutically acceptable” as used herein means being approved by a regulatory agency of the Federal or a state government, or listed in United States Pharmacopeia, European Pharmacopeia, or other generally recognized Pharmacopeia for use in animals, and more particularly in humans.

[0059] As used herein, the term “excipient” means a pharmaceutically-acceptable material, composition, or vehicle, such as a liquid or solid filler, diluent, solvent, or encapsulating material. Excipients include, for example, encapsulating materials or additives such as absorption accelerators, antioxidants, binders, buffers, carriers, coating agents, coloring agents, diluents, disintegrating agents, emulsifiers, extenders, fillers, flavoring agents, humectants, lubricants, perfumes, preservatives, propellants, releasing agents, sterilizing agents, sweeteners, solubilizers, wetting agents and mixtures thereof. The term “excipient” can also refer to a diluent, adjuvant (*e.g.*, Freund’s adjuvant (complete or incomplete) or vehicle.

[0060] In some embodiments, excipients are pharmaceutically acceptable excipients. Examples of pharmaceutically acceptable excipients include buffers, such as phosphate, citrate, and other organic acids; antioxidants, including ascorbic acid; low molecular weight (*e.g.*, fewer

than about 10 amino acid residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers, such as polyvinylpyrrolidone; amino acids, such as glycine, glutamine, asparagine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates, including glucose, mannose, or dextrans; chelating agents, such as EDTA; sugar alcohols, such as mannitol or sorbitol; salt-forming counterions, such as sodium; and/or nonionic surfactants, such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™. Other examples of pharmaceutically acceptable excipients are described in Remington and Gennaro, Remington's Pharmaceutical Sciences (18th ed. 1990).

[0061] In one embodiment, each component is “pharmaceutically acceptable” in the sense of being compatible with the other ingredients of a pharmaceutical formulation, and suitable for use in contact with the tissue or organ of humans and animals without excessive toxicity, irritation, allergic response, immunogenicity, or other problems or complications, commensurate with a reasonable benefit/risk ratio. *See, e.g.*, Lippincott Williams & Wilkins: Philadelphia, PA, 2005; Handbook of Pharmaceutical Excipients, 6th ed.; Rowe et al., Eds.; The Pharmaceutical Press and the American Pharmaceutical Association: 2009; Handbook of Pharmaceutical Additives, 3rd ed.; Ash and Ash Eds.; Gower Publishing Company: 2007; Pharmaceutical Preformulation and Formulation, 2nd ed.; Gibson Ed.; CRC Press LLC: Boca Raton, FL, 2009. In some embodiments, pharmaceutically acceptable excipients are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. In some embodiments, a pharmaceutically acceptable excipient is an aqueous pH buffered solution.

[0062] In some embodiments, excipients are 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 an exemplary excipient when a composition (*e.g.*, a pharmaceutical composition) is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid excipients, particularly for injectable solutions. An excipient can also 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. Compositions can take the form of solutions, suspensions, emulsion, tablets, pills,

capsules, powders, sustained-release formulations, and the like. Oral compositions, including formulations, can include standard excipients such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc.

[0063] As used herein, the terms “about” and “approximately” mean within 20%, within 15%, within 10%, within 9%, within 8%, within 7%, within 6%, within 5%, within 4%, within 3%, within 2%, within 1%, or less of a given value or range.

[0064] As used in the present disclosure and claims, the singular forms “a”, “an” and “the” include plural forms unless the context clearly dictates otherwise.

[0065] As used herein, the term “between” as used in a phrase as such “between A and B” or “between A-B” refers to a range including both A and B.

[0066] The term “and/or” as used in a phrase such as “A and/or B” herein is intended to include both A and B; A or B; A (alone); and B (alone). Likewise, the term “and/or” as used in a phrase such as “A, B, and/or C” is intended to encompass each of the following embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

5.2 Molecules Engineered to Form Oligomers

5.2.1 Molecules Comprising an Engineered Constant Region

[0067] In one aspect, provided herein is a molecule capable of forming connections with two or more other molecules of the same or similar structure, thus forming oligomers comprising two or more units. More specifically, in some embodiments, the molecule provided herein comprises an IgG C_{H2} region in which the amino acid residue at position 253 (according to EU numbering) is substituted to be a cysteine residue, thereby generating a site capable of forming a disulfide bond with another molecule having the same amino acid substitution.

[0068] In some embodiments, in addition to the cysteine residue at position 253 of C_{H2} region, the molecule provided herein comprises at least one other site capable of forming connection with other molecules of the same or similar structure. The type of connection provided herein can be covalent or non-covalent, and the connection provided herein can be direct or indirect. In some embodiments, the connection provided herein is a disulfide bond via cysteine residues. The cysteine residues can be introduced into the molecule, for example, by point mutations or by adding a tailpiece. Alternatively, the cysteine residues may be present

naturally in antibody region, such as a hinge region. In some embodiments, the above properties are presented in the molecule in combination.

[0069] In some embodiments, the type of connections between the molecules provided herein is via covalent bonds. In some specific embodiments, the type of connections between the molecules provided herein is via disulfide bonds. In some other specific embodiments, the type of connections between the molecules provided herein is via electron sharing. In other embodiments, the type of connections between the molecules provided herein is via non-covalent bonds. In some specific embodiments, the type of connections between the molecules provided herein is via hydrogen bonds. In some other specific embodiments, the type of connections between the molecules provided herein is via ionic interactions. In some other specific embodiments, the type of connections between the molecules provided herein is via Van der Waals forces. In some other specific embodiments, the type of connections between the molecules provided herein is via hydrophobic bonds.

[0070] In some embodiments, the molecule provided herein forms direct connections with each other. In other embodiments, the molecule provided herein forms indirect connections with each other. In some specific embodiments, the molecule provided herein connects with each other via directly formed disulfide bonds. In other embodiments, the molecule provided herein connects with each other via indirectly formed disulfide bonds. In some specific embodiments, the molecule provided herein connects with each other via one or more connecting sites, wherein the connecting molecule forms disulfide bonds with the molecules provided herein. In one specific embodiment, the connecting molecule is the J chain that is found in polymeric IgA and IgM.

[0071] In certain embodiments, provided herein is a molecule that forms connections with each other via disulfide bonds, which can be formed between two natural amino acids or unusual amino acids. In some embodiments, the connections formed between the molecules provided herein are disulfide bonds between two natural amino acids. In some specific embodiments, the connections formed between the molecules provided herein are disulfide bonds between two cysteine residues. In some embodiments, the connections formed between the molecules provided herein are disulfide bonds between two unusual amino acids. In some specific embodiments, the unusual amino acids each possess a reactive sulfhydryl group. In some

specific embodiments, the unusual amino acids are tyrosine derivatives with para-substituted aliphatic thiols of various lengths (see Liu et al., PNAS, 113 (21) 5910-5915 (2016)).

Point Mutations for Forming Disulfide Bonds

[0072] In some embodiments, provided herein is a molecule that forms connections with each other via disulfide bonds between introduced point mutations. In one embodiment, an alanine is substituted with a cysteine. In another embodiment, an arginine is substituted with a cysteine. In another embodiment, an asparagine is substituted with a cysteine. In another embodiment, an aspartic is substituted with a cysteine. In another embodiment, a glutamine is substituted with a cysteine. In another embodiment, a glutamic acid is substituted with a cysteine. In another embodiment, a glycine is substituted with a cysteine. In another embodiment, a histidine is substituted with a cysteine. In another embodiment, an isoleucine is substituted with a cysteine. In another embodiment, a leucine is substituted with a cysteine. In another embodiment, a lysine is substituted with a cysteine. In another embodiment, a methionine is substituted with a cysteine. In another embodiment, a phenylalanine is substituted with a cysteine. In another embodiment, a proline is substituted with a cysteine. In another embodiment, a serine is substituted with a cysteine. In another embodiment, a threonine is substituted with a cysteine. In another embodiment, a tryptophan is substituted with a cysteine. In another embodiment, a tyrosine is substituted with a cysteine. In another embodiment, a valine is substituted with a cysteine.

[0073] In some embodiments, provided herein is a molecule with an introduced point mutation in a location that facilitates the forming of stable disulfide bonds with each other. In some embodiments, the location of the introduced point mutation satisfies geometric constraints and parameters for stable disulfide bonds. In some specific embodiments, the location of the introduced point mutation ensures a close distance between $C\alpha - C\alpha'$ in the formed disulfide bond. In some specific embodiments, the location of the introduced point mutation ensures a close distance between $C\beta - C\beta'$ in the formed disulfide bond. In some specific embodiments, the location of the introduced point mutation ensures a proper bond angle between $C\alpha - C\beta - S\gamma$ in the formed disulfide bond. In other specific embodiments, the location of the introduced point mutation ensures a proper bond angle between $C\beta - S\gamma - S'\gamma$ in the formed disulfide bond. In yet other specific embodiments, the location of the introduced point mutation ensures a proper

rotation angle of the C β atoms about the S–S bond in the formed disulfide bond. In some specific embodiments, the molecule provided herein comprises in an IgG C_{H1} region with a point mutation to cysteine.

[0074] In some specific embodiments, the molecule provided herein comprises in an IgG C_{H2} region with a point mutation to cysteine. In some specific embodiments, the molecule provided herein comprises in an IgG C_{H3} region with a point mutation to cysteine. In some specific embodiments, the molecule provided herein comprises in a hinge region with a point mutation to cysteine. In some specific embodiments, the molecule provided herein comprises in an IgE C ϵ 1 region with a point mutation to cysteine. In some specific embodiments, the molecule provided herein comprises in an IgE C ϵ 2 region with a point mutation to cysteine. In some specific embodiments, the molecule provided herein comprises in an IgE C ϵ 3 region with a point mutation to cysteine. In some specific embodiments, the molecule provided herein comprises in an IgE C ϵ 4 region with a point mutation to cysteine. In some specific embodiments, the molecule provided herein comprises in an IgD C_{H1} region with a point mutation to cysteine. In some specific embodiments, the molecule provided herein comprises in an IgD C_{H2} region with a point mutation to cysteine. In some specific embodiments, the molecule provided herein comprises in an IgD C_{H3} region with a point mutation to cysteine. In some specific embodiments, the molecule provided herein comprises in an IgM C μ 1 region with a point mutation to cysteine. In some specific embodiments, the molecule provided herein comprises in an IgM C μ 2 region with a point mutation to cysteine. In some specific embodiments, the molecule provided herein comprises in an IgM C μ 3 region with a point mutation to cysteine. In some specific embodiments, the molecule provided herein comprises in an IgM C μ 4 region with a point mutation to cysteine. In some specific embodiments, the molecule provided herein comprises in an IgA C α 1 region with a point mutation to cysteine. In some specific embodiments, the molecule provided herein comprises in an IgA C α 2 region with a point mutation to cysteine. In some specific embodiments, the molecule provided herein comprises in an IgA C α 3 region with a point mutation to cysteine. In a specific embodiment, the molecule provided herein comprises in an IgG C_{H2} region with a point mutation at location Ile 253 by EU numbering to cysteine.

[0075] In some embodiments, the molecule provided herein comprises two or more the above described point mutations. In some embodiments, the molecule provided herein comprises a point mutation at location Ile 253 by EU numbering to cysteine in an IgG C_{H2} region and at least one other point mutation described above.

Added Tailpiece for Forming Disulfide Bonds

[0076] In certain embodiments, the molecule provided herein further comprises an added tailpiece which facilitates the forming of stable disulfide bonds with each other. In some embodiments, the added tailpiece comprises the eighteen amino acid long μ tailpiece (SEQ ID NO: 1). In other embodiments, the added tailpiece comprises 90% identity to SEQ ID NO: 1 and remains the cysteine residue in the SEQ ID NO: 1. In other embodiments, the added tailpiece comprises 85% identity to SEQ ID NO: 1 and remains the cysteine residue in the SEQ ID NO: 1. In other embodiments, the added tailpiece comprises 70% identity to SEQ ID NO: 1 and remains the cysteine residue in the SEQ ID NO: 1. In other embodiments, the added tailpiece comprises 65% identity to SEQ ID NO: 1 and remains the cysteine residue in the SEQ ID NO: 1. In other embodiments, the added tailpiece comprises 50% identity to SEQ ID NO: 1 and remains the cysteine residue in the SEQ ID NO: 1.

[0077] In some embodiments, the added tailpiece comprises the tailpiece in IgA α heavy chain (α tailpiece). In other embodiments, the added tailpiece comprises 85% identity to α tailpiece and remains the cysteine residue in the α tailpiece. In other embodiments, the added tailpiece comprises 70% identity to α tailpiece and remains the cysteine residue in the α tailpiece. In other embodiments, the added tailpiece comprises 65% identity to α tailpiece and remains the cysteine residue in the α tailpiece. In other embodiments, the added tailpiece comprises 50% identity to α tailpiece and remains the cysteine residue in the α tailpiece. In other embodiments, the added tailpiece comprises 35% identity to α tailpiece and remains the cysteine residue in the α tailpiece. In other embodiments, the added tailpiece comprises 20% identity to α tailpiece and remains the cysteine residue in the α tailpiece. In other embodiments, the added tailpiece comprises 5% identity to α tailpiece and remains the cysteine residue in the α tailpiece.

[0078] In certain embodiments, certain polypeptide described herein comprises amino acid sequences with certain percent identity relative to a reference polypeptide. The determination of percent identity between two sequences (*e.g.*, amino acid sequences or nucleic acid sequences)

can be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, Proc. Natl. Acad. Sci. U.S.A. 87:2264 2268 (1990), modified as in Karlin and Altschul, Proc. Natl. Acad. Sci. U.S.A. 90:5873 5877 (1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.*, J. Mol. Biol. 215:403 (1990). BLAST nucleotide searches can be performed with the NBLAST nucleotide program parameters set, *e.g.*, for score=100, word length=12 to obtain nucleotide sequences homologous to a nucleic acid molecules described herein. BLAST protein searches can be performed with the XBLAST program parameters set, *e.g.*, to score 50, word length=3 to obtain amino acid sequences homologous to a protein molecule described herein. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, Nucleic Acids Res. 25:3389 3402 (1997). Alternatively, PSI BLAST can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI Blast programs, the default parameters of the respective programs (*e.g.*, of XBLAST and NBLAST) can be used (see, *e.g.*, National Center for Biotechnology Information (NCBI) on the worldwide web, ncbi.nlm.nih.gov). Another non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, CABIOS 4:11-17 (1998). Such an algorithm is incorporated in the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically only exact matches are counted.

[0079] In other embodiments, provided herein is a molecule with an added short polypeptide containing one or more cysteine. In some specific embodiments, the added short polypeptide is less than five amino acids long. In some specific embodiments, the added short polypeptide is six, seven, eight, nine, or ten amino acids long. In some specific embodiments, the added short polypeptide is eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, or twenty amino acids long. In some specific embodiments, the added short polypeptide is twenty-one, twenty-two, twenty-three, twenty-four, twenty-five, twenty-six, twenty-seven,

twenty-eight, twenty-nine, thirty amino acids long. In some specific embodiments, the added short polypeptide is more than thirty amino acids long.

[0080] Provided herein is a molecule with an added tailpiece in a location which facilitates the forming of stable disulfide bonds with each other. In some embodiments, the tailpiece is added to the C-terminus of the molecule. In some specific embodiments, the tailpiece is added to the C-terminus of the C_{H1} region. In some specific embodiments, the tailpiece is added to the C-terminus of the hinge region. In some specific embodiments, the tailpiece is added to the C-terminus of the C_{H2} region. In some specific embodiments, the tailpiece is added to the C-terminus of the C_{H3} region. In some embodiments, the tailpiece is added to the N-terminus of the molecule. In some specific embodiments, the tailpiece is added to the N-terminus of the C_{H1} region. In some specific embodiments, the tailpiece is added to the N-terminus of the hinge region. In some specific embodiments, the tailpiece is added to the N-terminus of the C_{H2} region. In some specific embodiments, the tailpiece is added to the N-terminus of the C_{H3} region.

Configurations of the Engineered Molecule

[0081] In some embodiments, provided herein is a molecule comprising both a point mutation described above and an added tailpiece described above. In other embodiments, the molecule provided herein has both two or more point mutations described above and an added tailpiece described above. In yet other embodiments, the molecule provided herein has both a point mutation described above and two or more added tailpieces described above. In yet other embodiments, the molecule provided herein has both two or more point mutations described above and two or more added tailpieces described above.

[0082] In some embodiments, provided herein is a molecule comprising different antibody constant regions that are engineered for multimerization. In some embodiments, the molecules described herein comprise one or more point mutation(s) in C_{H2} region and thus engineered disulfide bond(s) can form between such molecules. In other embodiments, the molecules described herein comprise one or more added tailpiece(s) and thus engineered disulfide bond(s) can form between such molecules. In other embodiments, the molecules described herein comprise one or more point mutation(s) in C_{H2} region and one or more added tailpiece(s) and thus multiple engineered disulfide bonds can form between such molecules.

[0083] In some specific embodiments, the molecule described herein comprises an IgG C_{H2} region, wherein position 253 in the IgG C_{H2} region by EU numbering is substituted to be a cysteine. In some specific embodiments, the molecule described herein comprises an IgG C_{H2} region, wherein position 253 in the IgG C_{H2} region by EU numbering is substituted to be a cysteine, and an IgG hinge region. In some specific embodiments, the molecule described herein comprises an IgG C_{H2} region, wherein position 253 in the IgG C_{H2} region by EU numbering is substituted to be a cysteine, and an IgG C_{H3} region. In some specific embodiments, the molecule described herein comprises an IgG C_{H2} region, wherein position 253 in the IgG C_{H2} region by EU numbering is substituted to be a cysteine, and an IgG C_{H1} region.

[0084] In some specific embodiments, the molecule described herein comprises an IgG C_{H2} region, wherein position 253 in the IgG C_{H2} region by EU numbering is substituted to be a cysteine, an IgG hinge, and C_{H3} region. In some specific embodiments, the molecule described herein comprises an IgG C_{H2} region, wherein position 253 in the IgG C_{H2} region by EU numbering is substituted to be a cysteine, an IgG hinge, and an IgG C_{H1} region. In some specific embodiments, the molecule described herein comprises an IgG C_{H2} region, wherein position 253 in the IgG C_{H2} region by EU numbering is substituted to be a cysteine, an IgG C_{H3} region, and an IgG C_{H1} region. In some specific embodiments, the molecule described herein comprises an IgG C_{H2} region, wherein position 253 in the IgG C_{H2} region by EU numbering is substituted to be a cysteine, an IgG hinge, an IgG C_{H3} region, and an IgG C_{H1} region.

[0085] In the above embodiments, the order of different regions can follow the order in a wild-type IgG, or can be different from the order in a wild-type IgG.

[0086] In some embodiments, the molecules described herein further comprise one or more added tailpiece(s) as described above. In some specific embodiments, the molecule described herein comprises a human μ tailpiece (SEQ ID NO: 1) or a variant thereof. In some specific embodiments, the molecule described herein comprises a human μ tailpiece (SEQ ID NO: 1), and an IgG hinge region. In some specific embodiments, the molecule described herein comprises a human μ tailpiece (SEQ ID NO: 1), and an IgG C_{H2} region. In some specific embodiments, the molecule described herein comprises a human μ tailpiece (SEQ ID NO: 1), and an IgG C_{H3} region. In some specific embodiments, the molecule described herein comprises a human μ tailpiece (SEQ ID NO: 1), an IgG C_{H1} region.

[0087] In some specific embodiments, the molecule described herein comprises a human μ tailpiece (SEQ ID NO: 1), an IgG hinge, and an IgG C_{H2} region. In some specific embodiments, the molecule described herein comprises a human μ tailpiece (SEQ ID NO: 1), an IgG hinge region, and an IgG C_{H3} region. In some specific embodiments, the molecule described herein comprises a human μ tailpiece (SEQ ID NO: 1), an IgG hinge region, and an IgG C_{H1} region. In some specific embodiments, the molecule described herein comprises a human μ tailpiece (SEQ ID NO: 1), an IgG C_{H2} region, and an IgG C_{H3} region. In some specific embodiments, the molecule described herein comprises a human μ tailpiece (SEQ ID NO: 1), an IgG C_{H2} region, and an IgG C_{H1} region. In some specific embodiments, the molecule described herein comprises a human μ tailpiece (SEQ ID NO: 1), an IgG C_{H3} region, and an IgG C_{H1} region.

[0088] In some specific embodiments, the molecule described herein comprises a human μ tailpiece (SEQ ID NO: 1), an IgG hinge region, an IgG C_{H2} region, and an IgG C_{H3} region. In some specific embodiments, the molecule described herein comprises a human μ tailpiece (SEQ ID NO: 1), an IgG hinge region, an IgG C_{H2} region, and an IgG C_{H1} region. In some specific embodiments, the molecule described herein comprises a human μ tailpiece (SEQ ID NO: 1), an IgG hinge region, an IgG C_{H3} region, and an IgG C_{H1} region. In some specific embodiments, the molecule described herein comprises a human μ tailpiece (SEQ ID NO: 1), an IgG C_{H2} region, an IgG C_{H3} region, and an IgG C_{H1} region.

[0089] In some specific embodiments, the molecule described herein comprises a human μ tailpiece (SEQ ID NO: 1), an IgG hinge region, an IgG C_{H2} region, an IgG C_{H3} region, and an IgG C_{H1} region.

[0090] In the above embodiments, the order of different regions can follow the order in a wild-type IgG, or can be different from the order in a wild-type IgG, and the human μ tailpiece can be conjugated to the C-terminus or N-terminus of the present molecules.

[0091] In some embodiments, the molecules described herein comprise one or more point mutation(s) and one or more added tailpiece(s) and thus multiple engineered disulfide bonds can form between such molecules. In some specific embodiments, the molecule described herein comprises an IgG C_{H2} region, wherein position 253 in the IgG C_{H2} region by EU numbering is substituted to be a cysteine, and a human μ tailpiece (SEQ ID NO: 1). In some specific embodiments, the molecule described herein comprises an IgG C_{H2} region, wherein position 253

in the IgG C_{H2} region by EU numbering is substituted to be a cysteine, a human μ tailpiece (SEQ ID NO: 1), and an IgG hinge region. In some specific embodiments, the molecule described herein comprises an IgG C_{H2} region, wherein position 253 in the IgG C_{H2} region by EU numbering is substituted to be a cysteine, a human μ tailpiece (SEQ ID NO: 1), and an IgG C_{H3} region. In some specific embodiments, the molecule described herein comprises an IgG C_{H2} region, wherein position 253 in the IgG C_{H2} region by EU numbering is substituted to be a cysteine, a human μ tailpiece (SEQ ID NO: 1), and an IgG C_{H1} region. In some specific embodiments, the molecule described herein comprises an IgG C_{H2} region, wherein position 253 in the IgG C_{H2} region by EU numbering is substituted to be a cysteine, a human μ tailpiece (SEQ ID NO: 1), an IgG hinge region, and an IgG C_{H3} region. In some specific embodiments, the molecule described herein comprises an IgG C_{H2} region, wherein position 253 in the IgG C_{H2} region by EU numbering is substituted to be a cysteine, a human μ tailpiece (SEQ ID NO: 1), an IgG hinge region, and an IgG C_{H1} region. In some specific embodiments, the molecule described herein comprises an IgG C_{H2} region, wherein position 253 in the IgG C_{H2} region by EU numbering is substituted to be a cysteine, a human μ tailpiece (SEQ ID NO: 1), an IgG C_{H3} region, and an IgG C_{H1} region. In some specific embodiments, the molecule described herein comprises an IgG C_{H2} region, wherein position 253 in the IgG C_{H2} region by EU numbering is substituted to be a cysteine, a human μ tailpiece (SEQ ID NO: 1), an IgG hinge region, an IgG C_{H3} region, and an IgG C_{H1} region. In the above embodiments, the order of different regions can follow the order in a wild-type IgG, or can be different from the order in a wild-type IgG, and the human μ tailpiece can be conjugated to the C-terminus or N-terminus of the molecules.

[0092] For all of the IgG, IgE, IgD, IgM, IgA regions described herein, variations may be a substitution, deletion, or insertion of one or more codons encoding the antibody or polypeptide that results in a change in the amino acid sequence as compared with the native sequence antibody or polypeptide. Amino acid substitutions can be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, e.g., conservative amino acid replacements. Standard techniques known to those of skill in the art can be used to introduce mutations in the nucleotide sequence encoding a molecule provided herein, including, for example, site-directed mutagenesis and PCR-mediated mutagenesis which results in amino acid substitutions. Insertions or deletions

may optionally be in the range of about 1 to 5 amino acids. In certain embodiments, the substitution, deletion, or insertion includes fewer than 25 amino acid substitutions, fewer than 20 amino acid substitutions, fewer than 15 amino acid substitutions, fewer than 10 amino acid substitutions, fewer than 5 amino acid substitutions, fewer than 4 amino acid substitutions, fewer than 3 amino acid substitutions, or fewer than 2 amino acid substitutions relative to the original molecule. In a specific embodiment, the substitution is a conservative amino acid substitution made at one or more predicted non-essential amino acid residues. The variation allowed may be determined by systematically making insertions, deletions, or substitutions of amino acids in the sequence and testing the resulting variants for activity exhibited by the full-length or mature native sequence.

[0093] In addition to IgG C_{H2} region, the present molecules may further comprises other IgG regions. In some embodiments, the molecule provided herein comprises IgG C_{H1} region. In some embodiments, the molecule provided herein comprises IgG hinge region. In some embodiments, the molecule provided herein comprises IgG C_{H3} region. In some embodiments, the molecule provided herein comprises IgG C_{H1} and C_{H2} regions. In some embodiments, the molecule provided herein comprises IgG C_{H1} and C_{H3} regions. In some embodiments, the molecule provided herein comprises IgG hinge and C_{H2} regions. In some embodiments, the molecule provided herein comprises IgG hinge and C_{H3} regions. In some embodiments, the molecule provided herein comprises IgG C_{H2} and C_{H3} regions. In some embodiments, the molecule provided herein comprises IgG C_{H1}, hinge, and C_{H2} regions. In some embodiments, the molecule provided herein comprises IgG C_{H1}, hinge, and C_{H3} regions. In some embodiments, the molecule provided herein comprises IgG C_{H1}, C_{H2}, and C_{H3} regions. In some embodiments, the molecule provided herein comprises IgG C_{H1}, hinge, C_{H2}, and C_{H3} regions.

[0094] In some embodiments, the IgG regions disclosed in the preceding paragraph are from human IgG. In some specific embodiments, the IgG regions disclosed in the preceding paragraph are from human IgG1. In some specific embodiments, the IgG regions disclosed in the preceding paragraph are from human IgG2. In some specific embodiments, the IgG regions disclosed in the preceding paragraph are from human IgG3. In some specific embodiments, the IgG regions disclosed in the preceding paragraph are from human IgG4. In some specific embodiments, the IgG regions in one molecule disclosed in the preceding paragraph are mixed

with different human IgG isotypes. In other embodiments, the IgG regions disclosed in the preceding paragraph are from mouse IgG. In other embodiments, the IgG regions disclosed in the preceding paragraph are from rat IgG. In other embodiments, the IgG regions disclosed in the preceding paragraph are from monkey IgG, donkey IgG, sheep IgG, goat IgG, guinea pig IgG, camel IgG, horse IgG, or chicken IgG.

5.2.2 Binding Molecules

[0095] Besides the properties and structures for forming oligomers as disclosed above, the molecules provided herein comprises other properties and functions. In certain embodiments, the present molecules are binding molecules comprising a binding domain. In some embodiments, the present molecules are antibodies or fragments thereof. In other embodiments, the binding domain of the molecule provided herein comprises a non-immunoglobulin binding agent. In other embodiments, the binding domain of the molecule provided herein comprises a sequence of nucleic acids that forms specific binding to the target via reverse complementary binding.

[0096] In some embodiments, the binding domain of the molecule provided herein comprises an antibody fragment. Exemplary fragments include Fab fragments (*e.g.*, an antibody fragment that contains the antigen-binding domain and comprises a light chain and part of a heavy chain bridged by a disulfide bond); Fab' (*e.g.*, an antibody fragment containing a single antigen-binding domain comprising an Fab and an additional portion of the heavy chain through the hinge region); F(ab')₂ (*e.g.*, two Fab' molecules joined by inter-chain disulfide bonds in the hinge regions of the heavy chains; the Fab' molecules may be directed toward the same or different epitopes); a bispecific Fab (*e.g.*, a Fab molecule having two antigen binding domains, each of which may be directed to a different epitope); a single chain comprising a variable region, also known as, scFv (*e.g.*, the variable, antigen-binding determinative region of a single light and heavy chain of an antibody linked together by a chain of 10-25 amino acids); a disulfide-linked Fv, or dsFv (*e.g.*, the variable, antigen-binding determinative region of a single light and heavy chain of an antibody linked together by a disulfide bond); a camelized VH (*e.g.*, the variable, antigen-binding determinative region of a single heavy chain of an antibody in

which some amino acids at the VH interface are those found in the heavy chain of naturally occurring camel antibodies); a bispecific scFv (*e.g.*, an scFv or a dsFv molecule having two antigen-binding domains, each of which may be directed to a different epitope); a diabody (*e.g.*, a dimerized scFv formed when the VH domain of a first scFv assembles with the VL domain of a second scFv and the VL domain of the first scFv assembles with the VH domain of the second scFv; the two antigen-binding regions of the diabody may be directed towards the same or different epitopes); a triabody (*e.g.*, a trimerized scFv, formed in a manner similar to a diabody, but in which three antigen-binding domains are created in a single complex; the three antigen binding domains may be directed towards the same or different epitopes); and a tetrabody (*e.g.*, a tetramerized scFv, formed in a manner similar to a diabody, but in which four antigen-binding domains are created in a single complex; the four antigen binding domains may be directed towards the same or different epitopes).

[0097] Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (*see, e.g.*, Morimoto et al., 1992, J. Biochem. Biophys. Methods 24:107-17; and Brennan et al., 1985, Science 229:81-83). However, these fragments can now be produced directly by recombinant host cells. Fab, Fv, and scFv antibody fragments can all be expressed in and secreted from E. coli or yeast cells, thus allowing the facile production of large amounts of these fragments. Antibody fragments can be isolated from the antibody phage libraries. Alternatively, Fab'-SH fragments can be directly recovered from E. coli and chemically coupled to form F(ab')₂ fragments (Carter et al., 1992, Bio/Technology 10:163-67). According to another approach, F(ab')₂ fragments can be isolated directly from recombinant host cell culture. Fab and F(ab')₂ fragment with increased *in vivo* half-life comprising salvage receptor binding epitope residues are described in, for example, U.S. Pat. No. 5,869,046. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In certain embodiments, an antibody is a single chain Fv fragment (scFv) (*see, e.g.*, WO 93/16185; U.S. Pat. Nos. 5,571,894 and 5,587,458). Fv and scFv have intact combining sites that are devoid of constant regions; thus, they may be suitable for reduced nonspecific binding during *in vivo* use. scFv fusion proteins may be constructed to yield fusion of an effector protein at either the amino or the carboxy terminus of an scFv (*See, e.g.*, Borrebaeck ed., *supra*). The antibody fragment may also

be a “linear antibody,” for example, as described in the references cited above. Such linear antibodies may be monospecific or multi-specific, such as bispecific.

[0098] Smaller antibody-derived binding structures are the separate variable domains (V domains) also termed single variable domain antibodies (sdAbs). Certain types of organisms, the camelids and cartilaginous fish, possess high affinity single V-like domains mounted on an Fc equivalent domain structure as part of their immune system. (Woolven et al., 1999, Immunogenetics 50: 98-101; and Streltsov et al., 2004, Proc Natl Acad Sci USA. 101:12444-49). The V-like domains (called VhH in camelids and V-NAR in sharks) typically display long surface loops, which allow penetration of cavities of target antigens. They also stabilize isolated VH domains by masking hydrophobic surface patches.

[0099] These VhH and V-NAR domains have been used to engineer sdAbs. Human V domain variants have been designed using selection from phage libraries and other approaches that have resulted in stable, high binding VL- and VH-derived domains.

[00100] The antibody fragment in the molecule provided herein can be of any class (*e.g.*, IgG, IgE, IgM, IgD, and IgA) or any subclass (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2) of immunoglobulin molecule.

[00101] The antibodies described herein can, for example, include humanized antibodies, *e.g.*, deimmunized or composite human antibodies.

[00102] A humanized antibody can comprise human framework region and human constant region sequences. For example, a humanized antibody can comprise human constant region sequences. In certain embodiments, a humanized antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG1, IgG2, IgG3 and IgG4 (*e.g.*, variants of IgG4 and IgG4 nullbody). In certain embodiments, a humanized antibody can comprise kappa or lambda light chain constant sequences.

[00103] Humanized antibodies can be produced using a variety of techniques known in the art, including but not limited to, CDR-grafting (European Patent No. EP 239,400; International publication No. WO 91/09967; and U.S. Patent Nos. 5,225,539, 5,530,101, and 5,585,089), veneering or resurfacing (European Patent Nos. EP 592,106 and EP 519,596; Padlan, 1991, Molecular Immunology 28(4/5):489-498; Studnicka et al., 1994, Protein Engineering 7(6):805-814; and Roguska et al., 1994, PNAS 91:969-973), chain shuffling (U.S. Patent No. 5,565,332),

and techniques disclosed in, *e.g.*, U.S. Pat. No. 6,407,213, U.S. Pat. No. 5,766,886, WO 93/17105, Tan et al., J. Immunol. 169:1119-25 (2002), Caldas et al., Protein Eng. 13(5):353-60 (2000), Morea et al., Methods 20(3):267-79 (2000), Baca et al., J. Biol. Chem. 272(16):10678-84 (1997), Roguska et al., Protein Eng. 9(10):895-904 (1996), Couto et al., Cancer Res. 55 (23 Supp):5973s-5977s (1995), Couto et al., Cancer Res. 55(8):1717-22 (1995), Sandhu JS, Gene 150(2):409-10 (1994), and Pedersen et al., J. Mol. Biol. 235(3):959-73 (1994). *See also* U.S. Patent Pub. No. US 2005/0042664 A1 (Feb. 24, 2005), each of which is incorporated by reference herein in its entirety.

[00104] Various methods for humanizing non-human antibodies are known in the art. For example, a humanized antibody can have one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as “import” residues, which are typically taken from an “import” variable domain. Humanization may be performed, for example, following the method of Jones et al., 1986, Nature 321:522-25; Riechmann et al., 1988, Nature 332:323-27; and Verhoeyen et al., 1988, Science 239:1534-36), by substituting hypervariable region sequences for the corresponding sequences of a human antibody.

[00105] In some cases, the humanized antibodies are constructed by CDR grafting, in which the amino acid sequences of the six CDRs of the parent non-human antibody (*e.g.*, rodent) are grafted onto a human antibody framework. For example, Padlan et al. determined that only about one third of the residues in the CDRs actually contact the antigen, and termed these the “specificity determining residues,” or SDRs (Padlan et al., 1995, FASEB J. 9:133-39). In the technique of SDR grafting, only the SDR residues are grafted onto the human antibody framework (*see, e.g.*, Kashmiri et al., 2005, Methods 36:25-34).

[00106] The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies can be important to reduce antigenicity. For example, according to the so-called “best-fit” method, the sequence of the variable domain of a non-human (*e.g.*, rodent) antibody is screened against the entire library of known human variable-domain sequences. The human sequence that is closest to that of the rodent may be selected as the human framework for the humanized antibody (Sims et al., 1993, J. Immunol. 151:2296-308; and Chothia et al., 1987, J. Mol. Biol. 196:901-17). Another method uses a particular framework derived from the

consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter et al., 1992, Proc. Natl. Acad. Sci. USA 89:4285-89; and Presta et al., 1993, J. Immunol. 151:2623-32). In some cases, the framework is derived from the consensus sequences of the most abundant human subclasses, VL6 subgroup I (VL6I) and VH subgroup III (VHIII). In another method, human germline genes are used as the source of the framework regions.

[00107] In an alternative paradigm based on comparison of CDRs, called superhumanization, FR homology is irrelevant. The method consists of comparison of the non-human sequence with the functional human germline gene repertoire. Those genes encoding the same or closely related canonical structures to the murine sequences are then selected. Next, within the genes sharing the canonical structures with the non-human antibody, those with highest homology within the CDRs are chosen as FR donors. Finally, the non-human CDRs are grafted onto these FRs (*see, e.g.*, Tan et al., 2002, J. Immunol. 169:1119-25).

[00108] It is further generally desirable that antibodies be humanized with retention of their affinity for the antigen and other favorable biological properties. To achieve this goal, according to one method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. These include, for example, WAM (Whitelegg and Rees, 2000, Protein Eng. 13:819-24), Modeller (Sali and Blundell, 1993, J. Mol. Biol. 234:779-815), and Swiss PDB Viewer (Guex and Peitsch, 1997, Electrophoresis 18:2714-23). Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, *e.g.*, the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

[00109] Another method for antibody humanization is based on a metric of antibody humanness termed Human String Content (HSC). This method compares the mouse sequence with the repertoire of human germline genes, and the differences are scored as HSC. The target sequence is then humanized by maximizing its HSC rather than using a global identity measure to generate multiple diverse humanized variants (Lazar et al., 2007, *Mol. Immunol.* 44:1986-98).

[00110] In addition to the methods described above, empirical methods may be used to generate and select humanized antibodies. These methods include those that are based upon the generation of large libraries of humanized variants and selection of the best clones using enrichment technologies or high throughput screening techniques. Antibody variants may be isolated from phage, ribosome, and yeast display libraries as well as by bacterial colony screening (*see, e.g.*, Hoogenboom, 2005, *Nat. Biotechnol.* 23:1105-16; Dufner et al., 2006, *Trends Biotechnol.* 24:523-29; Feldhaus et al., 2003, *Nat. Biotechnol.* 21:163-70; and Schlapschy et al., 2004, *Protein Eng. Des. Sel.* 17:847-60).

[00111] In the FR library approach, a collection of residue variants are introduced at specific positions in the FR followed by screening of the library to select the FR that best supports the grafted CDR. The residues to be substituted may include some or all of the “Vernier” residues identified as potentially contributing to CDR structure (*see, e.g.*, Foote and Winter, 1992, *J. Mol. Biol.* 224:487-99), or from the more limited set of target residues identified by Baca et al. (1997, *J. Biol. Chem.* 272:10678-84).

[00112] In FR shuffling, whole FRs are combined with the non-human CDRs instead of creating combinatorial libraries of selected residue variants (*see, e.g.*, Dall’Acqua et al., 2005, *Methods* 36:43-60). The libraries may be screened for binding in a two-step process, first humanizing VL, followed by VH. Alternatively, a one-step FR shuffling process may be used. Such a process has been shown to be more efficient than the two-step screening, as the resulting antibodies exhibited improved biochemical and physicochemical properties including enhanced expression, increased affinity, and thermal stability (*see, e.g.*, Damschroder et al., 2007, *Mol. Immunol.* 44:3049-60).

[00113] The “humaneering” method is based on experimental identification of essential minimum specificity determinants (MSDs) and is based on sequential replacement of non-human fragments into libraries of human FRs and assessment of binding. It begins with regions of the

CDR3 of non-human VH and VL chains and progressively replaces other regions of the non-human antibody into the human FRs, including the CDR1 and CDR2 of both VH and VL. This methodology typically results in epitope retention and identification of antibodies from multiple subclasses with distinct human V-segment CDRs. Humaneering allows for isolation of antibodies that are 91-96% homologous to human germline gene antibodies (*see, e.g.*, Alfenito, Cambridge Healthtech Institute's Third Annual PEGS, The Protein Engineering Summit, 2007).

[00114] The "human engineering" method involves altering a non-human antibody or antibody fragment, such as a mouse or chimeric antibody or antibody fragment, by making specific changes to the amino acid sequence of the antibody so as to produce a modified antibody with reduced immunogenicity in a human that nonetheless retains the desirable binding properties of the original non-human antibodies. Generally, the technique involves classifying amino acid residues of a non-human (*e.g.*, mouse) antibody as "low risk," "moderate risk," or "high risk" residues. The classification is performed using a global risk/reward calculation that evaluates the predicted benefits of making particular substitution (*e.g.*, for immunogenicity in humans) against the risk that the substitution will affect the resulting antibody's folding. The particular human amino acid residue to be substituted at a given position (*e.g.*, low or moderate risk) of a non-human (*e.g.*, mouse) antibody sequence can be selected by aligning an amino acid sequence from the non-human antibody's variable regions with the corresponding region of a specific or consensus human antibody sequence. The amino acid residues at low or moderate risk positions in the non-human sequence can be substituted for the corresponding residues in the human antibody sequence according to the alignment. Techniques for making human engineered proteins are described in greater detail in Studnicka et al., 1994, Protein Engineering 7:805-14; U.S. Pat. Nos. 5,766,886; 5,770,196; 5,821,123; and 5,869,619; and PCT Publication WO 93/11794.

[00115] A composite human antibody can be generated using, for example, Composite Human Antibody™ technology (Antitope Ltd., Cambridge, United Kingdom). To generate composite human antibodies, variable region sequences are designed from fragments of multiple human antibody variable region sequences in a manner that avoids T cell epitopes, thereby minimizing the immunogenicity of the resulting antibody. Such antibodies can comprise human constant region sequences, *e.g.*, human light chain and/or heavy chain constant regions.

[00116] In some embodiments, the molecule provided herein contains a deimmunized antibody fragment whose T-cell epitopes have been removed. Methods for making deimmunized antibodies have been described. *See, e.g.*, Jones et al., *Methods Mol Biol.* 2009;525:405-23, xiv, and De Groot et al., *Cell. Immunol.* 244:148-153(2006)). Deimmunized antibodies comprise T-cell epitope-depleted variable regions and human constant regions. Briefly, VH and VL of an antibody are cloned and T-cell epitopes are subsequently identified by testing overlapping peptides derived from the VH and VL of the antibody in a T cell proliferation assay. T cell epitopes are identified via *in silico* methods to identify peptide binding to human MHC class II. Mutations are introduced in the VH and VL to abrogate binding to human MHC class II. Mutated VH and VL are then utilized to generate the deimmunized antibody

[00117] In specific embodiments, the molecule described herein is derived from a fully human anti-human antibody. Fully human antibodies may be produced by any method known in the art. Human antibody fragment provided herein can be constructed by combining Fv clone variable domain sequence(s) selected from human-derived phage display libraries with known human constant domain sequences(s). Alternatively, human monoclonal antibodies of the present disclosure can be made by the hybridoma method. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described, for example, by Kozbor, 1984, *J. Immunol.* 133:3001-05; Brodeur et al., *Monoclonal Antibody Production Techniques and Applications* 51-63 (1987); and Boerner et al., 1991, *J. Immunol.* 147:86-95.

[00118] It is also possible to produce transgenic animals (*e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. Transgenic mice that express human antibody repertoires have been used to generate high-affinity human sequence monoclonal antibodies against a wide variety of potential drug targets (*see, e.g.*, Jakobovits, A., 1995, *Curr. Opin. Biotechnol.* 6(5):561-66; Brüggemann and Taussing, 1997, *Curr. Opin. Biotechnol.* 8(4):455-58; U.S. Pat. Nos. 6,075,181 and 6,150,584; and Lonberg et al., 2005, *Nature Biotechnol.* 23:1117-25).

[00119] Alternatively, the human antibody may be prepared via immortalization of human B lymphocytes producing an antibody directed against a target antigen (*e.g.*, such B lymphocytes may be recovered from an individual or may have been immunized *in vitro*) (*see, e.g.*, Cole et

al., Monoclonal Antibodies and Cancer Therapy (1985); Boerner et al., 1991, J. Immunol. 147(1):86-95; and U.S. Pat. No. 5,750,373).

[00120] Gene shuffling can also be used to derive human antibodies from non-human, for example, rodent, antibodies, where the human antibody has similar affinities and specificities to the starting non-human antibody. According to this method, which is also called “epitope imprinting” or “guided selection,” either the heavy or light chain variable region of a non-human antibody fragment obtained by phage display techniques as described herein is replaced with a repertoire of human V domain genes, creating a population of non-human chain/human chain scFv or Fab chimeras. Selection with antigen results in isolation of a non-human chain/human chain chimeric scFv or Fab wherein the human chain restores the antigen binding site destroyed upon removal of the corresponding non-human chain in the primary phage display clone (*e.g.*, the epitope guides (imprints) the choice of the human chain partner). When the process is repeated in order to replace the remaining non-human chain, a human antibody is obtained (*see, e.g.*, PCT WO 93/06213; and Osbourn et al., 2005, Methods 36:61-68). Unlike traditional humanization of non-human antibodies by CDR grafting, this technique provides completely human antibodies, which have no FR or CDR residues of non-human origin. Examples of guided selection to humanize mouse antibodies towards cell surface antigens include the folate-binding protein present on ovarian cancer cells (*see, e.g.*, Figini et al., 1998, Cancer Res. 58:991-96) and CD147, which is highly expressed on hepatocellular carcinoma (*see, e.g.*, Bao et al., 2005, Cancer Biol. Ther. 4:1374-80).

[00121] A potential disadvantage of the guided selection approach is that shuffling of one antibody chain while keeping the other constant could result in epitope drift. In order to maintain the epitope recognized by the non-human antibody, CDR retention can be applied (*see, e.g.*, Klimka et al., 2000, Br. J. Cancer. 83:252-60; and Beiboer et al., 2000, J. Mol. Biol. 296:833-49). In this method, the non-human VH CDR3 is commonly retained, as this CDR may be at the center of the antigen-binding site and may be the most important region of the antibody for antigen recognition. In some instances, however, VH CDR3 and VL CDR3, as well as VH CDR2, VL CDR2, and VL CDR1 of the non-human antibody may be retained.

[00122] Multispecific antibodies such as bispecific antibodies are monoclonal antibodies that have binding specificities for at least two different antigens. In certain embodiments, the

multispecific antibodies provided herein are bispecific antibodies. In certain embodiments, bispecific antibodies are mouse, chimeric, human or humanized antibodies. In certain embodiments, one of the binding specificities is for one target/antigen and the other is for another target/antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of the same target/antigen. Bispecific antibodies can be prepared as full length antibodies or antibody fragments (*e.g.*, F(ab')₂ bispecific antibodies).

[00123] Methods for making multispecific antibodies are known in the art, such as, by co-expression of two immunoglobulin heavy chain-light chain pairs, where the two heavy chains have different specificities (*see, e.g.*, Milstein and Cuello, 1983, Nature 305:537-40). For further details of generating multispecific antibodies (*e.g.*, bispecific antibodies), *see*, for example, Bispecific Antibodies (Kontermann ed., 2011).

[00124] It may be desirable to modify the molecule described herein by Fc engineering, if the molecule described herein contains C_H1, hinge, C_H2, and/or C_H3 region(s). In certain embodiments, the modification to the above-mentioned regions in the molecule described herein results in the decrease or elimination of an effector function of the antibody. In certain embodiments, the effector function is antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and/or complement-dependent cytotoxicity (CDC). In some embodiments, the effector function is ADCC. In other embodiments, the effector function is ADCP. In other embodiments, the effector function is CDC. In one embodiment, the effector function is ADCC and ADCP. In one embodiment, the effector function is ADCC and CDC. In one embodiment, the effector function is ADCP and CDC. In one embodiment, the effector function is ADCC, ADCP and CDC. This may be achieved by introducing one or more amino acid substitutions in an Fc region of the molecule described herein.

[00125] To increase the serum half-life of the molecule described herein, one may incorporate a salvage receptor binding epitope into the molecule, for example, as described in U.S. Pat. No. 5,739,277. Term “salvage receptor binding epitope” refers to an epitope of the Fc region of an IgG molecule (*e.g.*, IgG1, IgG2, IgG3, or IgG4) that is responsible for increasing the *in vivo* serum half-life of the IgG molecule.

[00126] In some embodiments, amino acid sequence modification(s) of the molecule provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody, including but not limited to specificity, thermostability, expression level, effector functions, glycosylation, reduced immunogenicity, or solubility. Thus, in addition to the molecule and its antibody fragments described herein, it is contemplated that antibody variants can be prepared. For example, antibody variants can be prepared by introducing appropriate nucleotide changes into the encoding DNA, and/or by synthesis of the desired antibody or polypeptide. Those skilled in the art would appreciate that amino acid changes may alter post-translational processes of the antibody, such as changing the number or position of glycosylation sites or altering the membrane anchoring characteristics.

[00127] In some embodiments, the molecule provided herein is chemically modified, for example, by the covalent attachment of any type of other molecule(s) to the molecule. The antibody derivatives may include antibodies that have been chemically modified, for example, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to, specific chemical cleavage, acetylation, formulation, metabolic synthesis of tunicamycin, etc. Additionally, the antibody may contain one or more non-classical amino acids.

[00128] Variations may be a substitution, deletion, or insertion of one or more codons encoding the antibody or polypeptide that results in a change in the amino acid sequence as compared with the native sequence antibody or polypeptide. Amino acid substitutions can be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, *e.g.*, conservative amino acid replacements. Standard techniques known to those of skill in the art can be used to introduce mutations in the nucleotide sequence encoding a molecule provided herein, including, for example, site-directed mutagenesis and PCR-mediated mutagenesis which results in amino acid substitutions. Insertions or deletions may optionally be in the range of about 1 to 5 amino acids. In certain embodiments, the substitution, deletion, or insertion includes fewer than 25 amino acid substitutions, fewer than 20 amino acid substitutions, fewer than 15 amino acid substitutions, fewer than 10 amino acid substitutions, fewer than 5 amino acid substitutions,

fewer than 4 amino acid substitutions, fewer than 3 amino acid substitutions, or fewer than 2 amino acid substitutions relative to the original molecule. In a specific embodiment, the substitution is a conservative amino acid substitution made at one or more predicted non-essential amino acid residues. The variation allowed may be determined by systematically making insertions, deletions, or substitutions of amino acids in the sequence and testing the resulting variants for activity exhibited by the full-length or mature native sequence.

[00129] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (*e.g.*, for antibody-directed enzyme prodrug therapy) or a polypeptide which increases the serum half-life of the antibody.

[00130] A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a side chain with a similar charge. Families of amino acid residues having side chains with similar charges have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity. Following mutagenesis, the encoded protein can be expressed and the activity of the protein can be determined.

[00131] Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Alternatively, conservative (*e.g.*, within an amino acid group with

similar properties and/or side chains) substitutions may be made, so as to maintain or not significantly change the properties. Amino acids may be grouped according to similarities in the properties of their side chains (*see, e.g.,* Lehninger, Biochemistry 73-75 (2d ed. 1975)): (1) non-polar: Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M); (2) uncharged polar: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q); (3) acidic: Asp (D), Glu (E); and (4) basic: Lys (K), Arg (R), His(H).

[00132] Alternatively, naturally occurring residues may be divided into groups based on common side-chain properties: (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile; (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln; (3) acidic: Asp, Glu; (4) basic: His, Lys, Arg; (5) residues that influence chain orientation: Gly, Pro; and (6) aromatic: Trp, Tyr, Phe.

[00133] Any cysteine residue not involved in maintaining the proper conformation of the antibody provided herein also may be substituted, for example, with another amino acid, such as alanine or serine, to improve the oxidative stability of the molecule and to prevent aberrant crosslinking.

[00134] In some embodiments, the molecule with antibody variants having an improved property such as affinity, stability, or expression level as compared to a parent molecule may be prepared by *in vitro* affinity maturation. Like the natural prototype, *in vitro* affinity maturation is based on the principles of mutation and selection. Libraries of antibodies are displayed on the surface of an organism (*e.g.,* phage, bacteria, yeast, or mammalian cell) or in association (*e.g.,* covalently or non-covalently) with their encoding mRNA or DNA. Affinity selection of the displayed antibodies allows isolation of organisms or complexes carrying the genetic information encoding the antibodies. Two or three rounds of mutation and selection using display methods such as phage display usually results in antibody fragments with affinities in the low nanomolar range. The molecule with affinity matured antibodies can have nanomolar or even picomolar affinities for the target antigen.

[00135] Phage display is a widespread method for display and selection of antibodies. The antibodies are displayed on the surface of Fd or M13 bacteriophages as fusions to the bacteriophage coat protein. Selection involves exposure to antigen to allow phage-displayed antibodies to bind their targets, a process referred to as “panning.” Phage bound to antigen are recovered and used to infect bacteria to produce phage for further rounds of selection. For

review, *see*, for example, Hoogenboom, 2002, *Methods. Mol. Biol.* 178:1-37; and Bradbury and Marks, 2004, *J. Immunol. Methods* 290:29-49.

[00136] In a yeast display system (*see, e.g.*, Boder et al., 1997, *Nat. Biotech.* 15:553–57; and Chao et al., 2006, *Nat. Protocols* 1:755-68), the antibody may be fused to the adhesion subunit of the yeast agglutinin protein Aga2p, which attaches to the yeast cell wall through disulfide bonds to Aga1p. Display of a protein via Aga2p projects the protein away from the cell surface, minimizing potential interactions with other molecules on the yeast cell wall. Magnetic separation and flow cytometry are used to screen the library to select for antibodies with improved affinity or stability. Binding to a soluble antigen of interest is determined by labeling of yeast with biotinylated antigen and a secondary reagent such as streptavidin conjugated to a fluorophore. Variations in surface expression of the antibody can be measured through immunofluorescence labeling of either the hemagglutinin or c-Myc epitope tag flanking the scFv. Expression has been shown to correlate with the stability of the displayed protein, and thus antibodies can be selected for improved stability as well as affinity (*see, e.g.*, Shusta et al., 1999, *J. Mol. Biol.* 292:949-56). An additional advantage of yeast display is that displayed proteins are folded in the endoplasmic reticulum of the eukaryotic yeast cells, taking advantage of endoplasmic reticulum chaperones and quality-control machinery. Once maturation is complete, antibody affinity can be conveniently “titrated” while displayed on the surface of the yeast, eliminating the need for expression and purification of each clone. A theoretical limitation of yeast surface display is the potentially smaller functional library size than that of other display methods; however, a recent approach uses the yeast cells’ mating system to create combinatorial diversity estimated to be 10^{14} in size (*see, e.g.*, U.S. Pat. Publication 2003/0186374; and Blaise et al., 2004, *Gene* 342:211–18).

[00137] In ribosome display, antibody-ribosome-mRNA (ARM) complexes are generated for selection in a cell-free system. The DNA library coding for a particular library of antibodies is genetically fused to a spacer sequence lacking a stop codon. This spacer sequence, when translated, is still attached to the peptidyl tRNA and occupies the ribosomal tunnel, and thus allows the protein of interest to protrude out of the ribosome and fold. The resulting complex of mRNA, ribosome, and protein can bind to surface-bound ligand, allowing simultaneous isolation of the antibody and its encoding mRNA through affinity capture with the ligand. The ribosome-

bound mRNA is then reverse transcribed back into cDNA, which can then undergo mutagenesis and be used in the next round of selection (*see, e.g.*, Fukuda et al., 2006, Nucleic Acids Res. 34:e127). In mRNA display, a covalent bond between antibody and mRNA is established using puromycin as an adaptor molecule (Wilson et al., 2001, Proc. Natl. Acad. Sci. USA 98:3750-55).

[00138] As these methods are performed entirely *in vitro*, they provide two main advantages over other selection technologies. First, the diversity of the library is not limited by the transformation efficiency of bacterial cells, but only by the number of ribosomes and different mRNA molecules present in the test tube. Second, random mutations can be introduced easily after each selection round, for example, by non-proofreading polymerases, as no library must be transformed after any diversification step. In some embodiments, mammalian display systems may be used.

[00139] Diversity may also be introduced into the CDRs of the antibody libraries in a targeted manner or via random introduction. The former approach includes sequentially targeting all the CDRs of an antibody via a high or low level of mutagenesis or targeting isolated hot spots of somatic hypermutations (*see, e.g.*, Ho et al., 2005, J. Biol. Chem. 280:607-17) or residues suspected of affecting affinity on experimental basis or structural reasons. Diversity may also be introduced by replacement of regions that are naturally diverse via DNA shuffling or similar techniques (*see, e.g.*, Lu et al., 2003, J. Biol. Chem. 278:43496-507; U.S. Pat. Nos. 5,565,332 and 6,989,250). Alternative techniques target hypervariable loops extending into framework-region residues (*see, e.g.*, Bond et al., 2005, J. Mol. Biol. 348:699-709) employ loop deletions and insertions in CDRs or use hybridization-based diversification (*see, e.g.*, U.S. Pat. Publication No. 2004/0005709). Additional methods of generating diversity in CDRs are disclosed, for example, in U.S. Pat. No. 7,985,840. Further methods that can be used to generate antibody libraries and/or antibody affinity maturation are disclosed, *e.g.*, in U.S. Patent Nos. 8,685,897 and 8,603,930, and U.S. Publ. Nos. 2014/0170705, 2014/0094392, 2012/0028301, 2011/0183855, and 2009/0075378, each of which are incorporated herein by reference.

[00140] Screening of the libraries can be accomplished by various techniques known in the art. For example, the antibodies can be immobilized onto solid supports, columns, pins, or cellulose/poly(vinylidene fluoride) membranes/other filters, expressed on host cells affixed to

adsorption plates or used in cell sorting, or conjugated to biotin for capture with streptavidin-coated beads or used in any other method for panning display libraries.

[00141] For review of *in vitro* affinity maturation methods, *see, e.g.*, Hoogenboom, 2005, Nature Biotechnology 23:1105-16; Quiroz and Sinclair, 2010, Revista Ingenieria Biomedica 4:39-51; and references therein.

[00142] Covalent modifications of the antibody fragment(s) of the molecule provided herein are included within the scope of the present disclosure. Covalent modifications include reacting targeted amino acid residues of an antibody with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C- terminal residues of the antibody fragment(s) of the molecule. Other modifications include deamidation of glutaminyl and asparaginyl residues to the corresponding glutamyl and aspartyl residues, respectively, hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α -amino groups of lysine, arginine, and histidine side chains (*see, e.g.*, Creighton, Proteins: Structure and Molecular Properties 79-86 (1983)), acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

[00143] Other types of covalent modification of the antibody fragment(s) of the molecule provided herein included within the scope of this present disclosure include altering the native glycosylation pattern of the antibody or polypeptide (*see, e.g.*, Beck et al., 2008, Curr. Pharm. Biotechnol. 9:482-501; and Walsh, 2010, Drug Discov. Today 15:773-80), and linking the antibody to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol (PEG), polypropylene glycol, or polyoxyalkylenes, in the manner set forth, for example, in U.S. Pat. Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192; or 4,179,337.

[00144] An antibody fragment(s) of the molecule of the present disclosure may also be modified to form chimeric molecules comprising the antibody fragment(s) of the molecule fused to another, heterologous polypeptide or amino acid sequence, for example, an epitope tag (*see, e.g.*, Terpe, 2003, Appl. Microbiol. Biotechnol. 60:523-33) or another Fc region of an IgG molecule (*see, e.g.*, Aruffo, Antibody Fusion Proteins 221-42 (Chamow and Ashkenazi eds., 1999)).

[00145] The present disclosure also provides conjugates comprising any one of the antibodies of the present disclosure covalently bound by a synthetic linker to one or more non-antibody agents.

[00146] In some embodiments, antibody fragment(s) of the molecule provided herein are conjugated or recombinantly fused, *e.g.*, to a therapeutic agent (*e.g.*, a cytotoxic agent) or a diagnostic or detectable molecule. The conjugated or recombinantly fused antibody fragment(s) of the molecule can be useful, for example, for treating or preventing a disease or disorder. The conjugated or recombinantly fused antibodies can be useful, for example, for monitoring or prognosing the onset, development, progression, and/or severity of a disease.

[00147] Such diagnosis and detection can be accomplished, for example, by coupling the antibody fragment(s) of the molecule to detectable substances including, but not limited to, various enzymes, such as, but not limited to, horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; prosthetic groups, such as, but not limited to, streptavidin/biotin or avidin/biotin; fluorescent materials, such as, but not limited to, umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride, or phycoerythrin; luminescent materials, such as, but not limited to, luminol; bioluminescent materials, such as, but not limited to, luciferase, luciferin, or aequorin; chemiluminescent material, such as, but not limited to, an acridinium based compound or a HALOTAG; radioactive materials, such as, but not limited to, iodine (¹³¹I, ¹²⁵I, ¹²³I, and ¹²¹I), carbon (¹⁴C), sulfur (³⁵S), tritium (³H), indium (¹¹⁵In, ¹¹³In, ¹¹²In, and ¹¹¹In), technetium (⁹⁹Tc), thallium (²⁰¹Tl), gallium (⁶⁸Ga and ⁶⁷Ga), palladium (¹⁰³Pd), molybdenum (⁹⁹Mo), xenon (¹³³Xe), fluorine (¹⁸F), ¹⁵³Sm, ¹⁷⁷Lu, ¹⁵⁹Gd, ¹⁴⁹Pm, ¹⁴⁰La, ¹⁷⁵Yb, ¹⁶⁶Ho, ⁹⁰Y, ⁴⁷Sc, ¹⁸⁶Re, ¹⁸⁸Re, ¹⁴²Pr, ¹⁰⁵Rh, ⁹⁷Ru, ⁶⁸Ge, ⁵⁷Co, ⁶⁵Zn, ⁸⁵Sr, ³²P, ¹⁵³Gd, ¹⁶⁹Yb, ⁵¹Cr, ⁵⁴Mn, ⁷⁵Se, ¹¹³Sn, or ¹¹⁷Sn; positron emitting metals using various positron emission tomographies; and non-radioactive paramagnetic metal ions.

[00148] Also provided herein are antibody fragment(s) of the molecule that are recombinantly fused or chemically conjugated (covalent or non-covalent conjugations) to a heterologous protein or polypeptide (or fragment thereof, for example, to a polypeptide of about 10, about 20, about 30, about 40, about 50, about 60, about 70, about 80, about 90, or about 100 amino acids) to generate fusion proteins, as well as uses thereof. In particular, provided herein are fusion

proteins comprising a targeting region and a heterologous protein, polypeptide, or peptide. In one embodiment, the heterologous protein, polypeptide, or peptide that the antibody is fused to is useful for targeting the antibody to a particular cell type.

[00149] Moreover, antibodies provided herein can be fused to marker or “tag” sequences, such as a peptide, to facilitate purification. In specific embodiments, the marker or tag amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (*see, e.g.*, QIAGEN, Inc.), among others, many of which are commercially available. For example, as described in Gentz et al., 1989, Proc. Natl. Acad. Sci. USA 86:821-24, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the hemagglutinin (“HA”) tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., 1984, Cell 37:767-78), and the “FLAG” tag.

[00150] Methods for fusing or conjugating moieties (including polypeptides) to antibodies are known (*see, e.g.*, Arnon et al., Monoclonal Antibodies for Immunotargeting of Drugs in Cancer Therapy, in Monoclonal Antibodies and Cancer Therapy 243-56 (Reisfeld et al. eds., 1985); Hellstrom et al., Antibodies for Drug Delivery, in Controlled Drug Delivery 623-53 (Robinson et al. eds., 2d ed. 1987); Thorpe, Antibody Carriers of Cytotoxic Agents in Cancer Therapy: A Review, in Monoclonal Antibodies: Biological and Clinical Applications 475-506 (Pinchera et al. eds., 1985); Analysis, Results, and Future Prospective of the Therapeutic Use of Radiolabeled Antibody in Cancer Therapy, in Monoclonal Antibodies for Cancer Detection and Therapy 303-16 (Baldwin et al. eds., 1985); Thorpe et al., 1982, Immunol. Rev. 62:119-58; U.S. Pat. Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,723,125; 5,783,181; 5,908,626; 5,844,095; and 5,112,946; EP 307,434; EP 367,166; EP 394,827; PCT publications WO 91/06570, WO 96/04388, WO 96/22024, WO 97/34631, and WO 99/04813; Ashkenazi et al., 1991, Proc. Natl. Acad. Sci. USA, 88: 10535-39; Traunecker et al., 1988, Nature, 331:84-86; Zheng et al., 1995, J. Immunol. 154:5590-600; and Vil et al., 1992, Proc. Natl. Acad. Sci. USA 89:11337-41).

[00151] Fusion proteins may be generated, for example, through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as “DNA shuffling”). DNA shuffling may be employed to alter the activities of the antibodies as

provided herein, including, for example, antibodies with higher affinities and lower dissociation rates (*see, e.g.*, U.S. Pat. Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458; Patten et al., 1997, *Curr. Opin. Biotechnol.* 8:724-33; Harayama, 1998, *Trends Biotechnol.* 16(2):76-82; Hansson et al., 1999, *J. Mol. Biol.* 287:265-76; and Lorenzo and Blasco, 1998, *Biotechniques* 24(2):308-13). Antibodies, or the encoded antibodies, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion, or other methods prior to recombination. A polynucleotide encoding an antibody provided herein may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

[00152] The antibody fragment(s) of the molecule provided herein can also be conjugated to a second antibody to form an antibody heteroconjugate as described, for example, in U.S. Pat. No. 4,676,980.

[00153] The antibody fragment(s) of the molecule as provided herein may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride, or polypropylene.

[00154] The linker may be a “cleavable linker” facilitating release of the conjugated agent in the cell, but non-cleavable linkers are also contemplated herein. Linkers for use in the conjugates of the present disclosure include, without limitation, acid labile linkers (*e.g.*, hydrazone linkers), disulfide-containing linkers, peptidase-sensitive linkers (*e.g.*, peptide linkers comprising amino acids, for example, valine and/or citrulline such as citrulline-valine or phenylalanine-lysine), photolabile linkers, dimethyl linkers (*see, e.g.*, Chari et al., 1992, *Cancer Res.* 52:127-31; and U.S. Pat. No. 5,208,020), thioether linkers, or hydrophilic linkers designed to evade multidrug transporter-mediated resistance (*see, e.g.*, Kovtun et al., 2010, *Cancer Res.* 70:2528-37).

[00155] Conjugates of the antibody and an agent may be made using a variety of bifunctional protein coupling agents such as BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate). The present disclosure further contemplates that conjugates of

antibodies and agents may be prepared using any suitable methods as disclosed in the art (*see, e.g.*, Bioconjugate Techniques (Hermanson ed., 2d ed. 2008)).

[00156] Conventional conjugation strategies for antibodies and agents have been based on random conjugation chemistries involving the ϵ -amino group of Lys residues or the thiol group of Cys residues, which results in heterogeneous conjugates. Recently developed techniques allow site-specific conjugation to antibodies, resulting in homogeneous loading and avoiding conjugate subpopulations with altered antigen-binding or pharmacokinetics. These include engineering of “thiomabs” comprising cysteine substitutions at positions on the heavy and light chains that provide reactive thiol groups and do not disrupt immunoglobulin folding and assembly or alter antigen binding (*see, e.g.*, Junutula et al., 2008, J. Immunol. Meth. 332: 41-52; and Junutula et al., 2008, Nature Biotechnol. 26:925-32). In another method, selenocysteine is cotranslationally inserted into an antibody sequence by recoding the stop codon UGA from termination to selenocysteine insertion, allowing site specific covalent conjugation at the nucleophilic selenol group of selenocysteine in the presence of the other natural amino acids (*see, e.g.*, Hofer et al., 2008, Proc. Natl. Acad. Sci. USA 105:12451-56; and Hofer et al., 2009, Biochemistry 48(50):12047-57).

[00157] In some embodiments, the targeting region of the molecule provided herein comprises a non-immunoglobulin binding agent. The non-immunoglobulin binding agent forms specific binding to the target via enzyme-substrate, receptor-ligand or other protein-protein interactions. In some embodiments, a non-immunoglobulin binding agent is identified as an agent that displaces or is displaced by the molecule of the present disclosure in a competitive binding assay. These alternative binding agents may include, for example, any of the engineered protein scaffolds known in the art. Such scaffolds include, for example, anticalins, which are based upon the lipocalin scaffold, a protein structure characterized by a rigid beta-barrel that supports four hypervariable loops which form the ligand binding site. Novel binding specificities may be engineered by targeted random mutagenesis in the loop regions, in combination with functional display and guided selection (*see, e.g.*, Skerra, 2008, FEBS J. 275:2677-83). Other suitable scaffolds may include, for example, adnectins, or monobodies, based on the tenth extracellular domain of human fibronectin III (*see, e.g.*, Koide and Koide, 2007, Methods Mol. Biol. 352: 95-109); affibodies, based on the Z domain of staphylococcal protein A (*see, e.g.*, Nygren et al.,

2008, FEBS J. 275:2668-76); DARPins, based on ankyrin repeat proteins (*see, e.g.*, Stumpp et al., 2008, Drug. Discov. Today 13:695-701); fynomers, based on the SH3 domain of the human Fyn protein kinase (*see, e.g.*, Grabulovski et al., 2007, J. Biol. Chem. 282:3196-204); affitins, based on Sac7d from *Sulfolobus acidolarius* (*see, e.g.*, Krehenbrink et al., 2008, J. Mol. Biol. 383:1058-68); affilins, based on human γ -B-crystallin (*see, e.g.*, Ebersbach et al., 2007, J. Mol. Biol. 372:172-85); avimers, based on the A domain of membrane receptor proteins (*see, e.g.*, Silverman et al., 2005, Biotechnol. 23:1556-61); cysteine-rich knottin peptides (*see, e.g.*, Kolmar, 2008, FEBS J. 275:2684-90); and engineered Kunitz-type inhibitors (*see, e.g.*, Nixon and Wood, 2006, Curr. Opin. Drug. Discov. Dev. 9:261-68). For a review, *see, for example*, Gebauer and Skerra, 2009, Curr. Opin. Chem. Biol. 13:245-55.

[00158] In order to ensure the maximum level of yield of the molecules described herein secreted from host cells, a fragment of signal peptide can be added. In some embodiments, the molecule disclosed herein comprises a signal peptide (SEQ ID NO. 2). In other embodiments, the molecule disclosed herein comprises at least 95% identity to SEQ ID NO. 2. In other embodiments, the molecule disclosed herein comprises at least 90% identity to SEQ ID NO. 2. In other embodiments, the molecule disclosed herein comprises at least 85% identity to SEQ ID NO. 2. In other embodiments, the molecule disclosed herein comprises at least 80% identity to SEQ ID NO. 2. In other embodiments, the molecule disclosed herein comprises at least 70% identity to SEQ ID NO. 2. In other embodiments, the molecule disclosed herein comprises at least 60% identity to SEQ ID NO. 2. In other embodiments, the molecule disclosed herein comprises at least 50% identity to SEQ ID NO. 2.

[00159] The binding domain of the present molecules is capable of binding to one or more antigen(s). In some embodiments, the antigen can act as a cell surface marker on target cells associated with a special disease state. In some embodiments, the antigen is a tumor antigen.

[00160] In some embodiments, the antigen of a target cell is an antigen on the surface of the cancer cell. In some embodiments, the antigen is a tumor-specific antigen, a tumor-associated antigen, or a neoantigen. In some embodiments, the target cell is a cancer cell, *e.g.*, a cell of an adrenal cancer, anal cancer, appendix cancer, bile duct cancer, bladder cancer, bone cancer, brain cancer, breast cancer, cervical cancer, colorectal cancer, esophageal cancer, gallbladder cancer, gestational trophoblastic, head and neck cancer, Hodgkin lymphoma, intestinal cancer, kidney

cancer, leukemia, liver cancer, lung cancer, melanoma, mesothelioma, multiple myeloma (MM), neuroendocrine tumor, non-Hodgkin lymphoma, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, sinus cancer, skin cancer, soft tissue sarcoma spinal cancer, stomach cancer, testicular cancer, throat cancer, thyroid cancer, uterine cancer endometrial cancer, vaginal cancer, or vulvar cancer. In some embodiments, the cancer is an adrenal cancer, anal cancer, appendix cancer, bile duct cancer, bladder cancer, bone cancer, brain cancer, breast cancer, cervical cancer, colorectal cancer, esophageal cancer, gallbladder cancer, gestational trophoblastic, head and neck cancer, Hodgkin lymphoma, intestinal cancer, kidney cancer, leukemia, liver cancer, lung cancer, melanoma, mesothelioma, multiple myeloma (MM), neuroendocrine tumor, non-Hodgkin lymphoma, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, sinus cancer, skin cancer, soft tissue sarcoma spinal cancer, stomach cancer, testicular cancer, throat cancer, thyroid cancer, uterine cancer endometrial cancer, vaginal cancer, or vulvar cancer. In some embodiments, the cancer is a adrenal cancer. In some embodiments, the cancer is a anal cancer. In some embodiments, the cancer is an appendix cancer. In some embodiments, the cancer is a bile duct cancer. In some embodiments, the cancer is a bladder cancer. In some embodiments, the cancer is a bone cancer. In some embodiments, the cancer is a brain cancer. In some embodiments, the cancer is a breast cancer. In some embodiments, the cancer is a cervical cancer. In some embodiments, the cancer is a colorectal cancer. In some embodiments, the cancer is a esophageal cancer. In some embodiments, the cancer is a gallbladder cancer. In some embodiments, the cancer is a gestational trophoblastic. In some embodiments, the cancer is a head and neck cancer. In some embodiments, the cancer is a Hodgkin lymphoma. In some embodiments, the cancer is an intestinal cancer. In some embodiments, the cancer is a kidney cancer. In some embodiments, the cancer is a leukemia. In some embodiments, the cancer is a liver cancer. In some embodiments, the cancer is a lung cancer. In some embodiments, the cancer is a melanoma. In some embodiments, the cancer is a mesothelioma. In some embodiments, the cancer is a multiple myeloma (MM). In some embodiments, the cancer is a neuroendocrine tumor. In some embodiments, the cancer is a non-Hodgkin lymphoma. In some embodiments, the cancer is an oral cancer. In some embodiments, the cancer is an ovarian cancer. In some embodiments, the cancer is a pancreatic cancer. In some embodiments, the cancer is a prostate cancer. In some embodiments, the cancer is a sinus cancer.

In some embodiments, the cancer is a skin cancer. In some embodiments, the cancer is a soft tissue sarcoma spinal cancer. In some embodiments, the cancer is a stomach cancer. In some embodiments, the cancer is a testicular cancer. In some embodiments, the cancer is a throat cancer. In some embodiments, the cancer is a thyroid cancer. In some embodiments, the cancer is a uterine cancer endometrial cancer. In some embodiments, the cancer is a vaginal cancer. In some embodiments, the cancer is a vulvar cancer.

[00161] In some embodiments, the adrenal cancer is an adrenocortical carcinoma (ACC), adrenal cortex cancer, pheochromocytoma, or neuroblastoma. In some embodiments, the anal cancer is a squamous cell carcinoma, cloacogenic carcinoma, adenocarcinoma, basal cell carcinoma, or melanoma. In some embodiments, the appendix cancer is a neuroendocrine tumor (NET), mucinous adenocarcinoma, goblet cell carcinoid, intestinal-type adenocarcinoma, or signet-ring cell adenocarcinoma. In some embodiments, the bile duct cancer is an extrahepatic bile duct cancer, adenocarcinomas, hilar bile duct cancer, perihilar bile duct cancer, distal bile duct cancer, or intrahepatic bile duct cancer. In some embodiments, the bladder cancer is transitional cell carcinoma (TCC), papillary carcinoma, flat carcinoma, squamous cell carcinoma, adenocarcinoma, small-cell carcinoma, or sarcoma. In some embodiments, the bone cancer is a primary bone cancer, sarcoma, osteosarcoma, chondrosarcoma, sarcoma, fibrosarcoma, malignant fibrous histiocytoma, giant cell tumor of bone, chordoma, or metastatic bone cancer. In some embodiments, the brain cancer is an astrocytoma, brain stem glioma, glioblastoma, meningioma, ependymoma, oligodendroglioma, mixed glioma, pituitary carcinoma, pituitary adenoma, craniopharyngioma, germ cell tumor, pineal region tumor, medulloblastoma, or primary CNS lymphoma. In some embodiments, the breast cancer is a breast adenocarcinoma, invasive breast cancer, noninvasive breast cancer, breast sarcoma, metaplastic carcinoma, adenocystic carcinoma, phyllodes tumor, angiosarcoma, HER2-positive breast cancer, triple-negative breast cancer, or inflammatory breast cancer. In some embodiments, the cervical cancer is a squamous cell carcinoma, or adenocarcinoma. In some embodiments, the colorectal cancer is a colorectal adenocarcinoma, primary colorectal lymphoma, gastrointestinal stromal tumor, leiomyosarcoma, carcinoid tumor, mucinous adenocarcinoma, signet ring cell adenocarcinoma, gastrointestinal carcinoid tumor, or melanoma. In some embodiments, the esophageal cancer is an adenocarcinoma or squamous cell

carcinoma. In some embodiments, the gall bladder cancer is an adenocarcinoma, papillary adenocarcinoma, adenosquamous carcinoma, squamous cell carcinoma, small cell carcinoma, or sarcoma. In some embodiments, the gestational trophoblastic disease (GTD) is a hydatidiform mole, gestational trophoblastic neoplasia (GTN), choriocarcinoma, placental-site trophoblastic tumor (PSTT), or epithelioid trophoblastic tumor (ETT). In some embodiments, the head and neck cancer is a laryngeal cancer, nasopharyngeal cancer, hypopharyngeal cancer, nasal cavity cancer, paranasal sinus cancer, salivary gland cancer, oral cancer, oropharyngeal cancer, or tonsil cancer. In some embodiments, the Hodgkin lymphoma is a classical Hodgkin lymphoma, nodular sclerosis, mixed cellularity, lymphocyte-rich, lymphocyte-depleted, or nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL). In some embodiments, the intestinal cancer is a small intestine cancer, small bowel cancer, adenocarcinoma, sarcoma, gastrointestinal stromal tumors, carcinoid tumors, or lymphoma. In some embodiments, the kidney cancer is a renal cell carcinoma (RCC), clear cell RCC, papillary RCC, chromophobe RCC, collecting duct RCC, unclassified RCC, transitional cell carcinoma, urothelial cancer, renal pelvis carcinoma, or renal sarcoma. In some embodiments, the leukemia is an acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), hairy cell leukemia (HCL), or a myelodysplastic syndrome (MDS). In a specific embodiment, the leukemia is AML. In some embodiments, the liver cancer is a hepatocellular carcinoma (HCC), fibrolamellar HCC, cholangiocarcinoma, angiosarcoma, or liver metastasis. In some embodiments, the lung cancer is a small cell lung cancer, small cell carcinoma, combined small cell carcinoma, non-small cell lung cancer, lung adenocarcinoma, squamous cell lung cancer, large-cell undifferentiated carcinoma, pulmonary nodule, metastatic lung cancer, adenosquamous carcinoma, large cell neuroendocrine carcinoma, salivary gland-type lung carcinoma, lung carcinoid, mesothelioma, sarcomatoid carcinoma of the lung, or malignant granular cell lung tumor. In some embodiments, the melanoma is a superficial spreading melanoma, nodular melanoma, acral-lentiginous melanoma, lentigo maligna melanoma, amelanotic melanoma, desmoplastic melanoma, ocular melanoma, or metastatic melanoma. In some embodiments, the mesothelioma is a pleural mesothelioma, peritoneal mesothelioma, pericardial mesothelioma, or testicular mesothelioma. In some embodiments, the multiple myeloma is an active myeloma or smoldering myeloma. In some embodiments, the

neuroendocrine tumor is a gastrointestinal neuroendocrine tumor, pancreatic neuroendocrine tumor, or lung neuroendocrine tumor. In some embodiments, the non-Hodgkin's lymphoma is an anaplastic large-cell lymphoma, lymphoblastic lymphoma, peripheral T cell lymphoma, follicular lymphoma, cutaneous T cell lymphoma, lymphoplasmacytic lymphoma, marginal zone B-cell lymphoma, MALT lymphoma, small-cell lymphocytic lymphoma, Burkitt lymphoma, chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), precursor T-lymphoblastic leukemia/lymphoma, acute lymphocytic leukemia (ALL), adult T cell lymphoma/leukemia (ATLL), hairy cell leukemia, B-cell lymphomas, diffuse large B-cell lymphoma (DLBCL), primary mediastinal B-cell lymphoma, primary central nervous system (CNS) lymphoma, mantle cell lymphoma (MCL), marginal zone lymphomas, mucosa-associated lymphoid tissue (MALT) lymphoma, nodal marginal zone B-cell lymphoma, splenic marginal zone B-cell lymphoma, lymphoplasmacytic lymphoma, B-cell non-Hodgkin lymphoma, T cell non-Hodgkin lymphoma, natural killer cell lymphoma, cutaneous T cell lymphoma, Alibert-Bazin syndrome, Sezary syndrome, primary cutaneous anaplastic large-cell lymphoma, peripheral T cell lymphoma, angioimmunoblastic T cell lymphoma (AITL), anaplastic large-cell lymphoma (ALCL), systemic ALCL, enteropathy-type T cell lymphoma (EATL), or hepatosplenic gamma/delta T cell lymphoma. In some embodiments, the oral cancer is a squamous cell carcinoma, verrucous carcinoma, minor salivary gland carcinomas, lymphoma, benign oral cavity tumor, eosinophilic granuloma, fibroma, granular cell tumor, karatoacanthoma, leiomyoma, osteochondroma, lipoma, schwannoma, neurofibroma, papilloma, condyloma acuminatum, verruciform xanthoma, pyogenic granuloma, rhabdomyoma, odontogenic tumors, leukoplakia, erythroplakia, squamous cell lip cancer, basal cell lip cancer, mouth cancer, gum cancer, or tongue cancer. In some embodiments, the ovarian cancer is a ovarian epithelial cancer, mucinous epithelial ovarian cancer, endometrioid epithelial ovarian cancer, clear cell epithelial ovarian cancer, undifferentiated epithelial ovarian cancer, ovarian low malignant potential tumors, primary peritoneal carcinoma, fallopian tube cancer, germ cell tumors, teratoma, dysgerminoma ovarian germ cell cancer, endodermal sinus tumor, sex cord-stromal tumors, sex cord-gonadal stromal tumor, ovarian stromal tumor, granulosa cell tumor, granulosa-theca tumor, Sertoli-Leydig tumor, ovarian sarcoma, ovarian carcinosarcoma, ovarian adenocarcinoma, ovarian leiomyosarcoma, ovarian fibrosarcoma, Krukenberg tumor, or ovarian

cyst. In some embodiments, the pancreatic cancer is a pancreatic exocrine gland cancer, pancreatic endocrine gland cancer, or pancreatic adenocarcinoma, islet cell tumor, or neuroendocrine tumor. In some embodiments, the prostate cancer is a prostate adenocarcinoma, prostate sarcoma, transitional cell carcinoma, small cell carcinoma, or neuroendocrine tumor. In some embodiments, the sinus cancer is a squamous cell carcinoma, mucosa cell carcinoma, adenoid cystic cell carcinoma, acinic cell carcinoma, sinonasal undifferentiated carcinoma, nasal cavity cancer, paranasal sinus cancer, maxillary sinus cancer, ethmoid sinus cancer, or nasopharynx cancer. In some embodiments, the skin cancer is a basal cell carcinoma, squamous cell carcinoma, melanoma, Merkel cell carcinoma, Kaposi sarcoma (KS), actinic keratosis, skin lymphoma, or keratoacanthoma. In some embodiments, the soft tissue cancer is an angiosarcoma, dermatofibrosarcoma, epithelioid sarcoma, Ewing's sarcoma, fibrosarcoma, gastrointestinal stromal tumors (GISTs), Kaposi sarcoma, leiomyosarcoma, liposarcoma, dedifferentiated liposarcoma (DL), myxoid/round cell liposarcoma (MRCL), well-differentiated liposarcoma (WDL), malignant fibrous histiocytoma, neurofibrosarcoma, rhabdomyosarcoma (RMS), or synovial sarcoma. In some embodiments, the spinal cancer is a spinal metastatic tumor. In some embodiments, the stomach cancer is a stomach adenocarcinoma, stomach lymphoma, gastrointestinal stromal tumors, carcinoid tumor, gastric carcinoid tumors, Type I ECL-cell carcinoid, Type II ECL-cell carcinoid, or Type III ECL-cell carcinoid. In some embodiments, the testicular cancer is a seminoma, non-seminoma, embryonal carcinoma, yolk sac carcinoma, choriocarcinoma, teratoma, gonadal stromal tumor, leydig cell tumor, or sertoli cell tumor. In some embodiments, the throat cancer is a squamous cell carcinoma, adenocarcinoma, sarcoma, laryngeal cancer, pharyngeal cancer, nasopharynx cancer, oropharynx cancer, hypopharynx cancer, laryngeal cancer, laryngeal squamous cell carcinoma, laryngeal adenocarcinoma, lymphoepithelioma, spindle cell carcinoma, verrucous cancer, undifferentiated carcinoma, or lymph node cancer. In some embodiments, the thyroid cancer is a papillary carcinoma, follicular carcinoma, Hürthle cell carcinoma, medullary thyroid carcinoma, or anaplastic carcinoma. In some embodiments, the uterine cancer is an endometrial cancer, endometrial adenocarcinoma, endometroid carcinoma, serous adenocarcinoma, adenosquamous carcinoma, uterine carcinosarcoma, uterine sarcoma, uterine leiomyosarcoma, endometrial stromal sarcoma, or undifferentiated sarcoma. In some embodiments, the vaginal cancer is a

squamous cell carcinoma, adenocarcinoma, melanoma, or sarcoma. In some embodiments, the vulvar cancer is a squamous cell carcinoma or adenocarcinoma.

[00162] Tumor antigens are proteins that are produced by tumor cells that can elicit an immune response, particularly T-cell mediated immune responses. Exemplary tumor antigens include, but not limited to, a glioma-associated antigen, carcinoembryonic antigen (CEA), β -human chorionic gonadotropin, alphafetoprotein (AFP), lectin-reactive AFP, thyroglobulin, RAGE-1, MN-CAIX, human telomerase reverse transcriptase, RU1, RU2 (AS), intestinal carboxyl esterase, mut hsp70-2, M-CSF, prostase, prostate-specific antigen (PSA), PAP, NY-ESO-1, LAGE-1a, p53, prostein, PSMA, HER2/neu, survivin and telomerase, prostate-carcinoma tumor antigen-1 (PCTA-1), MAGE, ELF2M, neutrophil elastase, ephrinB2, insulin growth factor (IGF)-I, IGF-II, IGF-I receptor, and mesothelin.

[00163] In some embodiments, the cancer antigen is CEA, immature laminin receptor, TAG-72, HPV E6, HPV E7, BING-4, calcium-activated chloride channel 2, cyclin-B1, 9D7, EpCAM, EphA3, Her2/neu, telomerase, mesothelin, SAP-1, surviving, a BAGE family antigen, CAGE family antigen, GAGE family antigen, MAGE family antigen, SAGE family antigen, XAGE family antigen, NY-ESO-1/LAGE-1, PRAME, SSX-2, Melan-A, MART-1, Gp100, pmel17, tyrosinase, TRP-1, TRP-2, P. polypeptide, MC1R, prostate-specific antigen, β -catenin, BRCA1, BRCA2, CDK4, CML66, fibronectin, MART-2, p53, Ras, TGF- β RII, or MUC1. In some embodiments, the cancer antigen is CEA. In some embodiments, the cancer antigen is immature laminin receptor. In some embodiments, the cancer antigen is TAG-72. In some embodiments, the cancer antigen is HPV E6. In some embodiments, the cancer antigen is HPV E7. In some embodiments, the cancer antigen is BING-4. In some embodiments, the cancer antigen is calcium-activated chloride channel 2. In some embodiments, the cancer antigen is cyclin-B1. In some embodiments, the cancer antigen is 9D7. In some embodiments, the cancer antigen is EpCAM. In some embodiments, the cancer antigen is EphA3. In some embodiments, the cancer antigen is Her2/neu. In some embodiments, the cancer antigen is telomerase. In some embodiments, the cancer antigen is mesothelin. In some embodiments, the cancer antigen is SAP-1. In some embodiments, the cancer antigen is surviving. In some embodiments, the cancer antigen is a BAGE family antigen. In some embodiments, the cancer antigen is CAGE family antigen. In some embodiments, the cancer antigen is GAGE family antigen. In some

embodiments, the cancer antigen is MAGE family antigen. In some embodiments, the cancer antigen is SAGE family antigen. In some embodiments, the cancer antigen is XAGE family antigen. In some embodiments, the cancer antigen is NY-ESO-1/LAGE-1. In some embodiments, the cancer antigen is PRAME. In some embodiments, the cancer antigen is SSX-2. In some embodiments, the cancer antigen is Melan-A. In some embodiments, the cancer antigen is MART-1. In some embodiments, the cancer antigen is Gp100. In some embodiments, the cancer antigen is pmel17. In some embodiments, the cancer antigen is tyrosinase. In some embodiments, the cancer antigen is TRP-1. In some embodiments, the cancer antigen is TRP-2. In some embodiments, the cancer antigen is P. polypeptide. In some embodiments, the cancer antigen is MC1R. In some embodiments, the cancer antigen is prostate-specific antigen. In some embodiments, the cancer antigen is β -catenin. In some embodiments, the cancer antigen is BRCA1. In some embodiments, the cancer antigen is BRCA2. In some embodiments, the cancer antigen is CDK4. In some embodiments, the cancer antigen is CML66. In some embodiments, the cancer antigen is fibronectin. In some embodiments, the cancer antigen is MART-2. In some embodiments, the cancer antigen is p53. In some embodiments, the cancer antigen is Ras. In some embodiments, the cancer antigen is TGF- β RII. In some embodiments, the cancer antigen is MUC1.

[00164] In some embodiments, the tumor antigen comprises one or more antigenic cancer epitopes associated with a malignant tumor. Malignant tumors express a number of proteins that can serve as target antigens for an immune attack. These molecules include, but are not limited to, tissue-specific antigens such as MART-1, tyrosinase and gp100 in melanoma and prostatic acid phosphatase (PAP) and prostate-specific antigen (PSA) in prostate cancer. Other target molecules belong to the group of transformation-related molecules such as the oncogene HER2/Neu/ErbB-2. Yet another group of target antigens are onco-fetal antigens such as carcinoembryonic antigen (CEA).

[00165] In some embodiments, the tumor antigen is a tumor-specific antigen (TSA) or a tumor-associated antigen (TAA). A TSA is unique to tumor cells and does not occur on other cells in the body. A TAA associated antigen is not unique to a tumor cell, and instead is also expressed on a normal cell under conditions that fail to induce a state of immunologic tolerance to the antigen. The expression of the antigen on the tumor may occur under conditions that

enable the immune system to respond to the antigen. TAAs may be antigens that are expressed on normal cells during fetal development, when the immune system is immature, and unable to respond or they may be antigens that are normally present at extremely low levels on normal cells, but which are expressed at much higher levels on tumor cells.

[00166] Non-limiting examples of TSA or TAA antigens include: differentiation antigens such as MART-1/MelanA (MART-I), gp 100 (Pmel 17), tyrosinase, TRP-1, TRP-2 and tumor-specific multilineage antigens such as MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, pl5; overexpressed embryonic antigens such as CEA; overexpressed oncogenes and mutated tumor-suppressor genes such as p53, Ras, HER2/neu; unique tumor antigens resulting from chromosomal translocations; such as BCR-ABL, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR; and viral antigens, such as the Epstein Barr virus antigens EBVA and the human papillomavirus (HPV) antigens E6 and E7.

[00167] Other large, protein-based antigens include TSP-180, MAGE-4, MAGE-5, MAGE-6, RAGE, NY-ESO, pl85erbB2, pl80erbB-3, c-met, nm-23HI, PSA, TAG-72, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, beta-Catenin, CDK4, Mum-1, p 15, p 16, 43-9F, 5T4, 791Tgp72, alpha-fetoprotein, beta-HCG, BCA225, BTAA, CA 125, CA 15-3\CA 27.29\BCAA, CA 195, CA 242, CA-50, CAM43, CD68\PI, CO-029, FGF-5, G250, Ga733\EpCAM, HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB/70K, NY-CO- 1, RCAS 1, SDCCAG16, TA-90\Mac-2 binding protein\cyclophilin C-associated protein, TAAL6, TAG72, TLP, and TPS.

[00168] Additional non-limiting exemplary targets of the binding molecules provided herein include GPC2, CD276, Delta-like protein ligand 3 (DLL3), NY-ESO-1, melanoma associated antigen 4; survivin protein, synovial sarcoma X breakpoint protein 2, CD3, epidermal growth factor receptor (EGFR), erbb2 tyrosine kinase receptor, HER2, CEA, CD66, CD66e, ROR1, ntrkr1 tyrosine kinase receptor, GPC3, mesothelin, glutamate carboxypeptidase II, PMSA, PD-L1, folate receptor alpha, PSCA, Mucin 1, HLA antigen (such as HLA class I antigen A-2 alpha, HLA class I antigen A-11 alpha, and HLA class II antigen), c-Met, hepatocyte growth factor receptor, K-Ras GTPase (KRAS), IL-15 receptor, Kit tyrosine kinase, PDGF receptor beta, RET tyrosine kinase receptor; Raf 1 protein kinase, Raf B protein kinase, thymidylate synthase, topoisomerase II, Brachyury protein, Flt3 tyrosine kinase, VEGF, VEGF receptor (VEGF-1

receptor, VEGF-2 receptor, and VEGF-3 receptor), estrogen receptor, neoantigen, human papillomavirus E6, and heat shock protein.

[00169] In some embodiments, the binding molecule provided herein binds to a B cell antigen. In some embodiments, the B cell antigen is a CD1a, CD1b, CD1c, CD1d, CD2, CD5, CD6, CD9, CD11a, CD11b, CD11c, CD17, CD18, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD26, CD27, CD29, CD30, CD31, CD32a, CD32b, CD35, CD37, CD38, CD39, CD40, CD45, CD45RA, CD45RB, CD45RC, CD45RO, CD46, CD47, CD48, CD49b, CD49c, CD49d, CD50, CD52, CD53, CD54, CD55, CD58, CD60a, CD62L, CD63, CD68, CD69, CD70, CD72, CD73, CD74, CD75, CD75S, CD77, CD79a, CD79b, CD80, CD81, CD82, CD83, CD84, CD85E, CD85I, CD85J, CD86, CD92, CD95, CD97, CD98, CD99, CD100, CD102, CD108, CD119, CD120a, CD120b, CD121b, CD122, CD124, CD125, CD126, CD130, CD132, CD137, CD138, CD139, CD147, CD148, CD150, CD152, CD162, CD164, CD166, CD167a, CD170, CD171, CD175, CD175s, CD180, CD184, CD185, CD192, CD196, CD197, CD200, CD205, CD201a, CDw210b, CD212, CD213a1, CD213a2, CD 215, CD217, CD218a, CD218b, CD220, CD221, CD222, CD224, CD225, CD226, CD227, CD229, CD230, CD232, CD252, CD252, CD254, CD255, CD256, CD257 CD258, CD259, CD260, CD261, CD262, CD263, CD264, CD267, CD268, CD269, CD270, CD272, CD274, CD275, CD277, CD279, CD283, CD289, CD290, CD295, CD298, CD300, CD300c, CD305, CD306, CD307a, CD307b, CD307c, CD307d, CD307e, CD314, CD215, CD316, CD317, CD319, CD321, CD327, CD328, CD329, CD338, CD351, CD352, CD353, CD354, CD355, CD356, CD357, CD358, CD360, CD361, CD362, or CD363 antigen. In some embodiments, the B cell antigen is a CD1a antigen. In some embodiments, the B cell antigen is a CD1b antigen. In some embodiments, the B cell antigen is a CD1c antigen. In some embodiments, the B cell antigen is a CD1d antigen. In some embodiments, the B cell antigen is a CD2 antigen. In some embodiments, the B cell antigen is a CD5 antigen. In some embodiments, the B cell antigen is a CD6 antigen. In some embodiments, the B cell antigen is a CD9 antigen. In some embodiments, the B cell antigen is a CD11a antigen. In some embodiments, the B cell antigen is a CD11b antigen. In some embodiments, the B cell antigen is a CD11c antigen. In some embodiments, the B cell antigen is a CD17 antigen. In some embodiments, the B cell antigen is a CD18 antigen. In some embodiments, the B cell antigen is a CD19 antigen. In some embodiments, the B cell antigen is a CD20 antigen. In some

[illegible]

[illegible]

[illegible]

embodiments, the B cell antigen is a CD275 antigen. In some embodiments, the B cell antigen is a CD277 antigen. In some embodiments, the B cell antigen is a CD279 antigen. In some embodiments, the B cell antigen is a CD283 antigen. In some embodiments, the B cell antigen is a CD289 antigen. In some embodiments, the B cell antigen is a CD290 antigen. In some embodiments, the B cell antigen is a CD295 antigen. In some embodiments, the B cell antigen is a CD298 antigen. In some embodiments, the B cell antigen is a CD300 antigen. In some embodiments, the B cell antigen is a CD300c antigen. In some embodiments, the B cell antigen is a CD305 antigen. In some embodiments, the B cell antigen is a CD306 antigen. In some embodiments, the B cell antigen is a CD307a antigen. In some embodiments, the B cell antigen is a CD307b antigen. In some embodiments, the B cell antigen is a CD307c antigen. In some embodiments, the B cell antigen is a CD307d antigen. In some embodiments, the B cell antigen is a CD307e antigen. In some embodiments, the B cell antigen is a CD314 antigen. In some embodiments, the B cell antigen is a CD215 antigen. In some embodiments, the B cell antigen is a CD316 antigen. In some embodiments, the B cell antigen is a CD317 antigen. In some embodiments, the B cell antigen is a CD319 antigen. In some embodiments, the B cell antigen is a CD321 antigen. In some embodiments, the B cell antigen is a CD327 antigen. In some embodiments, the B cell antigen is a CD328 antigen. In some embodiments, the B cell antigen is a CD329 antigen. In some embodiments, the B cell antigen is a CD338 antigen. In some embodiments, the B cell antigen is a CD351 antigen. In some embodiments, the B cell antigen is a CD352 antigen. In some embodiments, the B cell antigen is a CD353 antigen. In some embodiments, the B cell antigen is a CD354 antigen. In some embodiments, the B cell antigen is a CD355 antigen. In some embodiments, the B cell antigen is a CD356 antigen. In some embodiments, the B cell antigen is a CD357 antigen. In some embodiments, the B cell antigen is a CD358 antigen. In some embodiments, the B cell antigen is a CD360 antigen. In some embodiments, the B cell antigen is a CD361 antigen. In some embodiments, the B cell antigen is a CD362 antigen. In some embodiments, the B cell antigen is a CD363 antigen.

[00170] In one embodiment, target of the present binding molecule is a pathogen. In certain embodiments, the target cell is a cell comprising a pathogen.

[00171] In some embodiments, the pathogen causes an infectious disease selected from the group consisting of an Acute Flaccid Myelitis (AFM), Anaplasmosis, Anthrax, Babesiosis,

Botulism, Brucellosis, Campylobacteriosis, Carbapenem-resistant Infection, Chancroid, Chikungunya Virus Infection, Chlamydia, Ciguatera, Difficile Infection, Perfringens, Coccidioidomycosis fungal infection, coronavirus infection, Covid-19 (SARS-CoV-2), Creutzfeldt-Jacob Disease/transmissible spongiform encephalopathy, Cryptosporidiosis (Crypto), Cyclosporiasis, Dengue 1,2,3 or 4, Diphtheria, E. coli infection/Shiga toxin-producing (STEC), Eastern Equine Encephalitis, Hemorrhagic Fever (Ebola), Ehrlichiosis, Encephalitis, Arboviral or parainfectious, Non-Polio Enterovirus, D68 Enterovirus (EV-D68), Giardiasis, Glanders, Gonococcal Infection, Granuloma inguinale, Haemophilus Influenza disease Type B (Hib or H-flu), Hantavirus Pulmonary Syndrome (HPS), Hemolytic Uremic Syndrome (HUS), Hepatitis A (Hep A), Hepatitis B (Hep B), Hepatitis C (Hep C), Hepatitis D (Hep D), Hepatitis E (Hep E), Herpes, Herpes Zoster (Shingles), Histoplasmosis infection, Human Immunodeficiency Virus/AIDS (HIV/AIDS), Human Papillomavirus (HPV), Influenza (Flu), Legionellosis (Legionnaires Disease), Leprosy (Hansens Disease), Leptospirosis, Listeriosis (Listeria), Lyme Disease, Lymphogranuloma venereum infection (LGV), Malaria, Measles, Melioidosis, Meningitis (Viral), Meningococcal Disease (Meningitis (Bacterial)), Middle East Respiratory Syndrome Coronavirus (MERS-CoV), Mumps, Norovirus, Pediculosis, Pelvic Inflammatory Disease (PID), Pertussis (Whooping Cough), Plague (Bubonic, Septicemic, Pneumonic), Pneumococcal Disease (Pneumonia), Poliomyelitis (Polio), Powassan, Psittacosis, Pthiriasis, Pustular Rash diseases (Small pox, monkeypox, cowpox), Q-Fever, Rabies, Rickettsiosis (Rocky Mountain Spotted Fever), Rubella (German Measles), Salmonellosis gastroenteritis (Salmonella), Scabies, Scombroid, Sepsis, Severe Acute Respiratory Syndrome (SARS), Shigellosis gastroenteritis (Shigella), Smallpox, Staphylococcal Infection Methicillin-resistant (MRSA), Staphylococcal Food Poisoning Enterotoxin B Poisoning (Staph Food Poisoning), Staphylococcal Infection Vancomycin Intermediate (VISA), Staphylococcal Infection Vancomycin Resistant (VRSA), Streptococcal Disease Group A (invasive) (Strep A (invasive), Streptococcal Disease, Group B (Strep-B), Streptococcal Toxic-Shock Syndrome STSS Toxic Shock, Syphilis (primary, secondary, early latent, late latent, congenital), Tetanus Infection, Trichomoniasis, Trichonosis Infection, Tuberculosis (TB), Tuberculosis Latent (LTBI), Tularemia, Typhoid Fever Group D, Vaginosis, Varicella (Chickenpox), Vibrio cholerae (Cholera), Vibriosis (Vibrio), Ebola Virus Hemorrhagic Fever, Lassa Virus Hemorrhagic Fever,

Marburg Virus Hemorrhagic Fever, West Nile Virus, Yellow Fever, Yersenia, and Zika Virus Infection. In some embodiments, the infectious disease is Acute Flaccid Myelitis (AFM). In some embodiments, the infectious disease is Anaplasmosis. In some embodiments, the infectious disease is Anthrax. In some embodiments, the infectious disease is Babesiosis. In some embodiments, the infectious disease is Botulism. In some embodiments, the infectious disease is Brucellosis. In some embodiments, the infectious disease is Campylobacteriosis. In some embodiments, the infectious disease is Carbapenem-resistant Infection. In some embodiments, the infectious disease is Chancroid. In some embodiments, the infectious disease is Chikungunya Virus Infection. In some embodiments, the infectious disease is Chlamydia. In some embodiments, the infectious disease is Ciguatera. In some embodiments, the infectious disease is Difficile Infection. In some embodiments, the infectious disease is Perfringens. In some embodiments, the infectious disease is Coccidioidomycosis fungal infection. In some embodiments, the infectious disease is coronavirus. In some embodiments, the infectious disease is Covid-19 (SARS-CoV-2). In some embodiments, the infectious disease is Creutzfeldt-Jacob Disease/transmissible spongiform encephalopathy. In some embodiments, the infectious disease is Cryptosporidiosis (Crypto). In some embodiments, the infectious disease is Cyclosporiasis. In some embodiments, the infectious disease is Dengue 1,2,3 or 4. In some embodiments, the infectious disease is Diphtheria. In some embodiments, the infectious disease is E. coli infection/Shiga toxin-producing (STEC). In some embodiments, the infectious disease is Eastern Equine Encephalitis. In some embodiments, the infectious disease is Hemorrhagic Fever (Ebola). In some embodiments, the infectious disease is Ehrlichiosis. In some embodiments, the infectious disease is Encephalitis. In some embodiments, the infectious disease is Arboviral or parainfectious. In some embodiments, the infectious disease is Non-Polio Enterovirus. In some embodiments, the infectious disease is D68 Enterovirus (EV-D68). In some embodiments, the infectious disease is Giardiasis. In some embodiments, the infectious disease is Glanders. In some embodiments, the infectious disease is Gonococcal Infection. In some embodiments, the infectious disease is Granuloma inguinale. In some embodiments, the infectious disease is Haemophilus Influenza disease Type B (Hib or H-flu). In some embodiments, the infectious disease is Hantavirus Pulmonary Syndrome (HPS). In some embodiments, the infectious disease is Hemolytic Uremic Syndrome (HUS). In some embodiments, the infectious disease is Hepatitis

A (Hep A). In some embodiments, the infectious disease is Hepatitis B (Hep B). In some embodiments, the infectious disease is Hepatitis C (Hep C). In some embodiments, the infectious disease is Hepatitis D (Hep D). In some embodiments, the infectious disease is Hepatitis E (Hep E). In some embodiments, the infectious disease is Herpes. In some embodiments, the infectious disease is Herpes Zoster (Shingles). In some embodiments, the infectious disease is Histoplasmosis infection. In some embodiments, the infectious disease is Human Immunodeficiency Virus/AIDS (HIV/AIDS). In some embodiments, the infectious disease is Human Papillomavirus (HPV). In some embodiments, the infectious disease is Influenza (Flu). In some embodiments, the infectious disease is Legionellosis (Legionnaires Disease). In some embodiments, the infectious disease is Leprosy (Hansens Disease). In some embodiments, the infectious disease is Leptospirosis. In some embodiments, the infectious disease is Listeriosis (Listeria). In some embodiments, the infectious disease is Lyme Disease. In some embodiments, the infectious disease is Lymphogranuloma venereum infection (LGV). In some embodiments, the infectious disease is Malaria. In some embodiments, the infectious disease is Measles. In some embodiments, the infectious disease is Melioidosis. In some embodiments, the infectious disease is Meningitis (Viral). In some embodiments, the infectious disease is Meningococcal Disease (Meningitis (Bacterial)). In some embodiments, the infectious disease is Middle East Respiratory Syndrome Coronavirus (MERS-CoV). In some embodiments, the infectious disease is Mumps. In some embodiments, the infectious disease is Norovirus. In some embodiments, the infectious disease is Pediculosis. In some embodiments, the infectious disease is Pelvic Inflammatory Disease (PID). In some embodiments, the infectious disease is Pertussis (Whooping Cough). In some embodiments, the infectious disease is Plague (Bubonic. In some embodiments, the infectious disease is Septicemic. In some embodiments, the infectious disease is Pneumonic). In some embodiments, the infectious disease is Pneumococcal Disease (Pneumonia). In some embodiments, the infectious disease is Poliomyelitis (Polio). In some embodiments, the infectious disease is Powassan. In some embodiments, the infectious disease is Psittacosis. In some embodiments, the infectious disease is Pthiriasis. In some embodiments, the infectious disease is Pustular Rash diseases (Small pox. In some embodiments, the infectious disease is monkeypox. In some embodiments, the infectious disease is cowpox). In some embodiments, the infectious disease is Q-Fever. In some embodiments, the infectious disease is

Rabies. In some embodiments, the infectious disease is Rickettsiosis (Rocky Mountain Spotted Fever). In some embodiments, the infectious disease is Rubella (German Measles). In some embodiments, the infectious disease is Salmonellosis gastroenteritis (Salmonella). In some embodiments, the infectious disease is Scabies. In some embodiments, the infectious disease is Scombroid. In some embodiments, the infectious disease is Sepsis. In some embodiments, the infectious disease is Severe Acute Respiratory Syndrome (SARS). In some embodiments, the infectious disease is Shigellosis gastroenteritis (Shigella). In some embodiments, the infectious disease is Smallpox. In some embodiments, the infectious disease is Staphylococcal Infection Methicillin-resistant (MRSA). In some embodiments, the infectious disease is Staphylococcal Food Poisoning Enterotoxin B Poisoning (Staph Food Poisoning). In some embodiments, the infectious disease is Saphylococcal Infection Vancomycin Intermediate (VISA). In some embodiments, the infectious disease is Staphylococcal Infection Vancomycin Resistant (VRSA). In some embodiments, the infectious disease is Streptococcal Disease Group A (invasive) (Strep A (invasive)). In some embodiments, the infectious disease is Streptococcal Disease. In some embodiments, the infectious disease is Group B (Strep-B). In some embodiments, the infectious disease is Streptococcal Toxic-Shock Syndrome STSS Toxic Shock. In some embodiments, the infectious disease is Syphilis (primary. In some embodiments, the infectious disease is secondary. In some embodiments, the infectious disease is early latent. In some embodiments, the infectious disease is late latent. In some embodiments, the infectious disease is congenital). In some embodiments, the infectious disease is Tetanus Infection. In some embodiments, the infectious disease is Trichomoniasis. In some embodiments, the infectious disease is Trichonosis Infection. In some embodiments, the infectious disease is Tuberculosis (TB). In some embodiments, the infectious disease is Tuberculosis Latent (LTBI). In some embodiments, the infectious disease is Tularemia. In some embodiments, the infectious disease is Typhoid Fever Group D. In some embodiments, the infectious disease is Vaginosis. In some embodiments, the infectious disease is Varicella (Chickenpox), Vibrio cholerae (Cholera). In some embodiments, the infectious disease is Vibriosis (Vibrio). In some embodiments, the infectious disease is Ebola Virus Hemorrhagic Fever. In some embodiments, the infectious disease is Lasa Virus Hemorrhagic Fever. In some embodiments, the infectious disease is Marburg Virus Hemorrhagic Fever. In some embodiments, the infectious disease is West Nile Virus. In some embodiments,

the infectious disease is Yellow Fever. In some embodiments, the infectious disease is Yersenia. In some embodiments, the infectious disease is and Zika Virus Infection.

[00172] In some embodiments, the pathogen is a bacteria. In some embodiments, the bacteria is a bacteria of a bacillus, bartonella, bordetella, borrelia, brucella, campylobacter, chlamydia, chlamydophila, clostridium, corynebacterium, enterococcus, escherichia, francisella, haemophilus, helicobacter, legionella, leptospira, listeria, mycobacterium, mycoplasma, neisseria, pseudomonas, rickettsia, salmonella, shigella, staphylococcus, streptococcus, treponema, ureaplasma, vibrio or yersinia genus. In some embodiments, the bacteria is a bacteria of the bacillus genus. In some embodiments, the bacteria is a bacteria of the bartonella genus. In some embodiments, the bacteria is a bacteria of the bordetella genus. In some embodiments, the bacteria is a bacteria of the borrelia genus. In some embodiments, the bacteria is a bacteria of the brucella genus. In some embodiments, the bacteria is a bacteria of the campylobacter genus. In some embodiments, the bacteria is a bacteria of the chlamydia genus. In some embodiments, the bacteria is a bacteria of the chlamydophila genus. In some embodiments, the bacteria is a bacteria of the clostridium genus. In some embodiments, the bacteria is a bacteria of the corynebacterium genus. In some embodiments, the bacteria is a bacteria of the enterococcus genus. In some embodiments, the bacteria is a bacteria of the escherichia genus. In some embodiments, the bacteria is a bacteria of the francisella genus. In some embodiments, the bacteria is a bacteria of the haemophilus genus. In some embodiments, the bacteria is a bacteria of the helicobacter genus. In some embodiments, the bacteria is a bacteria of the legionella genus. In some embodiments, the bacteria is a bacteria of the leptospira genus. In some embodiments, the bacteria is a bacteria of the listeria genus. In some embodiments, the bacteria is a bacteria of the mycobacterium genus. In some embodiments, the bacteria is a bacteria of the mycoplasma genus. In some embodiments, the bacteria is a bacteria of the neisseria genus. In some embodiments, the bacteria is a bacteria of the pseudomonas genus. In some embodiments, the bacteria is a bacteria of the rickettsia genus. In some embodiments, the bacteria is a bacteria of the salmonella genus. In some embodiments, the bacteria is a bacteria of the shigella genus. In some embodiments, the bacteria is a bacteria of the staphylococcus genus. In some embodiments, the bacteria is a bacteria of the streptococcus genus. In some embodiments, the bacteria is a bacteria of the treponema genus. In some embodiments, the bacteria is a bacteria of the

ureaplasma genus. In some embodiments, the bacteria is a bacteria of the vibrio genus. In some embodiments, the bacteria is a bacteria of the yersinia genus.

[00173] In some embodiments, the pathogen is a parasite. In some embodiments, the parasite is a protozoa, helminth, or ectoparasite. In some embodiments, the protozoa is an entamoeba, giardia, leishmania, balantidium, plasmodium, or cryptosporidium. In some embodiments, the helminth is a trematode, cestode, acanthocephalan, or round worm. In some embodiments, the ectoparasite is a arthropod.

[00174] In some embodiments, the pathogen is a virus. In some embodiments, the virus is a virus of the adenoviridae, arenaviridae, astroviridae, bunyaviridae, caliciviridae, coronaviridae, filoviridae, flaviviridae, hepadnaviridae, hepeviridae, orthomyxoviridae, papillomaviridae, paramyxoviridae, parvoviridae, picornaviridae, polyomaviridae, poxviridae, reoviridae, retroviridae, rhabdoviridae, or togaviridae family. In some embodiments, the virus is a virus of the virus is a virus of the adenoviridae family. In some embodiments, the virus is a virus of the arenaviridae family. In some embodiments, the virus is a virus of the astroviridae family. In some embodiments, the virus is a virus of the bunyaviridae family. In some embodiments, the virus is a virus of the caliciviridae family. In some embodiments, the virus is a virus of the coronaviridae family. In some embodiments, the virus is a virus of the filoviridae family. In some embodiments, the virus is a virus of the flaviviridae family. In some embodiments, the virus is a virus of the hepadnaviridae family. In some embodiments, the virus is a virus of the hepeviridae family. In some embodiments, the virus is a virus of the orthomyxoviridae family. In some embodiments, the virus is a virus of the papillomaviridae family. In some embodiments, the virus is a virus of the paramyxoviridae family. In some embodiments, the virus is a virus of the parvoviridae family. In some embodiments, the virus is a virus of the picornaviridae family. In some embodiments, the virus is a virus of the polyomaviridae family. In some embodiments, the virus is a virus of the poxviridae family. In some embodiments, the virus is a virus of the reoviridae family. In some embodiments, the virus is a virus of the retroviridae family. In some embodiments, the virus is a virus of the rhabdoviridae family. In some embodiments, the virus is a virus of the togaviridae family.

[00175] In some embodiments, the virus is an adenovirus, coronavirus, coxsackievirus, Epstein-Barr virus, hepatitis A virus, hepatitis B virus, hepatitis C virus, herpes simplex virus

type 2, cytomegalovirus, human herpes virus type 8, human immunodeficiency virus, influenza virus, measles virus, mumps virus, human papillomavirus, parainfluenza virus, poliovirus, rabies virus, respiratory syncytial virus, rubella virus, or varicella-zoster virus. In some embodiments, the virus is an adenovirus. In some embodiments, the virus is a coronavirus. In some embodiments, the coronavirus virus is Covid-19 (SARS-CoV-2). In some embodiments, the virus is a coxsackievirus. In some embodiments, the virus is a Epstein-Barr virus. In some embodiments, the virus is a hepatitis A virus. In some embodiments, the virus is a hepatitis B virus. In some embodiments, the virus is a hepatitis C virus. In some embodiments, the virus is a herpes simplex virus type 2. In some embodiments, the virus is a cytomegalovirus. In some embodiments, the virus is a human herpes virus type 8. In some embodiments, the virus is a human immunodeficiency virus. In some embodiments, the virus is an influenza virus. In some embodiments, the virus is a measles virus. In some embodiments, the virus is a mumps virus. In some embodiments, the virus is a human papillomavirus. In some embodiments, the virus is a parainfluenza virus. In some embodiments, the virus is a poliovirus. In some embodiments, the virus is a rabies virus. In some embodiments, the virus is a respiratory syncytial virus. In some embodiments, the virus is a rubella virus. In some embodiments, the virus is a varicella-zoster virus.

5.3 Oligomers

[00176] In another aspect, provided herein is an oligomer formed by the molecules provided herein such as those disclosed in Section 5.2. In some embodiments, the oligomer disclosed herein can be formed when the molecules are connected to two other molecules or more than two other molecules. The oligomer disclosed herein can be made up of homomeric or heteromeric molecules. The oligomer disclosed herein can be made up of different numbers of molecules. Thus, the present disclosure provides an effective platform for forming multispecific and/or multivalent binding oligomers.

[00177] In some embodiments, the oligomer provided herein is formed by the molecules each of which has one connection site, thus the oligomer is a dimer. In some embodiments, the oligomer provided herein is formed by the molecules each of which has two connection sites. In some specific embodiments, the two connection sites of one molecule are directed to two connection sites of one other molecule, thus the oligomer is a dimer. In some specific

embodiments, the two connection sites of one molecule are directed to connection sites of two other molecules, thus the oligomer is larger than a dimer. In some embodiments, the oligomer provided herein is formed by the molecules each of which has three connection sites. In some specific embodiments, the three connection sites of one molecule are directed to three connection sites of one other molecule, thus the oligomer is a dimer. In some specific embodiments, the three connection sites of one molecule are directed to connection sites of two other molecules, thus the oligomer is larger than a dimer. In some specific embodiments, the three connection sites of one molecule are directed to connection sites of three other molecules, thus the oligomer is larger than a dimer. In some embodiments, the oligomer provided herein comprises 3 units. In some embodiments, the oligomer provided herein comprises 4 units. In some embodiments, the oligomer provided herein comprises 5 units. In some embodiments, the oligomer provided herein comprises 6 units. In some embodiments, the oligomer provided herein comprises more than 6 units. In some embodiments, the oligomer is a trimer. In some embodiments, the oligomer is a tetramer. In some embodiments, the oligomer is a pentamer. In other embodiments, the oligomer is a hexamer. In other embodiments, the oligomer provided herein is a heptamer. In other embodiments, the oligomer provided herein is an octamer. In other embodiments, the oligomer provided herein is a nonamer. In other embodiments, the oligomer provided herein is a decamer. In other embodiments, the oligomer provided herein is an undecamer. In other embodiments, the oligomer provided herein is a twelve-mer. In other embodiments, the oligomer provided herein is a thirteen-mer. In other embodiments, the oligomer provided herein is a fourteen-mer. In other embodiments, the oligomer provided herein is a fifteen-mer. In other embodiments, the oligomer provided herein is a sixteen-mer. In other embodiments, the oligomer provided herein is a seventeen-mer. In other embodiments, the oligomer provided herein is an eighteen-mer. In other embodiments, the oligomer provided herein is a nineteen-mer. In other embodiments, the oligomer provided herein is a twenty-mer.

[00178] The oligomer provided herein can be formed by the same or different molecules. In some embodiments, the oligomer is homomeric. In other embodiments, the oligomer is heteromeric.

[00179] In some embodiments, the individual molecules in the oligomer provided herein all bind to the same antigen (or target), therefore the oligomer is monospecific; however at least two

molecules are different from each other, e.g., at the binding domain. For example, the molecules are different in terms of format or structure of the binding domains or the molecules are different in the sequences of the binding domains.

[00180] In some embodiments, all the molecules in the oligomer provided herein bind to the same antigen, and thus the oligomer is monospecific. In other embodiments, not all the molecules in the oligomer provided herein bind to the same antigen, and thus the oligomer is multispecific. In some embodiments, the oligomer is bispecific. In some embodiments, the oligomer is trispecific. In other embodiments, the oligomer is tetraspecific. In other embodiments, the oligomer is pentaspecific. In other embodiments, the oligomer is a hexaspecific.

[00181] In some embodiments, all the molecules in the oligomer provided herein bind to the same antigen but on different epitopes. In some embodiments, the oligomer provided herein is multivalent. In some embodiments, the oligomer is bivalent. In some embodiments, the oligomer is trivalent. In other embodiments, the oligomer is tetravalent. In other embodiments, the oligomer is pentavalent. In other embodiments, the oligomer is a hexavalent. In other embodiments, the oligomer provided herein is a heptavalent. In other embodiments, the oligomer provided herein is an octavalent. In other embodiments, the oligomer provided herein is a nonavalent. In other embodiments, the oligomer provided herein is a decavalent.

5.4 Pharmaceutical Composition

[00182] In one aspect, the present disclosure further provides pharmaceutical compositions comprising at least a molecule of the present disclosure. In some embodiments, a pharmaceutical composition comprises therapeutically effective amount of the molecules provided herein and a pharmaceutically acceptable excipient. In some specific embodiments, a pharmaceutical composition comprises therapeutically effective amount of oligomers provided herein and a pharmaceutically acceptable excipient.

[00183] In a specific embodiment, the term “excipient” can also refer to a diluent, adjuvant (e.g., Freund's adjuvant (complete or incomplete), carrier or vehicle. Pharmaceutical excipients 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. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid excipients.

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. Examples of suitable pharmaceutical excipients are described in Remington's Pharmaceutical Sciences (1990) Mack Publishing Co., Easton, PA. Such compositions will contain a prophylactically or therapeutically effective amount of the active ingredient provided herein, such as in purified form, together with a suitable amount of excipient so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

[00184] In some embodiments, the choice of excipient is determined in part by the particular cell, and/or by the method of administration. Accordingly, there are a variety of suitable formulations.

[00185] Typically, acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers, antioxidants including ascorbic acid, methionine, Vitamin E, sodium metabisulfite; preservatives, isotonicifiers, stabilizers, metal complexes (e.g. Zn-protein complexes); chelating agents such as EDTA and/or non-ionic surfactants.

[00186] Buffers may be used to control the pH in a range which optimizes the therapeutic effectiveness, especially if stability is pH dependent. Suitable buffering agents for use with the present disclosure include both organic and inorganic acids and salts thereof. For example, citrate, phosphate, succinate, tartrate, fumarate, gluconate, oxalate, lactate, acetate. Additionally, buffers may comprise histidine and trimethylamine salts such as Tris.

[00187] Preservatives may be added to retard microbial growth. Suitable preservatives for use with the present disclosure include octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium halides (e.g., chloride, bromide, iodide), benzethonium chloride; thimerosal, phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol, 3-pentanol, and m-cresol.

[00188] Tonicity agents, sometimes known as “stabilizers” can be present to adjust or maintain the tonicity of liquid in a composition. When used with large, charged biomolecules such as proteins and antibodies, they are often termed “stabilizers” because they can interact with the charged groups of the amino acid side chains, thereby lessening the potential for inter and intra-molecular interactions. Exemplary tonicity agents include polyhydric sugar alcohols, trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol.

[00189] Additional exemplary excipients include: (1) bulking agents, (2) solubility enhancers, (3) stabilizers and (4) agents preventing denaturation or adherence to the container wall. Such excipients include: polyhydric sugar alcohols (enumerated above); amino acids such as alanine, glycine, glutamine, asparagine, histidine, arginine, lysine, ornithine, leucine, 2-phenylalanine, glutamic acid, threonine, etc.; organic sugars or sugar alcohols such as sucrose, lactose, lactitol, trehalose, stachyose, mannose, sorbose, xylose, ribose, ribitol, myoinisitol, myoinisitol, galactose, galactitol, glycerol, cyclitols (e.g., inositol), polyethylene glycol; sulfur containing reducing agents, such as urea, glutathione, thiocetic acid, sodium thioglycolate, thioglycerol, α -monothioglycerol and sodium thio sulfate; low molecular weight proteins such as human serum albumin, bovine serum albumin, gelatin or other immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; monosaccharides (e.g., xylose, mannose, fructose, glucose; disaccharides (e.g., lactose, maltose, sucrose); trisaccharides such as raffinose; and polysaccharides such as dextrin or dextran.

[00190] Non-ionic surfactants or detergents (also known as “wetting agents”) may be present to help solubilize the therapeutic agent as well as to protect the therapeutic protein against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stress without causing denaturation of the active therapeutic protein or antibody. Suitable non-ionic surfactants include, e.g., polysorbates (20, 40, 60, 65, 80, etc.), polyoxamers (184, 188, etc.), PLURONIC® polyols, TRITON®, polyoxyethylene sorbitan monoethers (TWEEN®-20, TWEEN®-80, etc.), lauromacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose. Anionic detergents that can be used include sodium lauryl sulfate,

dioctyle sodium sulfosuccinate and dioctyl sodium sulfonate. Cationic detergents include benzalkonium chloride or benzethonium chloride.

[00191] The route of administration is in accordance with known and accepted methods, such as by single or multiple bolus or infusion over a long period of time in a suitable manner, e.g., injection or infusion by subcutaneous, intravenous, intraperitoneal, intramuscular, intraarterial, intralesional or intraarticular routes, topical administration, inhalation or by sustained release or extended-release means.

[00192] In another embodiment, a pharmaceutical composition can be provided as a controlled release or sustained release system. In one embodiment, a pump may be used to achieve controlled or sustained release (see, e.g., Sefton, *Crit. Ref. Biomed. Eng.* 14:201-40 (1987); Buchwald et al., *Surgery* 88:507-16 (1980); and Saudek et al., *N. Engl. J. Med.* 321:569-74 (1989)). In another embodiment, polymeric materials can be used to achieve controlled or sustained release of a prophylactic or therapeutic agent (e.g., a fusion protein as described herein) or a composition provided herein (see, e.g., *Medical Applications of Controlled Release* (Langer and Wise eds., 1974); *Controlled Drug Bioavailability, Drug Product Design and Performance* (Smolen and Ball eds., 1984); Ranger and Peppas, *J. Macromol. Sci. Rev. Macromol. Chem.* 23:61-126 (1983); Levy et al., *Science* 228:190-92 (1985); During et al., *Ann. Neurol.* 25:351-56 (1989); Howard et al., *J. Neurosurg.* 71:105-12 (1989); U.S. Pat. Nos. 5,679,377; 5,916,597; 5,912,015; 5,989,463; and 5,128,326; PCT Publication Nos. WO 99/15154 and WO 99/20253). Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In one embodiment, the polymer used in a sustained release formulation is inert, free of leachable impurities, stable on storage, sterile, and biodegradable. In yet another embodiment, a controlled or sustained release system can be placed in proximity of a particular target tissue, for example, the nasal passages or lungs, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, *Medical Applications of Controlled Release Vol. 2*, 115-38 (1984)). Controlled release systems are discussed, for example, by Langer, *Science* 249:1527-33

(1990). Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more agents as described herein (see, e.g., U.S. Pat. No. 4,526,938, PCT publication Nos. WO 91/05548 and WO 96/20698, Ning et al., *Radiotherapy & Oncology* 39:179-89 (1996); Song et al., *PDA J. of Pharma. Sci. & Tech.* 50:372-97 (1995); Cleek et al., *Pro. Int'l. Symp. Control. Rel. Bioact. Mater.* 24:853-54 (1997); and Lam et al., *Proc. Int'l. Symp. Control Rel. Bioact. Mater.* 24:759-60 (1997)).

[00193] The pharmaceutical compositions described herein may also contain more than one active compound or agent as necessary for the particular indication being treated. Alternatively, or in addition, the composition may comprise a cytotoxic agent, chemotherapeutic agent, cytokine, immunosuppressive agent, or growth inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[00194] The active ingredients may also be entrapped in microcapsules prepared, for example, by coascervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's *Pharmaceutical Sciences* 18th edition.

[00195] Various compositions and delivery systems are known and can be used with the therapeutic agents provided herein, including, but not limited to, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the antibody or therapeutic molecule provided herein, construction of a nucleic acid as part of a retroviral or other vector, etc.

[00196] In some embodiments, the pharmaceutical composition provided herein contains the binding molecules in amounts effective to treat or prevent the disease or disorder, such as a therapeutically effective or prophylactically effective amount. Therapeutic or prophylactic efficacy in some embodiments is monitored by periodic assessment of treated subjects. For repeated administrations over several days or longer, depending on the condition, the treatment is repeated until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful and can be determined.

[00197] In a specific embodiment, the term “excipient” can also refer to a diluent, adjuvant (e.g., Freund’s adjuvant (complete or incomplete), carrier or vehicle. Pharmaceutical excipients 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. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid excipients. 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. Examples of suitable pharmaceutical excipients are described in Remington’s Pharmaceutical Sciences (1990) Mack Publishing Co., Easton, PA. Such compositions will contain a prophylactically or therapeutically effective amount of the active ingredient provided herein, such as in purified form, together with a suitable amount of excipient so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

[00198] In some embodiments, the choice of excipient is determined in part by the particular cell, and/or by the method of administration. Accordingly, there are a variety of suitable formulations.

[00199] Typically, acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers, antioxidants including ascorbic acid, methionine, Vitamin E, sodium metabisulfite; preservatives, isotonicifiers, stabilizers, metal complexes (e.g. Zn-protein complexes); chelating agents such as EDTA and/or non-ionic surfactants.

[00200] Buffers may be used to control the pH in a range which optimizes the therapeutic effectiveness, especially if stability is pH dependent. Suitable buffering agents for use with the present disclosure include both organic and inorganic acids and salts thereof. For example, citrate, phosphate, succinate, tartrate, fumarate, gluconate, oxalate, lactate, acetate. Additionally, buffers may comprise histidine and trimethylamine salts such as Tris.

[00201] Preservatives may be added to retard microbial growth. Suitable preservatives for use with the present disclosure include octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium halides (e.g., chloride, bromide, iodide), benzethonium chloride; thimerosal, phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol, 3-pentanol, and m-cresol.

[00202] Tonicity agents, sometimes known as “stabilizers” can be present to adjust or maintain the tonicity of liquid in a composition. When used with large, charged biomolecules such as proteins and antibodies, they are often termed “stabilizers” because they can interact with the charged groups of the amino acid side chains, thereby lessening the potential for inter and intra-molecular interactions. Exemplary tonicity agents include polyhydric sugar alcohols, trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol.

[00203] Additional exemplary excipients include: (1) bulking agents, (2) solubility enhancers, (3) stabilizers and (4) agents preventing denaturation or adherence to the container wall. Such excipients include: polyhydric sugar alcohols (enumerated above); amino acids such as alanine, glycine, glutamine, asparagine, histidine, arginine, lysine, ornithine, leucine, 2-phenylalanine, glutamic acid, threonine, etc.; organic sugars or sugar alcohols such as sucrose, lactose, lactitol, trehalose, stachyose, mannose, sorbose, xylose, ribose, ribitol, myoinositol, myoinisitol, galactose, galactitol, glycerol, cyclitols (e.g., inositol), polyethylene glycol; sulfur containing reducing agents, such as urea, glutathione, thioctic acid, sodium thioglycolate, thioglycerol, α -monothioglycerol and sodium thio sulfate; low molecular weight proteins such as human serum albumin, bovine serum albumin, gelatin or other immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; monosaccharides (e.g., xylose, mannose, fructose, glucose; disaccharides (e.g., lactose, maltose, sucrose); trisaccharides such as raffinose; and polysaccharides such as dextrin or dextran.

[00204] Non-ionic surfactants or detergents (also known as “wetting agents”) may be present to help solubilize the therapeutic agent as well as to protect the therapeutic protein against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stress without causing denaturation of the active therapeutic protein or antibody. Suitable non-ionic surfactants include, e.g., polysorbates (20, 40, 60, 65, 80, etc.), polyoxamers (184, 188,

etc.), PLURONIC® polyols, TRITON®, polyoxyethylene sorbitan monoethers (TWEEN®-20, TWEEN®-80, etc.), laurmacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose. Anionic detergents that can be used include sodium lauryl sulfate, dioctyle sodium sulfosuccinate and dioctyl sodium sulfonate. Cationic detergents include benzalkonium chloride or benzethonium chloride.

[00205] The route of administration is in accordance with known and accepted methods, such as by single or multiple bolus or infusion over a long period of time in a suitable manner, e.g., injection or infusion by subcutaneous, intravenous, intraperitoneal, intramuscular, intraarterial, intralesional or intraarticular routes, topical administration, inhalation or by sustained release or extended-release means.

[00206] In another embodiment, a pharmaceutical composition can be provided as a controlled release or sustained release system. In one embodiment, a pump may be used to achieve controlled or sustained release (see, e.g., Sefton, Crit. Ref. Biomed. Eng. 14:201-40 (1987); Buchwald et al., Surgery 88:507-16 (1980); and Saudek et al., N. Engl. J. Med. 321:569-74 (1989)). In another embodiment, polymeric materials can be used to achieve controlled or sustained release of a prophylactic or therapeutic agent (e.g., a fusion protein as described herein) or a composition provided herein (see, e.g., Medical Applications of Controlled Release (Langer and Wise eds., 1974); Controlled Drug Bioavailability, Drug Product Design and Performance (Smolen and Ball eds., 1984); Ranger and Peppas, J. Macromol. Sci. Rev. Macromol. Chem. 23:61-126 (1983); Levy et al., Science 228:190-92 (1985); During et al., Ann. Neurol. 25:351-56 (1989); Howard et al., J. Neurosurg. 71:105-12 (1989); U.S. Pat. Nos. 5,679,377; 5,916,597; 5,912,015; 5,989,463; and 5,128,326; PCT Publication Nos. WO 99/15154 and WO 99/20253). Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In one embodiment, the polymer used in a sustained release formulation is inert, free of leachable impurities, stable on storage, sterile, and biodegradable. In yet another

embodiment, a controlled or sustained release system can be placed in proximity of a particular target tissue, for example, the nasal passages or lungs, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, *Medical Applications of Controlled Release* Vol. 2, 115-38 (1984)). Controlled release systems are discussed, for example, by Langer, *Science* 249:1527-33 (1990). Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more agents as described herein (see, e.g., U.S. Pat. No. 4,526,938, PCT publication Nos. WO 91/05548 and WO 96/20698, Ning et al., *Radiotherapy & Oncology* 39:179-89 (1996); Song et al., *PDA J. of Pharma. Sci. & Tech.* 50:372-97 (1995); Cleek et al., *Pro. Int'l. Symp. Control. Rel. Bioact. Mater.* 24:853-54 (1997); and Lam et al., *Proc. Int'l. Symp. Control Rel. Bioact. Mater.* 24:759-60 (1997)).

[00207] The pharmaceutical compositions described herein may also contain more than one active compound or agent as necessary for the particular indication being treated. Alternatively, or in addition, the composition may comprise a cytotoxic agent, chemotherapeutic agent, cytokine, immunosuppressive agent, or growth inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[00208] The active ingredients may also be entrapped in microcapsules prepared, for example, by coascervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's *Pharmaceutical Sciences* 18th edition.

[00209] Various compositions and delivery systems are known and can be used with the therapeutic agents provided herein, including, but not limited to, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the antibody or therapeutic molecule provided herein, construction of a nucleic acid as part of a retroviral or other vector, etc.

[00210] In some embodiments, the pharmaceutical composition provided herein contains the binding molecules in amounts effective to treat or prevent the disease or disorder, such as a therapeutically effective or prophylactically effective amount. Therapeutic or prophylactic efficacy in some embodiments is monitored by periodic assessment of treated subjects. For

repeated administrations over several days or longer, depending on the condition, the treatment is repeated until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful and can be determined.

5.5 Nucleic Acid Molecules, Vectors, and Host Cells

[00211] In certain embodiments, the disclosure encompasses nucleic acid molecules that encode the molecules described herein. The term “nucleic acid molecules that encode a polypeptide” encompasses a nucleic acid molecule that includes coding sequences for the polypeptide as well as a nucleic acid molecule which includes additional coding and/or non-coding sequences. The nucleic acid molecules of the disclosure can be in the form of RNA or in the form of DNA. DNA includes cDNA, genomic DNA, and synthetic DNA; and can be double-stranded or single-stranded, and if single stranded can be the coding strand or non-coding (anti-sense) strand.

[00212] In certain embodiments, a nucleic acid molecule comprises the coding sequence for a polypeptide fused in the same reading frame to a nucleic acid molecule which aids, for example, in expression and secretion of a polypeptide from a host cell (*e.g.*, a leader sequence which functions as a secretory sequence for controlling transport of a polypeptide). The nucleic acid molecule can have the leader sequence cleaved by the host cell to form a “mature” form of the polypeptide.

[00213] In certain embodiments, a nucleic acid molecule comprises the coding sequence for a polypeptide fused in the same reading frame to a marker or tag sequence. For example, in some embodiments, a marker sequence is a hexa-histidine tag supplied by a vector that allows efficient purification of the polypeptide fused to the marker in the case of a bacterial host. In some embodiments, a marker is used in conjunction with other affinity tags.

[00214] Recombinant expression of the molecule provided herein requires construction of an expression vector containing a polynucleotide that encodes the molecule. Once a polynucleotide encoding the molecule provided herein has been obtained, the vector for the production of the molecule may be produced by recombinant DNA technology using techniques well-known in the art. Thus, methods for preparing the molecule provided herein by expressing a polynucleotide containing nucleotide sequence encoding different portions of the molecule as described in Section 5.2 are described herein. Methods which are well known to those skilled in the art can

be used to construct expression vectors containing sequences encoding and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Also provided are replicable vectors comprising a nucleotide sequence encoding the molecule provided herein, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (*see, e.g.*, International Publication Nos. WO 86/05807 and WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy, the entire light chain, or both the entire heavy and light chains.

[00215] The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce the molecule provided herein. Thus, also provided herein are host cells containing a polynucleotide encoding the molecule provided herein, operably linked to a heterologous promoter. In certain embodiments for the expression of the molecules with antigen-binding fragments described herein, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

[00216] A variety of host-expression vector systems may be utilized to express the molecules provided herein (*see, e.g.*, U.S. Patent No. 5,807,715). Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express the molecule provided herein *in situ*. These include but are not limited to microorganisms such as bacteria (*e.g.*, *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing coding sequences; yeast (*e.g.*, *Saccharomyces Pichia*) transformed with recombinant yeast expression vectors containing coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing coding sequences; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV, tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing coding sequences; or mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, NS0, and 3T3 cells) harboring recombinant expression constructs containing promoters

derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter). Bacterial cells such as *Escherichia coli*, or, eukaryotic cells, especially for the expression of whole recombinant molecule, can be used for the expression of a recombinant molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., 1986, *Gene* 45:101; and Cockett et al., 1990, *Bio/Technology* 8:2). In some embodiments, the molecule provided herein is produced in CHO cells. In a specific embodiment, the expression of nucleotide sequences encoding the molecule provided herein is regulated by a constitutive promoter, inducible promoter or tissue specific promoter.

[00217] In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the molecule being expressed. For example, when a large quantity of such a molecule is to be produced, for the generation of pharmaceutical compositions of the molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited to, the *E. coli* expression vector pUR278 (Ruther et al., 1983, *EMBO* 12:1791), in which the coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, *Nucleic Acids Res.* 13:3101-3109; Van Heeke & Schuster, 1989, *J. Biol. Chem.* 24:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[00218] In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

[00219] In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the molecule in infected hosts (*e.g.*, *see* Logan & Shenk, 1984, Proc. Natl. Acad. Sci. USA 81:355-359). Specific initiation signals may also be required for efficient translation of inserted coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (*see, e.g.*, Bittner et al., 1987, Methods in Enzymol. 153:51-544).

[00220] In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells that possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, W138, BT483, Hs578T, HTB2, BT20 and T47D, NS0 (a murine myeloma cell line that does not endogenously produce any immunoglobulin chains), CRL7030 and HsS78Bst cells. In some embodiments, the molecule provided herein as a fully human antibody or a fragment thereof is produced in mammalian cells, such as CHO cells.

[00221] For long-term, high-yield production of recombinant proteins, stable expression can be utilized. For example, cell lines which stably express the molecule described herein may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci, which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines that express the molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compositions that interact directly or indirectly with the molecule.

[00222] A number of selection systems may be used, including but not limited to, the herpes simplex virus thymidine kinase (Wigler et al., 1977, Cell 11:223), hypoxanthineguanine phosphoribosyltransferase (Szybalska & Szybalski, 1992, Proc. Natl. Acad. Sci. USA 48:202), and adenine phosphoribosyltransferase (Lowy et al., 1980, Cell 22:8-17) genes can be employed in tk-, hgp^{rt}- or ap^{rt}-cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., 1980, Natl. Acad. Sci. USA 77:357; O'Hare et al., 1981, Proc. Natl. Acad. Sci. USA 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, Proc. Natl. Acad. Sci. USA 78:2072); neo, which confers resistance to the aminoglycoside G-418 (Wu and Wu, 1991, Biotherapy 3:87-95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, 1993, Science 260:926-932; and Morgan and Anderson, 1993, Ann. Rev. Biochem. 62:191-217; 1993, TIB TECH 11(5):155-2 15); and hyg^{ro}, which confers resistance to hygromycin (Santerre et al., 1984, Gene 30:147). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al.

(eds.), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., 1981, J. Mol. Biol. 150:1, which are incorporated by reference herein in their entireties.

[00223] The expression levels of the molecule described herein can be increased by vector amplification (for a review, *see* Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol. 3 (Academic Press, New York, 1987)). When a marker in the vector system is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the gene encoding the molecule described herein, production of the molecule will also increase (Crouse et al., 1983, Mol. Cell. Biol. 3:257).

[00224] For the molecule described in Section 5.2.2 which comprises an antigen-binding fragment, the host cell may be co-transfected with two expression vectors provided herein, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, 1986, Nature 322:52; and Kohler, 1980, Proc. Natl. Acad. Sci. USA 77:2197-2199). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

5.6 Methods of Generating Oligomerized Molecules

[00225] In another aspect, provided herein are methods for producing an oligomer. More specifically, the method provided herein comprises a first step for performing the function of introducing an amino acid substitution to one or more molecule(s) comprising an IgG C_{H2} region, e.g., substitution of the amino acid residue the position 253 by EU numbering with a cysteine residue; and a second step for performing the function of expressing such molecules. In some embodiments, the methods provided herein further comprises a step for performing the function of introducing a human μ tailpiece to the molecules. In some embodiments, the methods provided herein comprise further purifying or isolating oligomers comprising the

molecules. In some embodiments, the methods provided herein comprise constructing a vector encoding a molecule provided herein.

[00226] In some specific embodiments, provided herein is a method of producing oligomerized molecules, comprising: (a) introducing the vectors disclosed in Section 5.5 into a host cell; (b) cultivating the host cell under suitable conditions for the production of the oligomerized molecules; and (c) purifying the oligomerized molecules in the conditioned medium of the host cell. In some embodiments, prior to step (c), the methods provided herein may comprise detecting the existence of oligomerized molecules in the conditioned medium of the host cell, e.g., by SDS- polyacrylamide gel electrophoresis (PAGE). In some embodiments, the methods provided herein may further comprise characterizing the purified oligomerized molecules with intact mass spectrometry or high-performance liquid chromatography (HPLC)-size exclusion chromatography (SEC).

[00227] The point mutations described in Section 5.2.1 and other single residue modifications described in Section 5.2.2 can be made using methods known in the art such as oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis (*see, e.g.*, Carter, 1986, *Biochem J.* 237:1-7; and Zoller et al., 1982, *Nucl. Acids Res.* 10:6487-500), cassette mutagenesis (*see, e.g.*, Wells et al., 1985, *Gene* 34:315-23), or other known techniques can be performed on the cloned DNA to produce the targeted point mutations described in Section 5.2.1. The added tailpiece described in Section 5.2.1 and other genetic modifications described in Section 5.2.2 can be made using recombinant DNA techniques known to one skilled in the art (*see, e.g.*, Zyskind and Berstein, 1989, *Recombinant DNA laboratory Manual*; Rajagopal, 2012, *Recombinant DNA Technology and Genetic Engineering*; Sambrook and Russel, *Molecular Cloning, A Laboratory Manual*, 3rd ed., 2001, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; Kostelny et al., *Int. J. Cancer* 93:556-565, 2001; Cole et al., *J. Immunol.* 159:3613-3621, 1997 and Tsurushita et al., *Methods* 36:69-83, 2005.).

[00228] After obtaining the vectors encoding the engineered molecules as disclosed in Section 5.5, cell transfection or transduction can be performed in host cells. Introducing polynucleotides into a host cell can be done by any known method, including, for example, packaging the polynucleotide in a virus (or into a viral vector) and transducing a host cell with the virus (or vector) or by transfection procedures known in the art. Methods for introducing heterologous

polynucleotides into mammalian cells are well known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene-mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei. Mammalian cell lines available as hosts for expression are well known in the art and include, but are not limited to CHO cells, HeLa cells, and human hepatocellular carcinoma cells.

[00229] After being introduced vectors of interest, host cells secrete mixture, which contains the monomeric form of the molecule and different levels of oligomers of the molecules, into culture media. In some embodiments, culture media is collected 12 hours after introducing vectors to host cells. In some embodiments, culture media is collected 24 hours after introducing vectors to host cells. In some embodiments, culture media is collected 36 hours after introducing vectors to host cells. In some embodiments, culture media is collected 48 hours after introducing vectors to host cells. In some embodiments, culture media is collected 60 hours after introducing vectors to host cells. In some embodiments, culture media is collected 72 hours after introducing vectors to host cells. In some embodiments, culture media is collected 84 hours after introducing vectors to host cells. In some embodiments, culture media is collected 96 hours after introducing vectors to host cells.

[00230] As an initial step to confirm the existence of oligomerized molecules in the conditioned medium before further purification and characterization, detection of a molecular-weight profile of different components of the mixture is performed. Since monomeric and oligomeric forms of the molecule described herein at least differ in terms of mass, the detection technologies based on mass are well known in the art and include, but not limited to, gel electrophoresis, such as SDS-PAGE, centrifugation, and chromatography, such as gel filtration columns.

[00231] Once the molecule provided herein has been produced by recombinant expression, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Further, the

molecule provided herein can be fused to heterologous polypeptide sequences described herein or otherwise known in the art to facilitate purification.

[00232] The purified oligomerized molecules need to be characterized and confirmed its identity. Different methods are known in the art. In some embodiments, the purified oligomerized molecules can be characterized by mass spectrometry. In specific embodiments, the purified oligomerized molecules can be characterized by intact mass spectrometry. In other embodiments, the purified oligomerized molecules can be characterized by HPLC. In specific embodiments, the purified oligomerized molecules can be characterized by HPLC-SEC. In other embodiments, the purified oligomerized molecules can be characterized by x-ray crystallography. In other embodiments, the purified oligomerized molecules can be characterized by NMR spectroscopy. In other embodiments, the purified oligomerized molecules can be characterized by cryoelectron microscopy.

Recombinant Production in Prokaryotic Cells

[00233] Polynucleic acid sequences encoding the molecules (such as antibodies or fragments thereof) of the present disclosure can be obtained using standard recombinant techniques. Desired polynucleic acid sequences may be isolated and sequenced from antibody producing cells such as hybridoma cells. Alternatively, polynucleotides can be synthesized using nucleotide synthesizer or PCR techniques. Once obtained, sequences encoding the polypeptides are inserted into a recombinant vector capable of replicating and expressing heterologous polynucleotides in prokaryotic hosts. Many vectors that are available and known in the art can be used for the purpose of the present disclosure. Selection of an appropriate vector will depend mainly on the size of the nucleic acids to be inserted into the vector and the particular host cell to be transformed with the vector. Each vector contains various components, depending on its function (amplification or expression of heterologous polynucleotide, or both) and its compatibility with the particular host cell in which it resides. The vector components generally include, but are not limited to, an origin of replication, a selection marker gene, a promoter, a ribosome binding site (RBS), a signal sequence, the heterologous nucleic acid insert and a transcription termination sequence.

[00234] In general, plasmid vectors containing replicon and control sequences which are derived from species compatible with the host cell are used in connection with these hosts. The

vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells. For example, *E. coli* is typically transformed using pBR322, a plasmid derived from an *E. coli* species. Examples of pBR322 derivatives used for expression of particular antibodies are described in detail in Carter *et al.*, U.S. Pat. No. 5,648,237.

[00235] In addition, phage vectors containing replicon and control sequences that are compatible with the host microorganism can be used as transforming vectors in connection with these hosts. For example, bacteriophage such as GEM™-11 may be utilized in making a recombinant vector which can be used to transform susceptible host cells such as *E. coli* LE392.

[00236] The expression vector of the present application may comprise two or more promoter-cistron pairs, encoding each of the polypeptide components. A promoter is an untranslated regulatory sequence located upstream (5') to a cistron that modulates its expression. Prokaryotic promoters typically fall into two classes, inducible and constitutive. Inducible promoter is a promoter that initiates increased levels of transcription of the cistron under its control in response to changes in the culture condition, *e.g.* the presence or absence of a nutrient or a change in temperature.

[00237] A large number of promoters recognized by a variety of potential host cells are well known. The selected promoter can be operably linked to cistron DNA encoding the present antibody by removing the promoter from the source DNA via restriction enzyme digestion and inserting the isolated promoter sequence into the vector of the present application. Both the native promoter sequence and many heterologous promoters may be used to direct amplification and/or expression of the target genes. In some embodiments, heterologous promoters are utilized, as they generally permit greater transcription and higher yields of expressed target gene as compared to the native target polypeptide promoter.

[00238] Promoters suitable for use with prokaryotic hosts include the PhoA promoter, the -galactamase and lactose promoter systems, a tryptophan (*trp*) promoter system and hybrid promoters such as the *tac* or the *trc* promoter. However, other promoters that are functional in bacteria (such as other known bacterial or phage promoters) are suitable as well. Their nucleic acid sequences have been published, thereby enabling a skilled worker operably to ligate them to

cistrons encoding the target peptide (Siebenlist *et al. Cell* 20: 269 (1980)) using linkers or adaptors to supply any required restriction sites.

[00239] In one aspect, each cistron within the recombinant vector comprises a secretion signal sequence component that directs translocation of the expressed polypeptides across a membrane. In general, the signal sequence may be a component of the vector, or it may be a part of the target polypeptide DNA that is inserted into the vector. The signal sequence selected for the purpose of this invention should be one that is recognized and processed (*i.e.* cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process the signal sequences native to the heterologous polypeptides, the signal sequence can be substituted by a prokaryotic signal sequence selected, for example, from the group consisting of the alkaline phosphatase, penicillinase, Ipp, or heat-stable enterotoxin II (STII) leaders, LamB, PhoE, PelB, OmpA and MBP.

[00240] In some embodiments, the production of the antibodies according to the present disclosure can occur in the cytoplasm of the host cell, and therefore does not require the presence of secretion signal sequences within each cistron. Certain host strains (*e.g.*, the *E. coli* trxB⁻ strains) provide cytoplasm conditions that are favorable for disulfide bond formation, thereby permitting proper folding and assembly of expressed protein subunits.

[00241] Prokaryotic host cells suitable for expressing the molecules (such as antibodies or fragments thereof) of the present disclosure include Archaeobacteria and Eubacteria, such as Gram-negative or Gram-positive organisms. Examples of useful bacteria include *Escherichia* (*e.g.*, *E. coli*), *Bacilli* (*e.g.*, *B. subtilis*), Enterobacteria, *Pseudomonas* species (*e.g.*, *P. aeruginosa*), *Salmonella typhimurium*, *Serratia marcescans*, *Klebsiella*, *Proteus*, *Shigella*, *Rhizobia*, *Vitreoscilla*, or *Paracoccus*. In some embodiments, gram-negative cells are used. In one embodiment, *E. coli* cells are used as hosts. Examples of *E. coli* strains include strain W3110 (Bachmann, *Cellular and Molecular Biology*, vol. 2 (Washington, D.C.: American Society for Microbiology, 1987), pp. 1190-1219; ATCC Deposit No. 27,325) and derivatives thereof, including strain 33D3 having genotype W3110 AfhuA (AtonA) ptr3 lac Iq lacL8 AompT A(nmpc-fepE) degP41 kan^R (U.S. Pat. No. 5,639,635). Other strains and derivatives thereof, such as *E. coli* 294 (ATCC 31,446), *E. coli* B, *E. coli* 1776 (ATCC 31,537) and *E. coli* RV308 (ATCC 31,608) are also suitable. These examples are illustrative rather than limiting. Methods for

constructing derivatives of any of the above-mentioned bacteria having defined genotypes are known in the art and described in, for example, Bass *et al.*, *Proteins*, 8:309-314 (1990). It is generally necessary to select the appropriate bacteria taking into consideration replicability of the replicon in the cells of a bacterium. For example, *E. coli*, *Serratia*, or *Salmonella* species can be suitably used as the host when well known plasmids such as pBR322, pBR325, pACYC177, or pKN410 are used to supply the replicon.

[00242] Typically the host cell should secrete minimal amounts of proteolytic enzymes, and additional protease inhibitors may desirably be incorporated in the cell culture.

[00243] Host cells are transformed with the above-described expression vectors and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. Transformation means introducing DNA into the prokaryotic host so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride is generally used for bacterial cells that contain substantial cell-wall barriers. Another method for transformation employs polyethylene glycol/DMSO. Yet another technique used is electroporation.

[00244] Prokaryotic cells used to produce the antibodies of the present application are grown in media known in the art and suitable for culture of the selected host cells. Examples of suitable media include luria broth (LB) plus necessary nutrient supplements. In some embodiments, the media also contains a selection agent, chosen based on the construction of the expression vector, to selectively permit growth of prokaryotic cells containing the expression vector. For example, ampicillin is added to media for growth of cells expressing ampicillin resistant gene.

[00245] Any necessary supplements besides carbon, nitrogen, and inorganic phosphate sources may also be included at appropriate concentrations introduced alone or as a mixture with another supplement or medium such as a complex nitrogen source. Optionally the culture medium may contain one or more reducing agents selected from the group consisting of glutathione, cysteine, cystamine, thioglycollate, dithioerythritol and dithiothreitol. The prokaryotic host cells are cultured at suitable temperatures and pHs.

[00246] If an inducible promoter is used in the expression vector of the present application, protein expression is induced under conditions suitable for the activation of the promoter. In one aspect of the present application, PhoA promoters are used for controlling transcription of the polypeptides. Accordingly, the transformed host cells are cultured in a phosphate-limiting medium for induction. Preferably, the phosphate-limiting medium is the C.R.A.P medium (see, e.g., Simmons *et al.*, *J. Immunol. Methods* 263:133-147 (2002)). A variety of other inducers may be used, according to the vector construct employed, as is known in the art.

[00247] The expressed antibodies of the present disclosure are secreted into and recovered from the periplasm of the host cells. Protein recovery typically involves disrupting the microorganism, generally by such means as osmotic shock, sonication or lysis. Once cells are disrupted, cell debris or whole cells may be removed by centrifugation or filtration. The proteins may be further purified, for example, by affinity resin chromatography. Alternatively, proteins can be transported into the culture media and isolated therein. Cells may be removed from the culture and the culture supernatant being filtered and concentrated for further purification of the proteins produced. The expressed polypeptides can be further isolated and identified using commonly known methods such as polyacrylamide gel electrophoresis (PAGE) and Western blot assay.

[00248] Alternatively, protein production is conducted in large quantity by a fermentation process. Various large-scale fed-batch fermentation procedures are available for production of recombinant proteins. To improve the production yield and quality of the antibodies of the present disclosure, various fermentation conditions can be modified. For example, the chaperone proteins have been demonstrated to facilitate the proper folding and solubility of heterologous proteins produced in bacterial host cells. Chen *et al.* *J Bio Chem* 274:19601-19605 (1999); U.S. Pat. No. 6,083,715; U.S. Pat. No. 6,027,888; Bothmann and Pluckthun, *J. Biol. Chem.* 275:17100-17105 (2000); Ramm and Pluckthun, *J. Biol. Chem.* 275:17106-17113 (2000); Arie *et al.*, *Mol. Microbiol.* 39:199-210 (2001).

[00249] To minimize proteolysis of expressed heterologous proteins (especially those that are proteolytically sensitive), certain host strains deficient for proteolytic enzymes can be used for the present invention, as described in, for example, U.S. Pat. No. 5,264,365; U.S. Pat. No. 5,508,192; Hara *et al.*, *Microbial Drug Resistance*, 2:63-72 (1996). *E. coli* strains deficient for

proteolytic enzymes and transformed with plasmids overexpressing one or more chaperone proteins may be used as host cells in the expression system encoding the antibodies of the present application.

[00250] The antibodies produced herein can be further purified to obtain preparations that are substantially homogeneous for further assays and uses. Standard protein purification methods known in the art can be employed. The following procedures are exemplary of suitable purification procedures: fractionation on immunoaffinity or ion-exchange columns, ethanol precipitation, reverse phase HPLC, chromatography on silica or on a cation-exchange resin such as DEAE, chromatofocusing, SDS-PAGE, ammonium sulfate precipitation, and gel filtration using, for example, Sephadex G-75. Protein A immobilized on a solid phase for example can be used in some embodiments for immunoaffinity purification of binding molecules of the present disclosure. The solid phase to which Protein A is immobilized is preferably a column comprising a glass or silica surface, more preferably a controlled pore glass column or a silicic acid column. In some embodiments, the column has been coated with a reagent, such as glycerol, in an attempt to prevent nonspecific adherence of contaminants. The solid phase is then washed to remove contaminants non-specifically bound to the solid phase. Finally the antibodies of interest is recovered from the solid phase by elution.

Recombinant Production in Eukaryotic Cells

[00251] For eukaryotic expression, the vector components generally include, but are not limited to, one or more of the following, a signal sequence, an origin of replication, one or more marker genes, and enhancer element, a promoter, and a transcription termination sequence.

[00252] A vector for use in a eukaryotic host may also an insert that encodes a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. The heterologous signal sequence selected preferably is one that is recognized and processed (*i.e.*, cleaved by a signal peptidase) by the host cell. In mammalian cell expression, mammalian signal sequences as well as viral secretory leaders, for example, the herpes simplex gD signal, are available. The DNA for such precursor region can be ligated in reading frame to DNA encoding the antibodies of the present application.

[00253] Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

[00254] Expression and cloning vectors may contain a selection gene, also termed a selectable marker. Selection genes may encode proteins that confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, neomycin, methotrexate, or tetracycline; complement auxotrophic deficiencies; or supply critical nutrients not available from complex media.

[00255] One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin, mycophenolic acid and hygromycin.

[00256] Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up nucleic acid encoding the antibodies of the present application. For example, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. An exemplary appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity. Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with the polypeptide encoding-DNA sequences, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3'-phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic.

[00257] Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to the nucleic acid encoding the desired polypeptide sequences. Eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of the transcription of many genes may be included. The 3' end of most eukaryotic may be the signal for addition of the poly A tail to the 3' end of the coding sequence. All of these sequences may be inserted into eukaryotic expression vectors.

[00258] Polypeptide transcription from vectors in mammalian host cells can be controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), from heterologous mammalian promoters, *e.g.*, the actin promoter or an immunoglobulin promoter, from heat-shock promoters, provided such promoters are compatible with the host cell systems.

[00259] Transcription of a DNA encoding the antibodies of the present disclosure by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α -fetoprotein, and insulin). Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. *See also* Yaniv, *Nature* 297:17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the polypeptide encoding sequence, but is preferably located at a site 5' from the promoter.

[00260] Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the polypeptide-encoding mRNA. One useful transcription termination component is the bovine growth hormone polyadenylation region.

[00261] Suitable host cells for cloning or expressing the DNA in the vectors herein include higher eukaryote cells described herein, including vertebrate host cells. Propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub *et al.*, *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); mouse sertoli cells (TM4, Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney

cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TR1 cells (Mather *et al.*, *Annals N.Y. Acad. Sci.* 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

[00262] Host cells can be transformed with the above-described expression or cloning vectors for antibodies production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

[00263] The host cells used to produce the antibodies of the present application may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham *et al.*, *Meth. Enz.* 58:44 (1979), Barnes *et al.*, *Anal. Biochem.* 102:255 (1980), U.S. Pat. No. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Pat. Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as GENTAMYCIN™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

[00264] When using recombinant techniques, the antibodies can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the antibody is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, are removed, for example, by centrifugation or ultrafiltration. Where the antibody is secreted into the

medium, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

[00265] The protein composition prepared from the cells can be purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being the preferred purification technique. The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly (styrene-divinyl) benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSE™ chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered. Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to low pH hydrophobic interaction chromatography.

5.7 Methods of Uses

5.7.1. Therapeutic Methods and Uses

[00266] In another aspect, provided herein are methods for using and uses of the molecules and oligomers provided herein. Such methods and uses include therapeutic methods and uses, for example, involving administration of the molecules or the oligomers, or compositions containing the same, to a subject having a disease or disorder. In some embodiments, the composition is administered in an effective amount to effect treatment of the disease or disorder. Uses include uses of the compositions in such methods and treatments, and in the preparation of a medicament in order to carry out such therapeutic methods. In some embodiments, the methods are carried out by administering the compositions to the subject having or suspected of having the disease or condition. In some embodiments, the methods thereby treat the disease or disorder in the subject.

[00267] In some embodiments, the treatment provided herein cause complete or partial amelioration or reduction of a disease or disorder, or a symptom, adverse effect or outcome, or phenotype associated therewith. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. The terms include, but do not imply, complete curing of a disease or complete elimination of any symptom or effect(s) on all symptoms or outcomes.

[00268] As used herein, in some embodiments, the treatment provided herein delay development of a disease or disorder, e.g., defer, hinder, slow, retard, stabilize, suppress and/or postpone development of the disease (such as cancer). This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease or disorder. For example, a late stage cancer, such as development of metastasis, may be delayed. In other embodiments, the method or the use provided herein prevents a disease or disorder.

[00269] In some embodiments, the present molecules or oligomers are used for treating solid tumor cancer. In other embodiments, the present molecules or oligomers are used for treating blood cancer. In other embodiments, the disease or disorder is an autoimmune and inflammatory disease. In other embodiments, the disease or disorder is an infectious disease.

[00270] In some embodiments, the disease or disorder is a disease of abnormal cell growth and/or dysregulated apoptosis. Examples of such diseases include, but are not limited to, cancer, mesothelioma, bladder cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, ovarian cancer, breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, bone cancer, colon cancer, rectal cancer, cancer of the anal region, stomach cancer, gastrointestinal (gastric, colorectal and/or duodenal) cancer, chronic lymphocytic leukemia, acute lymphocytic leukemia, esophageal cancer, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the

penis, testicular cancer, hepatocellular (hepatic and/or biliary duct) cancer, primary or secondary central nervous system tumor, primary or secondary brain tumor, Hodgkin's disease, chronic or acute leukemia, chronic myeloid leukemia, lymphocytic lymphoma, lymphoblastic leukemia, follicular lymphoma, lymphoid malignancies of T-cell or B-cell origin, melanoma, multiple myeloma, oral cancer, non-small-cell lung cancer, prostate cancer, small-cell lung cancer, cancer of the kidney and/or ureter, renal cell carcinoma, carcinoma of the renal pelvis, neoplasms of the central nervous system, primary central nervous system lymphoma, non-Hodgkin's lymphoma, spinal axis tumors, brain stem glioma, pituitary adenoma, adrenocortical cancer, gall bladder cancer, cancer of the spleen, cholangiocarcinoma, fibrosarcoma, neuroblastoma, retinoblastoma or a combination thereof.

[00271] In some embodiments, the disease or disorder is selected from the group consisting of bladder cancer, brain cancer, breast cancer, bone marrow cancer, cervical cancer, chronic lymphocytic leukemia, acute lymphocytic leukemia, colorectal cancer, esophageal cancer, hepatocellular cancer, lymphoblastic leukemia, follicular lymphoma, lymphoid malignancies of T-cell or B-cell origin, melanoma, myelogenous leukemia, myeloma, oral cancer, ovarian cancer, non-small-cell lung cancer, prostate cancer, small-cell lung cancer and spleen cancer.

[00272] In some embodiments, the disease or disorder is a hematological cancer, such as leukemia, lymphoma, or myeloma. In some embodiments, the cancer is selected from a group consisting of Hodgkin's lymphoma, non-Hodgkin's lymphoma (NHL), cutaneous B-cell lymphoma, activated B-cell lymphoma, diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), follicular center lymphoma, transformed lymphoma, lymphocytic lymphoma of intermediate differentiation, intermediate lymphocytic lymphoma (ILL), diffuse poorly differentiated lymphocytic lymphoma (PDL), centrocytic lymphoma, diffuse small-cleaved cell lymphoma (DSCCL), peripheral T-cell lymphomas (PTCL), cutaneous T-Cell lymphoma, mantle zone lymphoma, low grade follicular lymphoma, multiple myeloma (MM), chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DLBCL), myelodysplastic syndrome (MDS), acute T cell leukemia, acute myeloid leukemia (AML), acute promyelocytic leukemia, acute myeloblastic leukemia, acute megakaryoblastic leukemia, precursor B acute lymphoblastic leukemia, precursor T acute lymphoblastic leukemia, Burkitt's leukemia (Burkitt's lymphoma),

acute biphenotypic leukemia, chronic myeloid lymphoma, chronic myelogenous leukemia (CML), and chronic monocytic leukemia.

[00273] In other embodiments, the disease or disorder is a solid tumor cancer. In some embodiments, the solid tumor cancer is selected from a group consisting of a carcinoma, an adenocarcinoma, an adrenocortical carcinoma, a colon adenocarcinoma, a colorectal adenocarcinoma, a colorectal carcinoma, a ductal cell carcinoma, a lung carcinoma, a thyroid carcinoma, a nasopharyngeal carcinoma, a melanoma, a non-melanoma skin carcinoma, a liver cancer and a lung cancer.

[00274] In some embodiments, the cancer is an adrenal cancer. In some embodiments, the cancer is an anal cancer. In some embodiments, the cancer is an appendix cancer. In some embodiments, the cancer is a bile duct cancer. In some embodiments, the cancer is a bladder cancer. In some embodiments, the cancer is a bone cancer. In some embodiments, the cancer is a brain cancer. In some embodiments, the cancer is a breast cancer. In some embodiments, the cancer is a cervical cancer. In some embodiments, the cancer is a colorectal cancer. In some embodiments, the cancer is an esophageal cancer. In some embodiments, the cancer is a gallbladder cancer. In some embodiments, the cancer is a gestational trophoblastic. In some embodiments, the cancer is a head and neck cancer. In some embodiments, the cancer is a Hodgkin lymphoma. In some embodiments, the cancer is an intestinal cancer. In some embodiments, the cancer is a kidney cancer. In some embodiments, the cancer is a leukemia. In some embodiments, the cancer is a liver cancer. In some embodiments, the cancer is a lung cancer. In some embodiments, the cancer is a melanoma. In some embodiments, the cancer is a mesothelioma. In some embodiments, the cancer is a multiple myeloma (MM). In some embodiments, the cancer is a neuroendocrine tumor. In some embodiments, the cancer is a non-Hodgkin lymphoma. In some embodiments, the cancer is an oral cancer. In some embodiments, the cancer is an ovarian cancer. In some embodiments, the cancer is a pancreatic cancer. In some embodiments, the cancer is a prostate cancer. In some embodiments, the cancer is a sinus cancer. In some embodiments, the cancer is a skin cancer. In some embodiments, the cancer is a soft tissue sarcoma spinal cancer. In some embodiments, the cancer is a stomach cancer. In some embodiments, the cancer is a testicular cancer. In some embodiments, the cancer is a throat cancer. In some embodiments, the cancer is a thyroid cancer. In some embodiments, the cancer is

a uterine cancer endometrial cancer. In some embodiments, the cancer is a vaginal cancer. In some embodiments, the cancer is a vulvar cancer.

[00275] In some embodiments, the adrenal cancer is an adrenocortical carcinoma (ACC), adrenal cortex cancer, pheochromocytoma, or neuroblastoma. In some embodiments, the anal cancer is a squamous cell carcinoma, cloacogenic carcinoma, adenocarcinoma, basal cell carcinoma, or melanoma. In some embodiments, the appendix cancer is a neuroendocrine tumor (NET), mucinous adenocarcinoma, goblet cell carcinoid, intestinal-type adenocarcinoma, or signet-ring cell adenocarcinoma. In some embodiments, the bile duct cancer is an extrahepatic bile duct cancer, adenocarcinomas, hilar bile duct cancer, perihilar bile duct cancer, distal bile duct cancer, or intrahepatic bile duct cancer. In some embodiments, the bladder cancer is transitional cell carcinoma (TCC), papillary carcinoma, flat carcinoma, squamous cell carcinoma, adenocarcinoma, small-cell carcinoma, or sarcoma. In some embodiments, the bone cancer is a primary bone cancer, sarcoma, osteosarcoma, chondrosarcoma, sarcoma, fibrosarcoma, malignant fibrous histiocytoma, giant cell tumor of bone, chordoma, or metastatic bone cancer. In some embodiments, the brain cancer is an astrocytoma, brain stem glioma, glioblastoma, meningioma, ependymoma, oligodendroglioma, mixed glioma, pituitary carcinoma, pituitary adenoma, craniopharyngioma, germ cell tumor, pineal region tumor, medulloblastoma, or primary CNS lymphoma. In some embodiments, the breast cancer is a breast adenocarcinoma, invasive breast cancer, noninvasive breast cancer, breast sarcoma, metaplastic carcinoma, adenocystic carcinoma, phyllodes tumor, angiosarcoma, HER2-positive breast cancer, triple-negative breast cancer, or inflammatory breast cancer. In some embodiments, the cervical cancer is a squamous cell carcinoma, or adenocarcinoma. In some embodiments, the colorectal cancer is a colorectal adenocarcinoma, primary colorectal lymphoma, gastrointestinal stromal tumor, leiomyosarcoma, carcinoid tumor, mucinous adenocarcinoma, signet ring cell adenocarcinoma, gastrointestinal carcinoid tumor, or melanoma. In some embodiments, the esophageal cancer is an adenocarcinoma or squamous cell carcinoma. In some embodiments, the gall bladder cancer is an adenocarcinoma, papillary adenocarcinoma, adenosquamous carcinoma, squamous cell carcinoma, small cell carcinoma, or sarcoma. In some embodiments, the gestational trophoblastic disease (GTD) is a hydatidiform mole, gestational trophoblastic neoplasia (GTN), choriocarcinoma, placental-site trophoblastic

tumor (PSTT), or epithelioid trophoblastic tumor (ETT). In some embodiments, the head and neck cancer is a laryngeal cancer, nasopharyngeal cancer, hypopharyngeal cancer, nasal cavity cancer, paranasal sinus cancer, salivary gland cancer, oral cancer, oropharyngeal cancer, or tonsil cancer. In some embodiments, the Hodgkin lymphoma is a classical Hodgkin lymphoma, nodular sclerosis, mixed cellularity, lymphocyte-rich, lymphocyte-depleted, or nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL). In some embodiments, the intestinal cancer is a small intestine cancer, small bowel cancer, adenocarcinoma, sarcoma, gastrointestinal stromal tumors, carcinoid tumors, or lymphoma. In some embodiments, the kidney cancer is a renal cell carcinoma (RCC), clear cell RCC, papillary RCC, chromophobe RCC, collecting duct RCC, unclassified RCC, transitional cell carcinoma, urothelial cancer, renal pelvis carcinoma, or renal sarcoma. In some embodiments, the leukemia is an acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), hairy cell leukemia (HCL), or a myelodysplastic syndrome (MDS). In a specific embodiment, the leukemia is AML. In some embodiments, the liver cancer is a hepatocellular carcinoma (HCC), fibrolamellar HCC, cholangiocarcinoma, angiosarcoma, or liver metastasis. In some embodiments, the lung cancer is a small cell lung cancer, small cell carcinoma, combined small cell carcinoma, non-small cell lung cancer, lung adenocarcinoma, squamous cell lung cancer, large-cell undifferentiated carcinoma, pulmonary nodule, metastatic lung cancer, adenosquamous carcinoma, large cell neuroendocrine carcinoma, salivary gland-type lung carcinoma, lung carcinoid, mesothelioma, sarcomatoid carcinoma of the lung, or malignant granular cell lung tumor. In some embodiments, the melanoma is a superficial spreading melanoma, nodular melanoma, acral-lentiginous melanoma, lentigo maligna melanoma, amelanotic melanoma, desmoplastic melanoma, ocular melanoma, or metastatic melanoma. In some embodiments, the mesothelioma is a pleural mesothelioma, peritoneal mesothelioma, pericardial mesothelioma, or testicular mesothelioma. In some embodiments, the multiple myeloma is an active myeloma or smoldering myeloma. In some embodiments, the neuroendocrine tumor is a gastrointestinal neuroendocrine tumor, pancreatic neuroendocrine tumor, or lung neuroendocrine tumor. In some embodiments, the non-Hodgkin's lymphoma is an anaplastic large-cell lymphoma, lymphoblastic lymphoma, peripheral T cell lymphoma, follicular lymphoma, cutaneous T cell lymphoma, lymphoplasmacytic lymphoma, marginal zone

B-cell lymphoma, MALT lymphoma, small-cell lymphocytic lymphoma, Burkitt lymphoma, chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), precursor T-lymphoblastic leukemia/lymphoma, acute lymphocytic leukemia (ALL), adult T cell lymphoma/leukemia (ATLL), hairy cell leukemia, B-cell lymphomas, diffuse large B-cell lymphoma (DLBCL), primary mediastinal B-cell lymphoma, primary central nervous system (CNS) lymphoma, mantle cell lymphoma (MCL), marginal zone lymphomas, mucosa-associated lymphoid tissue (MALT) lymphoma, nodal marginal zone B-cell lymphoma, splenic marginal zone B-cell lymphoma, lymphoplasmacytic lymphoma, B-cell non-Hodgkin lymphoma, T cell non-Hodgkin lymphoma, natural killer cell lymphoma, cutaneous T cell lymphoma, Alibert-Bazin syndrome, Sezary syndrome, primary cutaneous anaplastic large-cell lymphoma, peripheral T cell lymphoma, angioimmunoblastic T cell lymphoma (AITL), anaplastic large-cell lymphoma (ALCL), systemic ALCL, enteropathy-type T cell lymphoma (EATL), or hepatosplenic gamma/delta T cell lymphoma. In some embodiments, the oral cancer is a squamous cell carcinoma, verrucous carcinoma, minor salivary gland carcinomas, lymphoma, benign oral cavity tumor, eosinophilic granuloma, fibroma, granular cell tumor, keratoacanthoma, leiomyoma, osteochondroma, lipoma, schwannoma, neurofibroma, papilloma, condyloma acuminatum, verruciform xanthoma, pyogenic granuloma, rhabdomyoma, odontogenic tumors, leukoplakia, erythroplakia, squamous cell lip cancer, basal cell lip cancer, mouth cancer, gum cancer, or tongue cancer. In some embodiments, the ovarian cancer is a ovarian epithelial cancer, mucinous epithelial ovarian cancer, endometrioid epithelial ovarian cancer, clear cell epithelial ovarian cancer, undifferentiated epithelial ovarian cancer, ovarian low malignant potential tumors, primary peritoneal carcinoma, fallopian tube cancer, germ cell tumors, teratoma, dysgerminoma ovarian germ cell cancer, endodermal sinus tumor, sex cord-stromal tumors, sex cord-gonadal stromal tumor, ovarian stromal tumor, granulosa cell tumor, granulosa-theca tumor, Sertoli-Leydig tumor, ovarian sarcoma, ovarian carcinosarcoma, ovarian adenosarcoma, ovarian leiomyosarcoma, ovarian fibrosarcoma, Krukenberg tumor, or ovarian cyst. In some embodiments, the pancreatic cancer is a pancreatic exocrine gland cancer, pancreatic endocrine gland cancer, or pancreatic adenocarcinoma, islet cell tumor, or neuroendocrine tumor. In some embodiments, the prostate cancer is a prostate adenocarcinoma, prostate sarcoma, transitional cell carcinoma, small cell carcinoma, or neuroendocrine tumor. In

some embodiments, the sinus cancer is a squamous cell carcinoma, mucosa cell carcinoma, adenoid cystic cell carcinoma, acinic cell carcinoma, sinonasal undifferentiated carcinoma, nasal cavity cancer, paranasal sinus cancer, maxillary sinus cancer, ethmoid sinus cancer, or nasopharynx cancer. In some embodiments, the skin cancer is a basal cell carcinoma, squamous cell carcinoma, melanoma, Merkel cell carcinoma, Kaposi sarcoma (KS), actinic keratosis, skin lymphoma, or keratoacanthoma. In some embodiments, the soft tissue cancer is an angiosarcoma, dermatofibrosarcoma, epithelioid sarcoma, Ewing's sarcoma, fibrosarcoma, gastrointestinal stromal tumors (GISTs), Kaposi sarcoma, leiomyosarcoma, liposarcoma, dedifferentiated liposarcoma (DL), myxoid/round cell liposarcoma (MRCL), well-differentiated liposarcoma (WDL), malignant fibrous histiocytoma, neurofibrosarcoma, rhabdomyosarcoma (RMS), or synovial sarcoma. In some embodiments, the spinal cancer is a spinal metastatic tumor. In some embodiments, the stomach cancer is a stomach adenocarcinoma, stomach lymphoma, gastrointestinal stromal tumors, carcinoid tumor, gastric carcinoid tumors, Type I ECL-cell carcinoid, Type II ECL-cell carcinoid, or Type III ECL-cell carcinoid. In some embodiments, the testicular cancer is a seminoma, non-seminoma, embryonal carcinoma, yolk sac carcinoma, choriocarcinoma, teratoma, gonadal stromal tumor, leydig cell tumor, or sertoli cell tumor. In some embodiments, the throat cancer is a squamous cell carcinoma, adenocarcinoma, sarcoma, laryngeal cancer, pharyngeal cancer, nasopharynx cancer, oropharynx cancer, hypopharynx cancer, laryngeal cancer, laryngeal squamous cell carcinoma, laryngeal adenocarcinoma, lymphoepithelioma, spindle cell carcinoma, verrucous cancer, undifferentiated carcinoma, or lymph node cancer. In some embodiments, the thyroid cancer is a papillary carcinoma, follicular carcinoma, Hürthle cell carcinoma, medullary thyroid carcinoma, or anaplastic carcinoma. In some embodiments, the uterine cancer is an endometrial cancer, endometrial adenocarcinoma, endometroid carcinoma, serous adenocarcinoma, adenosquamous carcinoma, uterine carcinosarcoma, uterine sarcoma, uterine leiomyosarcoma, endometrial stromal sarcoma, or undifferentiated sarcoma. In some embodiments, the vaginal cancer is a squamous cell carcinoma, adenocarcinoma, melanoma, or sarcoma. In some embodiments, the vulvar cancer is a squamous cell carcinoma or adenocarcinoma.

[00276] In some embodiments, the disease or disorder is caused by a pathogen. In some embodiments, the pathogen causes an infectious disease selected from the group consisting of an

Acute Flaccid Myelitis (AFM), Anaplasmosis, Anthrax, Babesiosis, Botulism, Brucellosis, Campylobacteriosis, Carbapenem-resistant Infection, Chancroid, Chikungunya Virus Infection, Chlamydia, Ciguatera, Difficile Infection, Perfringens, Coccidioidomycosis fungal infection, coronavirus infection, Covid-19 (SARS-CoV-2), Creutzfeldt-Jacob Disease/transmissible spongiform encephalopathy, Cryptosporidiosis (Crypto), Cyclosporiasis, Dengue 1,2,3 or 4, Diphtheria, E. coli infection/Shiga toxin-producing (STEC), Eastern Equine Encephalitis, Hemorrhagic Fever (Ebola), Ehrlichiosis, Encephalitis, Arboviral or parainfectious, Non-Polio Enterovirus, D68 Enterovirus (EV-D68), Giardiasis, Glanders, Gonococcal Infection, Granuloma inguinale, Haemophilus Influenza disease Type B (Hib or H-flu), Hantavirus Pulmonary Syndrome (HPS), Hemolytic Uremic Syndrome (HUS), Hepatitis A (Hep A), Hepatitis B (Hep B), Hepatitis C (Hep C), Hepatitis D (Hep D), Hepatitis E (Hep E), Herpes, Herpes Zoster (Shingles), Histoplasmosis infection, Human Immunodeficiency Virus/AIDS (HIV/AIDS), Human Papillomavirus (HPV), Influenza (Flu), Legionellosis (Legionnaires Disease), Leprosy (Hansen's Disease), Leptospirosis, Listeriosis (Listeria), Lyme Disease, Lymphogranuloma venereum infection (LGV), Malaria, Measles, Melioidosis, Meningitis (Viral), Meningococcal Disease (Meningitis (Bacterial)), Middle East Respiratory Syndrome Coronavirus (MERS-CoV), Mumps, Norovirus, Pediculosis, Pelvic Inflammatory Disease (PID), Pertussis (Whooping Cough), Plague (Bubonic, Septicemic, Pneumonic), Pneumococcal Disease (Pneumonia), Poliomyelitis (Polio), Powassan, Psittacosis, Pthiriasis, Pustular Rash diseases (Small pox, monkeypox, cowpox), Q-Fever, Rabies, Rickettsiosis (Rocky Mountain Spotted Fever), Rubella (German Measles), Salmonellosis gastroenteritis (Salmonella), Scabies, Scombroid, Sepsis, Severe Acute Respiratory Syndrome (SARS), Shigellosis gastroenteritis (Shigella), Smallpox, Staphylococcal Infection Methicillin-resistant (MRSA), Staphylococcal Food Poisoning Enterotoxin B Poisoning (Staph Food Poisoning), Staphylococcal Infection Vancomycin Intermediate (VISA), Staphylococcal Infection Vancomycin Resistant (VRSA), Streptococcal Disease Group A (invasive) (Strep A (invasive), Streptococcal Disease, Group B (Strep-B), Streptococcal Toxic-Shock Syndrome STSS Toxic Shock, Syphilis (primary, secondary, early latent, late latent, congenital), Tetanus Infection, Trichomoniasis, Trichonosis Infection, Tuberculosis (TB), Tuberculosis Latent (LTBI), Tularemia, Typhoid Fever Group D, Vaginosis, Varicella (Chickenpox), Vibrio cholerae (Cholera), Vibriosis (Vibrio), Ebola Virus Hemorrhagic

Fever, Lassa Virus Hemorrhagic Fever, Marburg Virus Hemorrhagic Fever, West Nile Virus, Yellow Fever, Yersenia, and Zika Virus Infection.

[00277] In some embodiments, the pathogen is a bacteria. In some embodiments, the bacteria is a bacteria of a bacillus, bartonella, bordetella, borrelia, brucella, campylobacter, chlamydia, chlamydophila, clostridium, corynebacterium, enterococcus, escherichia, francisella, haemophilus, helicobacter, legionella, leptospira, listeria, mycobacterium, mycoplasma, neisseria, pseudomonas, rickettsia, salmonella, shigella, staphylococcus, streptococcus, treponema, ureaplasma, vibrio or yersinia genus.

[00278] In some embodiments, the pathogen is a parasite. In some embodiments, the parasite is a protozoa, helminth, or ectoparasite. In some embodiments, the protozoa is an entamoeba, giardia, leishmania, balantidium, plasmodium, or cryptosporidium. In some embodiments, the helminth is a trematode, cestode, acanthocephalan, or round worm. In some embodiments, the ectoparasite is an arthropod.

[00279] In some embodiments, the pathogen is a virus. In some embodiments, the virus is a virus of the adenoviridae, arenaviridae, astroviridae, bunyaviridae, caliciviridae, coronaviridae, filoviridae, flaviviridae, hepadnaviridae, hepeviridae, orthomyxoviridae, papillomaviridae, paramyxoviridae, parvoviridae, picornaviridae, polyomaviridae, poxviridae, reoviridae, retroviridae, rhabdoviridae, or togaviridae family.

[00280] In some embodiments, the virus is an adenovirus, coronavirus, coxsackievirus, Epstein-Barr virus, hepatitis A virus, hepatitis B virus, hepatitis C virus, herpes simplex virus type 2, cytomegalovirus, human herpes virus type 8, human immunodeficiency virus, influenza virus, measles virus, mumps virus, human papillomavirus, parainfluenza virus, poliovirus, rabies virus, respiratory syncytial virus, rubella virus, or varicella-zoster virus.

[00281] In other embodiments, the disease or disorder is an immune or autoimmune disorder. Such disorders include autoimmune bullous disease, abetalipoproteinemia, acquired immunodeficiency-related diseases, acute immune disease associated with organ transplantation, acquired acrocyanosis, acute and chronic parasitic or infectious processes, acute pancreatitis, acute renal failure, acute rheumatic fever, acute transverse myelitis, adenocarcinomas, aerial ectopic beats, adult (acute) respiratory distress syndrome, AIDS dementia complex, alcoholic cirrhosis, alcohol-induced liver injury, alcohol-induced hepatitis, allergic conjunctivitis, allergic

contact dermatitis, allergic rhinitis, allergy and asthma, allograft rejection, alpha-1-antitrypsin deficiency, Alzheimer's disease, amyotrophic lateral sclerosis, anemia, angina pectoris, ankylosing spondylitis-associated lung disease, anterior horn cell degeneration, antibody mediated cytotoxicity, antiphospholipid syndrome, anti-receptor hypersensitivity reactions, aortic and peripheral aneurysms, aortic dissection, arterial hypertension, arteriosclerosis, arteriovenous fistula, arthropathy, asthenia, asthma, ataxia, atopic allergy, atrial fibrillation (sustained or paroxysmal), atrial flutter, atrioventricular block, atrophic autoimmune hypothyroidism, autoimmune haemolytic anaemia, autoimmune hepatitis, type-1 autoimmune hepatitis (classical autoimmune or lupoid hepatitis), autoimmune mediated hypoglycemia, autoimmune neutropenia, autoimmune thrombocytopenia, autoimmune thyroid disease, B-cell lymphoma, bone graft rejection, bone marrow transplant (BMT) rejection, bronchiolitis obliterans, bundle branch block, burns, cachexia, cardiac arrhythmias, cardiac stun syndrome, cardiac tumors, cardiomyopathy, cardiopulmonary bypass inflammation response, cartilage transplant rejection, cerebellar cortical degenerations, cerebellar disorders, chaotic or multifocal atrial tachycardia, chemotherapy-associated disorders, chlamydia, cholestatitis, chronic alcoholism, chronic active hepatitis, chronic fatigue syndrome, chronic immune disease associated with organ transplantation, chronic eosinophilic pneumonia, chronic inflammatory pathologies, chronic mucocutaneous candidiasis, chronic obstructive pulmonary disease (COPD), chronic salicylate intoxication, colorectal common variable immunodeficiency (common variable hypogammaglobulinemia), conjunctivitis, connective tissue disease-associated interstitial lung disease, contact dermatitis, Coombs-positive hemolytic anemia, cor pulmonale, Creutzfeldt-Jakob disease, cryptogenic autoimmune hepatitis, cryptogenic fibrosing alveolitis, culture-negative sepsis, cystic fibrosis, cytokine therapy-associated disorders, Crohn's disease, dementia pugilistica, demyelinating diseases, dengue hemorrhagic fever, dermatitis, dermatitis scleroderma, dermatologic conditions, dermatomyositis/ polymyositis-associated lung disease, diabetes, diabetic arteriosclerotic disease, diabetes mellitus, diffuse Lewy body disease, dilated cardiomyopathy, dilated congestive cardiomyopathy, discoid lupus erythematosus, disorders of the basal ganglia, disseminated intravascular coagulation, Down's Syndrome in middle age, drug-induced interstitial lung disease, drug-induced hepatitis, drug-induced movement disorders induced by drugs which block CNS dopamine receptors, drug sensitivity, eczema, encephalomyelitis,

endocarditis, endocrinopathy, enteropathic synovitis, epiglottitis, Epstein-Barr virus infection, erythromelalgia, extrapyramidal and cerebellar disorders, familial hemaphagocytic lymphohistiocytosis, fetal thymus implant rejection, Friedreich's ataxia, functional peripheral arterial disorders, female infertility, fibrosis, fibrotic lung disease, fungal sepsis, gas gangrene, gastric ulcer, giant cell arteritis, glomerular nephritis, glomerulonephritides, Goodpasture's syndrome, goitrous autoimmune hypothyroidism (Hashimoto's disease), gouty arthritis, graft rejection of any organ or tissue, graft versus host disease, gram-negative sepsis, gram-positive sepsis, granulomas due to intracellular organisms, group B streptococci (GBS) infection, Graves' disease, hemosiderosis-associated lung disease, hairy cell leukemia, Hallerorden- Spatz disease, Hashimoto's thyroiditis, hay fever, heart transplant rejection, hemachromatosis, hematopoietic malignancies (leukemia and lymphoma), hemolytic anemia, hemolytic uremic syndrome/thrombolytic thrombocytopenic purpura, hemorrhage, Henoch-Schoenlein purpura, hepatitis A, hepatitis B, hepatitis C, HIV infection/HIV neuropathy, Hodgkin's disease, hypoparathyroidism, Huntington's chorea, hyperkinetic movement disorders, hypersensitivity reactions, hypersensitivity pneumonitis, hyperthyroidism, hypokinetic movement disorders, hypothalamic-pituitary-adrenal axis evaluation, idiopathic Addison's disease, idiopathic leucopenia, idiopathic pulmonary fibrosis, idiopathic thrombocytopenia, idiosyncratic liver disease, infantile spinal muscular atrophy, infectious diseases, inflammation of the aorta, inflammatory bowel disease, insulin dependent diabetes mellitus, interstitial pneumonitis, iridocyclitis/uveitis/optic neuritis, ischemia-reperfusion injury, ischemic stroke, juvenile pernicious anemia, juvenile rheumatoid arthritis, juvenile spinal muscular atrophy, Kaposi's sarcoma, Kawasaki's disease, kidney transplant rejection, legionella, leishmaniasis, leprosy, lesions of the corticospinal system, linear IgA disease, lipidema, liver transplant rejection, Lyme disease, lymphoderma, lymphocytic infiltrative lung disease, malaria, male infertility idiopathic or NOS, malignant histiocytosis, malignant melanoma, meningitis, meningococcemia, microscopic vasculitis of the kidneys, migraine headache, mitochondrial multisystem disorder, mixed connective tissue disease, mixed connective tissue disease- associated lung disease, monoclonal gammopathy, multiple myeloma, multiple systems degenerations (Mencel, Dejerine-Thomas, Shy-Drager and Machado-Joseph), myalgic encephalitis/Royal Free Disease, myasthenia gravis, microscopic vasculitis of the kidneys, mycobacterium avium intracellulare,

mycobacterium tuberculosis, myelodysplastic syndrome, myocardial infarction, myocardial ischemic disorders, nasopharyngeal carcinoma, neonatal chronic lung disease, nephritis, nephrosis, nephrotic syndrome, neurodegenerative diseases, neurogenic I muscular atrophies, neutropenic fever, non-alcoholic steatohepatitis, occlusion of the abdominal aorta and its branches, occlusive arterial disorders, organ transplant rejection, orchitis/epididymitis, orchitis/vasectomy reversal procedures, organomegaly, osteoarthritis, osteoporosis, ovarian failure, pancreas transplant rejection, parasitic diseases, parathyroid transplant rejection, Parkinson's disease, pelvic inflammatory disease, pemphigus vulgaris, pemphigus foliaceus, pemphigoid, perennial rhinitis, pericardial disease, peripheral atherosclerotic disease, peripheral vascular disorders, peritonitis, pernicious anemia, phacogenic uveitis, Pneumocystis carinii pneumonia, pneumonia, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes syndrome), post-perfusion syndrome, post-pump syndrome, post-MI cardiomyopathy syndrome, postinfectious interstitial lung disease, premature ovarian failure, primary biliary cirrhosis, primary sclerosing hepatitis, primary myxoedema, primary pulmonary hypertension, primary sclerosing cholangitis, primary vasculitis, progressive supranuclear palsy, psoriasis, psoriasis type 1, psoriasis type 2, psoriatic arthropathy, pulmonary hypertension secondary to connective tissue disease, pulmonary manifestation of polyarteritis nodosa, post-inflammatory interstitial lung disease, radiation fibrosis, radiation therapy, Raynaud's phenomenon and disease, Raynaud's disease, Refsum's disease, regular narrow QRS tachycardia, Reiter's disease, renal disease NOS, renovascular hypertension, reperfusion injury, restrictive cardiomyopathy, rheumatoid arthritis-associated interstitial lung disease, rheumatoid spondylitis, sarcoidosis, Schmidt's syndrome, scleroderma, senile chorea, senile dementia of Lewy body type, sepsis syndrome, septic shock, seronegative arthropathies, shock, sickle cell anemia, T-cell or FAB ALL, Takayasu's disease/arteritis, telangiectasia, Th2-type and Th1-type mediated diseases, thromboangitis obliterans, thrombocytopenia, thyroiditis, toxicity, toxic shock syndrome, transplants, trauma/hemorrhage, type-2 autoimmune hepatitis (anti-LKM antibody hepatitis), type B insulin resistance with acanthosis nigricans, type III hypersensitivity reactions, type IV hypersensitivity, ulcerative colitic arthropathy, ulcerative colitis, unstable angina, uremia, urosepsis, urticaria, uveitis, valvular heart diseases, varicose veins, vasculitis, vasculitic diffuse lung disease, venous diseases, venous thrombosis, ventricular fibrillation, vitiligo acute

liver disease, viral and fungal infections, viral encephalitis/aseptic meningitis, vital- associated hemaphagocytic syndrome, Wegener's granulomatosis, Wernicke-Korsakoff syndrome, Wilson's disease, xenograft rejection of any organ or tissue, yersinia and salmonella-associated arthropathy, acquired immunodeficiency disease syndrome (AIDS), autoimmune lymphoproliferative syndrome, hemolytic anemia, inflammatory diseases, thrombocytopenia, acute and chronic immune diseases associated with organ transplantation, Addison's disease, allergic diseases, alopecia, alopecia areata, atheromatous disease/arteriosclerosis, atherosclerosis, arthritis (including osteoarthritis, juvenile chronic arthritis, septic arthritis, Lyme arthritis, psoriatic arthritis and reactive arthritis), Sjogren's disease-associated lung disease, Sjogren's syndrome, skin allograft rejection, skin changes syndrome, small bowel transplant rejection, sperm autoimmunity, multiple sclerosis (all subtypes), spinal ataxia, spinocerebellar degenerations, spondyloarthropathy, sporadic polyglandular deficiency type I, sporadic polyglandular deficiency type II, Still's disease, streptococcal myositis, stroke, structural lesions of the cerebellum, subacute sclerosing panencephalitis, sympathetic ophthalmia, syncope, syphilis of the cardiovascular system, systemic anaphylaxis, systemic inflammatory response syndrome, systemic onset juvenile rheumatoid arthritis, systemic lupus erythematosus, systemic lupus erythematosus-associated lung disease, lupus nephritis, systemic sclerosis, and systemic sclerosis-associated interstitial lung disease.

[00282] In some embodiments, the disease or disorder is an inflammatory disease.

Inflammation plays a fundamental role in host defenses and the progression of immune-mediated diseases. The inflammatory response is initiated in response to injury (*e.g.*, trauma, ischemia, and foreign particles) and infection (*e.g.*, bacterial or viral infection) by a complex cascade of events, including chemical mediators (*e.g.*, cytokines and prostaglandins) and inflammatory cells (*e.g.*, leukocytes). The inflammatory response is characterized by increased blood flow, increased capillary permeability, and the influx of phagocytic cells. These events result in swelling, redness, warmth (altered heat patterns), and pus formation at the site of injury or infection.

[00283] Cytokines and prostaglandins control the inflammatory response, and are released in an ordered and self-limiting cascade into the blood or affected tissues. This release of cytokines and prostaglandins increases the blood flow to the area of injury or infection, and may result in

redness and warmth. Some of these chemicals cause a leak of fluid into the tissues, resulting in swelling. This protective process may stimulate nerves and cause pain. These changes, when occurring for a limited period in the relevant area, work to the benefit of the body. A delicate well-balanced interplay between the humoral and cellular immune elements in the inflammatory response enables the elimination of harmful agents and the initiation of the repair of damaged tissue. When this delicately balanced interplay is disrupted, the inflammatory response may result in considerable damage to normal tissue and may be more harmful than the original insult that initiated the reaction. In these cases of uncontrolled inflammatory responses, clinical intervention is needed to prevent tissue damage and organ dysfunction. Diseases such as psoriasis, rheumatoid arthritis, osteoarthritis, psoriatic arthritis, Crohn's disease, asthma, allergies or inflammatory bowel disease, are characterized by chronic inflammation. Inflammatory diseases such as arthritis, related arthritic conditions (e.g., osteoarthritis, rheumatoid arthritis, and psoriatic arthritis), inflammatory bowel disease (e.g., Crohn's disease and ulcerative colitis), sepsis, psoriasis, atopic dermatitis, contact dermatitis, and chronic obstructive pulmonary disease, chronic inflammatory pulmonary diseases are also prevalent and problematic ailments.

5.7.2. Diagnostic and Detection Methods and Uses

[00284] In another aspect, provided herein are methods involving use of the binding molecules provided herein for detection, prognosis, diagnosis, staging, determining binding of a particular treatment to one or more tissues or cell types, and/or informing treatment decisions in a subject, such as by the detection of an antigen and/or the presence of an epitope thereof recognized by the antibody.

[00285] In some embodiments, a sample, such as a cell, tissue sample, lysate, composition, or other sample derived therefrom is contacted with a binding molecule provided herein and the binding is determined or detected. When binding in the test sample is demonstrated or detected as compared to a reference cell of the same tissue type, it may indicate the presence of an associated disease or disorder, and/or that a therapeutic containing the antibody will specifically bind to a tissue or cell that is the same as or is of the same type as the tissue or cell or other biological material from which the sample is derived. In some embodiments, the sample is from human tissues and may be from diseased and/or normal tissue, *e.g.*, from a subject having the

disease or disorder to be treated and/or from a subject of the same species as such subject but that does not have the disease or disorder to be treated. In some cases, the normal tissue or cell is from a subject having the disease or disorder to be treated but is not itself a diseased cell or tissue, such as a normal tissue from the same or a different organ than a cancer that is present in a given subject.

[00286] Various methods known in the art for detecting specific antibody-antigen binding can be used. Exemplary immunoassays include fluorescence polarization immunoassay (FPIA), fluorescence immunoassay (FIA), enzyme immunoassay (EIA), nephelometric inhibition immunoassay (NIA), enzyme linked immunosorbent assay (ELISA), and radioimmunoassay (RIA). An indicator moiety, or label group, can be used so as to meet the needs of various uses of the method which are often dictated by the availability of assay equipment and compatible immunoassay procedures. Exemplary labels include radionuclides (*e.g.* ^{125}I , ^{131}I , ^{35}S , ^3H , or ^{32}P and/or chromium (^{51}Cr), cobalt (^{57}Co), fluorine (^{18}F), gadolinium (^{153}Gd , ^{159}Gd), germanium (^{68}Ge), holmium (^{166}Ho), indium (^{115}In , ^{113}In , ^{112}In , ^{111}In), iodine (^{125}I , ^{123}I , ^{121}I), lanthanum (^{140}La), lutetium (^{177}Lu), manganese (^{54}Mn), molybdenum (^{99}Mo), palladium (^{103}Pd), phosphorous (^{32}P), praseodymium (^{142}Pr), promethium (^{149}Pm), rhenium (^{186}Re , ^{188}Re), rhodium (^{105}Rh), ruthenium (^{97}Ru), samarium (^{153}Sm), scandium (^{47}Sc), selenium (^{75}Se), (^{85}Sr), sulphur (^{35}S), technetium (^{99}Tc), thallium (^{201}Tl), tin (^{113}Sn , ^{117}Sn), tritium (^3H), xenon (^{133}Xe), ytterbium (^{169}Yb , ^{175}Yb), yttrium (^{90}Y), enzymes (*e.g.*, alkaline phosphatase, horseradish peroxidase, luciferase, or β -galactosidase), fluorescent moieties or proteins (*e.g.*, fluorescein, rhodamine, phycoerythrin, GFP, or BFP), or luminescent moieties (*e.g.*, QdotTM nanoparticles supplied by the Quantum Dot Corporation, Palo Alto, Calif.). Various general techniques to be used in performing the various immunoassays noted above are known.

[00287] In certain embodiments, labeled antibodies are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, *e.g.*, through an enzymatic reaction or molecular interaction. In other embodiments, antibodies are not labeled, and the presence thereof can be detected using a labeled antibody which binds to any of the antibodies.

5.8 Kits and Articles of Manufacture

[00288] Further provided are kits, unit dosages, and articles of manufacture comprising any of the molecules and oligomers described herein. In some embodiments, a kit is provided which contains any one of the pharmaceutical compositions described herein and preferably provides instructions for its use.

[00289] The kits of the present application are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (*e.g.*, sealed Mylar or plastic bags), and the like. Kits may optionally provide additional components such as buffers and interpretative information. The present application thus also provides articles of manufacture, which include vials (such as sealed vials), bottles, jars, flexible packaging, and the like.

[00290] The article of manufacture can comprise a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, *etc.* The containers may be formed from a variety of materials such as glass or plastic. Generally, the container holds a composition which is effective for treating a disease or disorder (such as cancer) described herein, and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The label or package insert indicates that the composition is used for treating the particular condition in an individual. The label or package insert will further comprise instructions for administering the composition to the individual. The label may indicate directions for reconstitution and/or use. The container holding the pharmaceutical composition may be a multi-use vial, which allows for repeat administrations (*e.g.* from 2-6 administrations) of the reconstituted formulation. Package insert refers to instructions customarily included in commercial packages of therapeutic products that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products. Additionally, the article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[00291] The kits or article of manufacture may include multiple unit doses of the pharmaceutical composition and instructions for use, packaged in quantities sufficient for storage and use in pharmacies, for example, hospital pharmacies and compounding pharmacies.

[00292] For the sake of conciseness, certain abbreviations are used herein. One example is the single letter abbreviation to represent amino acid residues. The amino acids and their corresponding three letter and single letter abbreviations are as follows:

alanine	Ala	(A)
arginine	Arg	(R)
asparagine	Asn	(N)
aspartic acid	Asp	(D)
cysteine	Cys	(C)
glutamic acid	Glu	(E)
glutamine	Gln	(Q)
glycine	Gly	(G)
histidine	His	(H)
isoleucine	Ile	(I)
leucine	Leu	(L)
lysine	Lys	(K)
methionine	Met	(M)
phenylalanine	Phe	(F)
proline	Pro	(P)
serine	Ser	(S)
threonine	Thr	(T)
tryptophan	Trp	(W)
tyrosine	Tyr	(Y)
valine	Val	(V)

[00293] The disclosure is generally disclosed herein using affirmative language to describe the numerous embodiments. The disclosure also specifically includes embodiments in which particular subject matter is excluded, in full or in part, such as substances or materials, method steps and conditions, protocols, procedures, assays or analysis. Thus, even though the disclosure

is generally not expressed herein in terms of what the disclosure does not include, aspects that are not expressly included in the disclosure are nevertheless disclosed herein.

[00294] A number of embodiments of the disclosure have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the disclosure. Accordingly, the following examples are intended to illustrate but not limit the scope of disclosure described in the claims.

6. EXAMPLES

[00295] The following is a description of various methods and materials used in the studies, and are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below were performed and are all of the experiments that may be performed. It is to be understood that exemplary descriptions written in the present tense were not necessarily performed, but rather that the descriptions can be performed to generate the data and the like associated with the teachings of the present invention. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, percentages, etc.), but some experimental errors and deviations should be accounted for.

6.1 Example 1 – Plasmid Construction for huIgG1 Fc- μ tp Variants.

[00296] This example provides the design of plasmid constructs for candidates with different point mutations in the constant region to test for formation of hexamers.

[00297] Constructs were made where each of the following groups of mutations were introduced into the human IgG1 Fc region based on the EU numbering. Ile 253 was substituted with cysteine (I253C). Tyr 436 was substituted with cysteine (Y436C). Gln 438 was substituted with cysteine (Q438C). His 310 was substituted with cysteine (H310C). Leu 251 was substituted with cysteine (L251C), Ile 253 was substituted with glycine (I253G), and Ser 254 was substituted with cysteine (S254C). Ser 254 was substituted with cysteine (S254C) and Asn 434 was substituted with cysteine (N434C). Leu 251 was substituted with cysteine (L251C) and Ser 254 was substituted with cysteine (S254C). Asn 286 was substituted with cysteine (N286C).

[00298] For each of the above constructs, a μ tp (SEQ ID NO: 1) was added to the C-terminus of the CH_3 region. For each of the above constructs, a signal peptide (SEQ ID NO: 2) was added

to the N-terminus of the C_H2 region. The amino acid sequence of huIgG1 Fc- μ tp with I253C mutation is SEQ ID NO: 3. The amino acid sequence of huIgG1 Fc- μ tp with Y436C mutation is SEQ ID NO: 4. The amino acid sequence of huIgG1 Fc- μ tp with Q438C mutation is SEQ ID NO: 5. The amino acid sequence of huIgG1 Fc- μ tp with H310C mutation is SEQ ID NO: 6. The amino acid sequence of huIgG1 Fc- μ tp with L251C, I253G, and S254C mutations is SEQ ID NO: 7. The amino acid sequence of huIgG1 Fc- μ tp with S254C and N434C mutations is SEQ ID NO: 8. The amino acid sequence of huIgG1 Fc- μ tp with L251C and S254C mutations is SEQ ID NO: 9. The amino acid sequence of huIgG1 Fc- μ tp with N286C mutations is SEQ ID NO: 10. The various sequences described above are shown in Table 1 below.

Table 1. Construct Sequences

SEQ ID NO.	Description	Sequence
1	immunoglobulin μ chain tail piece (μ tp)	PTLYNVSLVMSDTAGTCY
2	signal peptide	MDMRVPAQLLGLLLLWLRGARC
3	huIgG1 Fc- μ tp with I253C mutation	MDMRVPAQLLGLLLLWLRGARCCPPCPAPELL GGPSVFLFPPKPKDTLMCSRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDS DGSFFLYSKLTVDKSRWQQGNVFCFSVMHE ALHNHYTQKSLSLSPGKPTLYNVSLVMSDTAGT CY
4	huIgG1 Fc- μ tp with Y436C mutation	MDMRVPAQLLGLLLLWLRGARCCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDS DGSFFLYSKLTVDKSRWQQGNVFCFSVMHE

SEQ ID NO.	Description	Sequence
		ALHNHCTQKSLSLSPGKPTLYNVSLVMSDTAGT CY
5	huIgG1 Fc- μ tp with Q438C mutation	MDMRVPAQLLGLLLLWLRGARCCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDS DGSFFLYSKLTVDKSRWQQGNVFCFSVMHE ALHNHYTCKSLSLSPGKPTLYNVSLVMSDTAGT CY
6	huIgG1 Fc- μ tp with H310C mutation	MDMRVPAQLLGLLLLWLRGARCCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLCQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDS DGSFFLYSKLTVDKSRWQQGNVFCFSVMHE ALHNHYTQKSLSLSPGKPTLYNVSLVMSDTAGT CY
7	huIgG1 Fc- μ tp with L251C, I253G, and S254C mutations	MDMRVPAQLLGLLLLWLRGARCCPPCPAPELL GGPSVFLFPPKPKDTCMGCRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDS DGSFFLYSKLTVDKSRWQQGNVFCFSVMHE ALHNHYTQKSLSLSPGKPTLYNVSLVMSDTAGT CY
8	huIgG1 Fc- μ tp with S254C and N434C mutations	MDMRVPAQLLGLLLLWLRGARCCPPCPAPELL GGPSVFLFPPKPKDTLMICRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPV L

SEQ ID NO.	Description	Sequence
		DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHCHYTQKSLSLSPGKPTLYNVSLVMSDTAGT CY
9	huIgG1 Fc- μ tp with L251C and S254C mutations	MDMRVPAQLLGLLLLWLRGARCCPPCPAPELL GGPSVFLFPPKPKDTCMICRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPV DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGKPTLYNVSLVMSDTAGT CY
10	huIgG1 Fc- μ tp with N286C mutations	MDMRVPAQLLGLLLLWLRGARCCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHCAKTKPREEQYNSTYR VVS LTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGKPTLYNVSLVMSDTAGTC Y

[00299] Gene cloning, site mutation, and plasmid construction were carried out with standard molecular biology techniques well known in the art (*see, e.g.*, Sambrook and Russel, Molecular Cloning, A Laboratory Manual, 3rd ed., 2001, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; Kostelny et al., Int. J. Cancer 93:556-565, 2001; Cole et al., J. Immunol. 159:3613-3621, 1997 and Tsurushita et al., Methods 36:69-83, 2005.). In particular, the amino acid sequences of the huIgG1 Fc- μ tp variants were codon optimized for mammalian expression. The codon optimized DNA was synthesized and sub-cloned into mammalian expression vector pTT5.

6.2 Example 2 – Production, Purification, and Characterization of Hexamers of huIgG1 Fc- μ tp Variants.

[00300] The various huIgG1 Fc- μ tp constructs were transiently expressed using the Expi293 expression system (Thermo Fisher Scientific). Transfection was done following manufacturer's protocol. The transfected cell culture was incubated at 37°C, 5% CO₂ for 4 days. 10 μ L of conditioned medium from each of huIgG1 Fc- μ tp constructs and controls was mixed with 3.3 μ L 4X Laemmli buffer and was heated at 95°C for 5 min. NuPAGE™ 3-8%, Tris-Acetate protein gel was prepared, and used to visualize the transient expression of target proteins in the conditioned medium (*see* **FIG. 1**).

[00301] As shown in **FIG. 1A**, wild type huIgG1- μ tp appeared mainly as monomeric form (54kDa, lane 12). Surprisingly, strong hexamer formation was observed for huIgG1 Fc (I253C)- μ tp (324kDa, lane 6). However, the majority of huIgG1 Fc (Q438C)- μ tp and huIgG1 Fc (Y436C)- μ tp were expressed as a monomer (lane 8 and 10). As shown in **FIG. 1B**, huIgG1 Fc (H310C)- μ tp, huIgG1 Fc (L251C, I253G, and S254C)- μ tp, huIgG1 Fc (S254C and N434C)- μ tp, and huIgG1 Fc (L251C and S254C)- μ tp also were expressed as a monomer (lanes 5, 6, 7, and 8). As shown in **FIG. 1C**, huIgG1 Fc (N286C)- μ tp was expressed as monomer (lanes 5). Therefore, huIgG1 Fc (I253C)- μ tp was used for further purification and analysis.

[00302] Purification of huIgG1 Fc (I253C)- μ tp was initiated manually by affinity pulldown using MabSelect™ protein A resin and CaptureSelect™ FcXL Affinity Matrix (Thermo Fisher Scientific) side by side. 200 μ L of protein A and FcXL resin were packed in small spin columns. Culture media with the expressed proteins were applied to the column by pipetting. After binding, the flow-through, wash and elution processes were performed by centrifugation at 700 g for 0.5 min per spin. Based on manufacturer's recommendation, Buffer 1 (50 mM sodium acetate, pH 3.5) was used for protein A elution while Buffer 2 (20 mM acetic acid, 100 mM glycine, pH 3.5) was used for FcXL elution. PBS buffer (pH 7.5) was used as the wash buffer for both resins. Each elution fraction was at a volume of 200 μ L and neutralized immediately after elution. To analyze the purification by electrophoresis, 20 μ L of sample was mixed with 7 μ L 4X Laemmli buffer (non-reducing), and heat denatured at 95°C for 5 min. 4-20% Criterion™ TGX Stain-Free™ Protein Gel was used to resolve and visualize the purification products. Hexamers of huIgG1 Fc (I253C)- μ tp was not detected in any elution fraction of protein A resin (*see* lanes E1-E4 of **FIG. 2A**) but existed in the flow-through fraction (data not shown). In contrast, hexamers of huIgG1 Fc (I253C)- μ tp was detected in the elution fraction of FcXL resin

(see lane E1 of **FIG. 2B**). Such observation suggests that huIgG1 Fc (I253C)- μ tp completely lost binding to protein A, which requires C_H2 interaction. huIgG1 Fc (I253C)- μ tp can bind to FcXL resin via C_H3 interaction.

[00303] Alternative to the affinity purification method described above, huIgG1 Fc (I253C)- μ tp was captured and purified using diafiltration followed by anion exchange chromatography (AIEX). Such an example is described in detail below. 5 ml culture media with the expressed protein was filtered and buffer exchanged to Buffer A (50 mM Tris, 50 mM NaCl, pH 8.0) via diafiltration using a 30 kDa cutoff concentrator (MilliporeSigma™ Amicon™ Ultra). The protein was then loaded to a 1 ml HiTrap™ Q XL column (Cytiva) on a ÄKTA Avant FPLC system at a flow rate of 1 ml/min. The column with bound protein was washed by 10 column volume (CV) of Buffer A and eluted using a liner gradient from 100% Buffer A towards 100% Buffer B with high salt (50 mM Tris, 500 mM NaCl, pH 8.0). The elution fractions were collected at 250 μ l volume. Hexameric huIgG1 Fc (I253C)- μ tp exhibited strong binding to Q XL resin and eluted at a gradient of 75% Buffer B or higher (note that 25% Buffer A/75% Buffer B contains 370 mM NaCl, and the conductivity was equivalent to 18.45 mS/cm). Elution fractions were analyzed by electrophoresis using a 4-20% Criterion™ TGX Stain-Free™ Protein Gel as shown in **FIG. 2C**. The pooled fractions (right to the marker lane in **FIG. 2C**) were buffer exchanged to standard HBS buffer for further characterization.

The purified huIgG1 Fc (I253C)- μ tp hexamer was subjected to characterization by Mass spectrometry, HPLC-SEC and nano DSF. The precise molecular weight of huIgG1 Fc (I253C)- μ tp single chain (the only sub-unit of the homo-hexamer) was measured by intact mass spectrometry after de-glycosylation and DTT reduction. Upon the complete removal of N-glycosylation and reducing of disulfide bonds, a single huIgG1 Fc (I253C)- μ tp chain has a detected molecular weight of 26905.70 Da, as shown in **FIG. 3A**, which matches the calculated molar mass of a single chain without post translational modification (26904.50 Da). The purified huIgG1 Fc (I253C)- μ tp hexamer was also analyzed under non-denaturing conditions by HPLC-SEC (Agilent). 15 μ l of huIgG1 Fc (I253C)- μ tp (1 mg/ml) was injected to a TSKgel G3000SW column (TOSOH) for a 30-minute run at a flow rate of 0.5 ml/min using 2X PBS as the mobile phase. BioRad gel filtration standard was used before and after the sample runs. As shown in **FIG. 3B**, the retention time of bovine thyroglobulin (670 kDa) was 13 minutes, the retention

time of bovine γ -globulin (158 kDa) was 18 minutes, the retention time of chicken ovalbumin (44 kDa) was 20 minutes. As confirmed by intact mass spectrometry, huIgG1 Fc (I253C)- μ tp monomer comprised 2 Fc chains and had an estimated size of 54 kDa, while the hexamer comprised 12 Fc chains and had a size of 324 kDa. It is unambiguous that on the HPLC-SEC chromatogram (**FIG. 3B and 3C**), huIgG1 Fc (I253C)- μ tp hexamer had a retention time of 15 minutes and the monomer had a retention time of 19 minutes, while the higher oligomer had a retention time of 13 minutes. Quantitative analysis of the HPLC-SEC (**FIG. 3C**) showed that 94.2% of huIgG1 Fc (I253C)- μ tp formed hexamers (from a one-step AIEX). To evaluate the thermal stability of huIgG1 Fc (I253C)- μ tp hexamer, 50 μ l of the protein (1 mg/ml) was subjected to standard analysis by nano DSF. The intrinsic fluorescence changes resulting from thermal unfolding (**FIG. 3D**) demonstrated that hexameric huIgG1 Fc (I253C)- μ tp had a single melting temperature of 79.54 °C. The above characterization suggests that huIgG1 Fc (I253C)- μ tp forms the desired homo-hexamer, it is feasible to purify, and it has exceptional thermal stability.

6.3 Example 3 – Production and Purification of IgG hexamers .

[00304] Exemplary hexameric IgG antibodies comprising the huIgG1 Fc (I253C)- μ tp were expressed and purified. The antigen binding portion of the exemplary hexameric IgG antibodies comprised a VH and VL from anti-beta klotho, anti-GFRAL or anti-VEGF as shown in Table 2. Table 2.

SEQ ID NO.	Description	Sequence
11	anti-beta klotho VH	QVQLQQSGAEVKKPGASVKVSCASGYTFTSY DINWVRQAPGQGLEWIGWIYPGDGSTKYNEKF KGKATITRDTSASTAYMELSSLRSED TAVYFCA RSDYYGSRSFAYWGQGT LTVSS
12	anti-beta klotho VL	DIVMTQSPDSLAVSLGERATINCRASKSVSTSGY VYMHWYQQKPGQP KLLIYLASYLESGVPDRF SGSGSGTDFTLTISSVQAEDVAVYYCQHSDLT FPFGGGTKLEIK
13	anti-GFRAL VH	QIQLVQSGPELKKPGETVKISCKASGYTFTDYG VIWVKQAPGKALKWMGWINTYTGEPTYADDL KGRFAFSLETSASSASLQINN LKNEDTATYFCAR RYGPEDIDYWGGQGTTLTVSS

SEQ ID NO.	Description	Sequence
14	anti-GFRAL VL	DIVLTQSPVSLAVSLGQRATISCRASESVDNYGI SFMSWFQQKPGQPPKLLIYAASHQSGVPARFS GSGSGTDFSLNIHPMEEDDSAMYFCLQSKEVPW TFGGGTKLEIK
15	anti-VEGF VH	EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYG MNWVRQAPGKGLEWVGWINTYTGEPTYAADF KRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYC AKYPHYYGSSHWYFDVWGQGTLLTVSS
16	anti-VEGF VL	DIQMTQSPSSLSASVGDRVTITCSASQDISNYLN WYQQKPGKAPKVLIIYFTSSLHSGVPSRFSGSGS GTDFTLTISLQPEDFATYYCQQYSTVPWTFGQ GTKVEIK

[00305] Proteins were transiently expressed using the Expi293 expression system and analyzed by electrophoresis as described above. IgG hexamers comprising an huIgG1 Fc (I253C)- μ tp and a VH and VL of anti-beta klotho are shown in lane 4; IgG hexamers comprising an huIgG1 Fc (I253C)- μ tp and a VH and VL of anti-GFRAL are shown in lane 5; and IgG hexamers comprising an huIgG1 Fc (I253C)- μ tp and a VH and VL of anti-VEGF are shown in lane 6 of **FIG. 4**.

[00306] These hexamers were purified by protein L affinity chromatography followed by mixed mode chromatography. The protein sample was filtered through a 0.2 μ m sterile filter unit and purified by affinity chromatography. Under the right buffer condition (Buffer A: 1xPBS) the sample was loaded to a 5 ml HiTrap ProL column using AKTA AVANT FPLC system at a flow rate of 5 ml/min. The bound protein was washed by 5 CV of Buffer A and eluted from the resin using 15 CV of 100 % Buffer B1 (0.1 M Glycine, 20 mM acetate, pH 3.5) and then 15 CV of Buffer B2 (0.1 M Glycine, 20 mM acetate, pH 3.0). The eluted fractions were pooled and neutralized separately. The neutralized pooled fractions were then filtered through a 0.2 μ m sterile filter unit and purified by mixed mode chromatography. Under the right buffer condition (Buffer C: 50 mM HEPES, 30 mM NaCl, 5 mM PO₄) the sample was loaded to a EconoFit CHT Type II, 40 μ m prepacked column using AKTA AVANT FPLC system at a flow rate of 5 ml/min. The bound protein was washed by 5 CV of Buffer C and eluted with a 20 CV linear gradient from 0% to 100% Buffer D1 (50 mM HEPES, 2 M NaCl, 5 mM PO₄), then with a

10 CV linear gradient back to 0% Buffer D1. This was repeated with Buffer D2 (50 mM HEPES, 30 mM NaCl, 500 mM PO₄). The fractions were collected in a 96 deep well plate. The monomer was eluted in the NaCl gradient, while the hexamer was eluted in the PO₄ gradient.

[00307] From the foregoing, it will be appreciated that, although specific embodiments have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of what is provided herein. All of the references referred to above are incorporated herein by reference in their entireties.

WHAT IS CLAIMED IS:

1. A molecule comprising an IgG C_H2 region, wherein position 253 in the IgG C_H2 region by EU numbering is substituted to be a cysteine.
2. The molecule of claim 1, wherein the molecule further comprises an IgG hinge region.
3. The molecule of claim 1 or claim 2, further comprising an IgG C_H3 region.
4. The molecule of any one of claims 1 to 3, further comprising a human μ tailpiece.
5. The molecule of claim 4, wherein the human μ tailpiece comprises an amino acid sequence of SEQ ID NO: 1 or an amino acid sequence having at least 75%, 80%, 85%, or 90% identity to SEQ ID NO: 1.
6. The molecule of claim 4 or claim 5, wherein the human μ tailpiece is conjugated to the C-terminus of the IgG C_H2 region.
7. The molecule of claim 4 or claim 5, wherein the human μ tailpiece is conjugated to the C-terminus of the IgG C_H3 region.
8. The molecule of any one of claims 1 to 7, wherein the molecule further comprises an IgG C_H1 region.
9. The molecule of any one of claims 1 to 8, wherein the IgG is a human IgG.
10. The molecule of claim 9, wherein the human IgG is a human IgG1.
11. The molecule of claim 9, wherein the human IgG is a human IgG2.
12. The molecule of claim 9, wherein the human IgG is a human IgG3.

13. The molecule of claim 9, wherein the human IgG is a human IgG4.
14. The molecule of any one of claims 1 to 13, wherein the molecule further comprises a binding domain that specifically binds to a target.
15. The molecule of claim 14, wherein the binding domain is an antibody fragment.
16. The molecule of any one of claims 1 to 14, wherein the molecule is an antibody or antigen binding fragment thereof.
17. An oligomer comprising two or more molecules of any one of claims 1 to 16.
18. An oligomer comprising two or more molecules, each molecule comprising an IgG C_H2 region, wherein position 253 in the IgG C_H2 region by EU numbering is substituted to be a cysteine.
19. The oligomer of claim 18, wherein the molecule further comprises an IgG hinge region.
20. The oligomer of claim 18 or claim 19, further comprising an IgG C_H3 region.
21. The oligomer of any one of claims 18 to 20, further comprising a human μ tailpiece.
22. The oligomer of claim 21, wherein the human μ tailpiece comprises an amino acid sequence of SEQ ID NO: 1 or an amino acid sequence having at least 80%, 85%, 90%, or 95% identity to SEQ ID NO: 1.
23. The oligomer of claim 21 or claim 22, wherein the human μ tailpiece is conjugated to the C-terminus of the IgG C_H2 region.

24. The oligomer of claim 21 or claim 22, wherein the human μ tailpiece is conjugated to the C-terminus of the IgG C_H3 region.
25. The oligomer of any one of claims 18 to 24, wherein the molecule further comprises an IgG C_H1 region.
26. The oligomer of any one of claims 18 to 25, wherein the IgG is a human IgG.
27. The oligomer of claim 26, wherein the human IgG is a human IgG1.
28. The oligomer of claim 26, wherein the human IgG is a human IgG2.
29. The oligomer of claim 26, wherein the human IgG is a human IgG3.
30. The oligomer of claim 26, wherein the human IgG is a human IgG4.
31. The oligomer of any one of claims 18 to 30, wherein the molecule further comprises a binding domain that specifically binds to a target.
32. The oligomer of claim 31, wherein the binding domain is an antibody fragment.
33. The oligomer of any one of claims 18 to 32, wherein the molecule is an antibody or antigen binding fragment thereof.
34. The oligomer of any one of claims 18 to 33, wherein the oligomer is a pentamer.
35. The oligomer of any one of claims 18 to 33, wherein the oligomer is a hexamer.
36. The oligomer of any one of claims 18 to 35, wherein the oligomer is homomeric and the two or more molecules bind to the same target.
37. The oligomer of any one of claims 18 to 35, wherein the oligomer is heteromeric.

38. The oligomer of claim 37, wherein the two or more molecules bind to two or more different targets.

39. An isolated nucleic acid encoding the molecule of any one of claims 1 to 16.

40. A vector comprising the nucleic acid of claim 39.

41. A pharmaceutical composition, comprising the molecule of any one of claims 1 to 16, the oligomer of any one of claims 17 to 38, the isolated nucleic acid of claim 39, or the vector of claim 40, and a pharmaceutically acceptable excipient.

42. A method for treating a disease or disorder in a subject comprising administering to the subject the pharmaceutical composition of claim 41.

43. A method of making an oligomer comprising two or more molecules each comprising an IgG C_H2 region, comprising introducing into each molecule cysteine amino acid substitution at position 253 by EU numbering in the IgG C_H2 region.

44. A method of producing oligomerized molecules, comprising:

- i. introducing the vector of claim 40 to a host cell;
- ii. cultivating the host cell under suitable conditions for the production of the oligomerized molecules; and
- iii. purifying the oligomerized molecules.

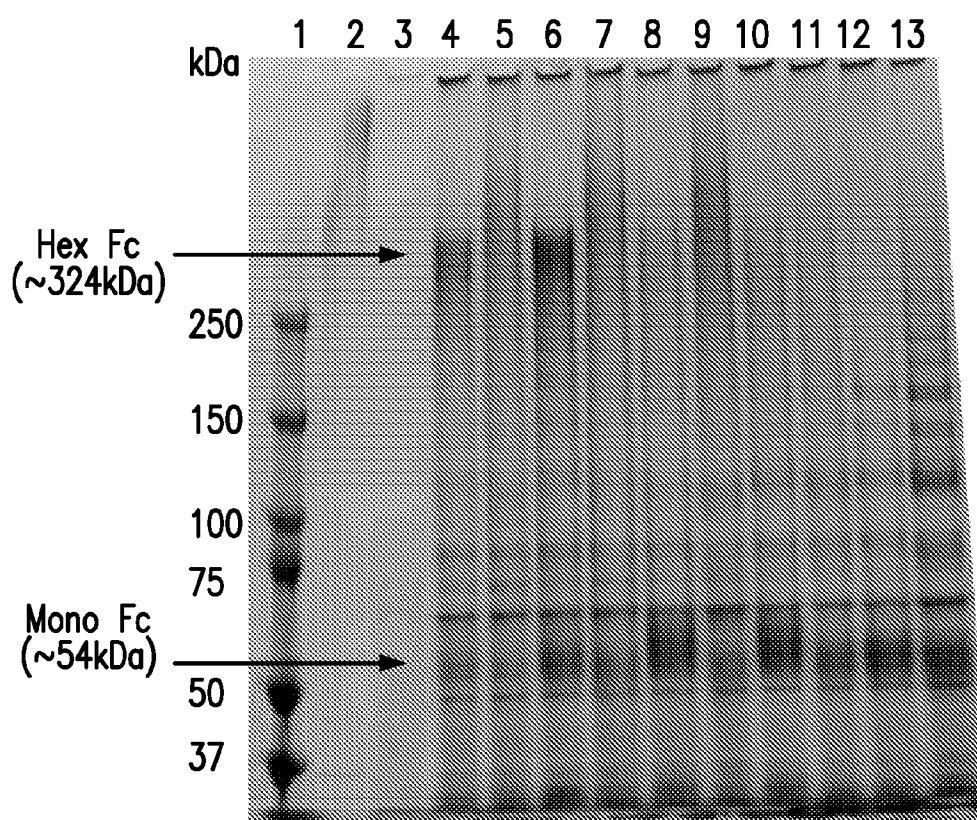


FIG. 1A

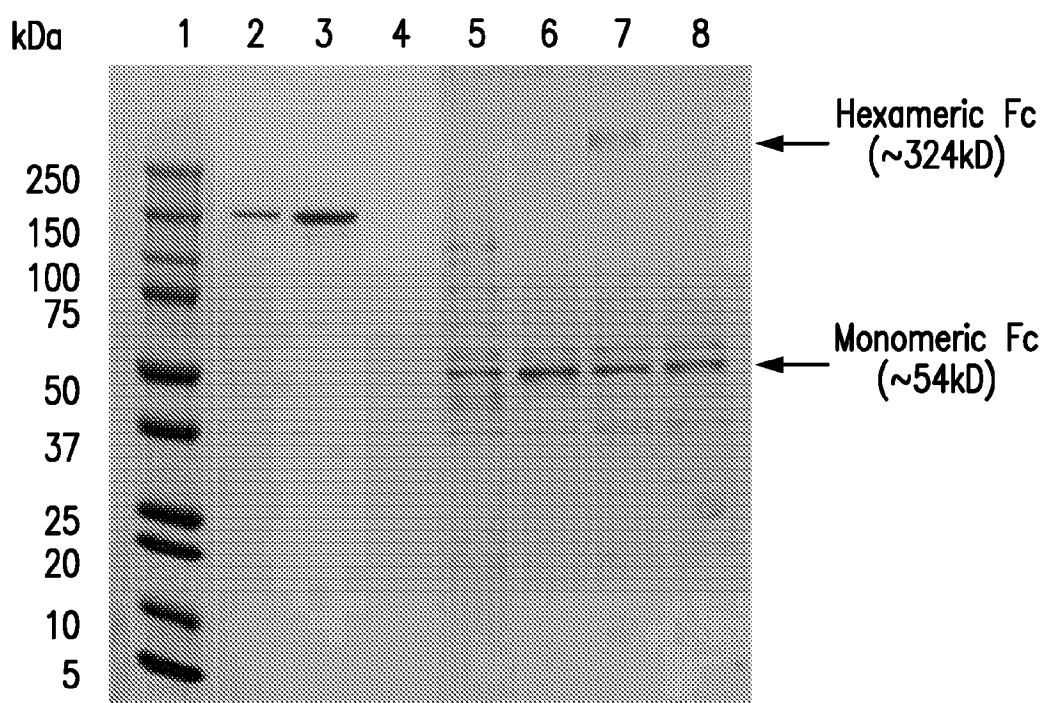


FIG. 1B

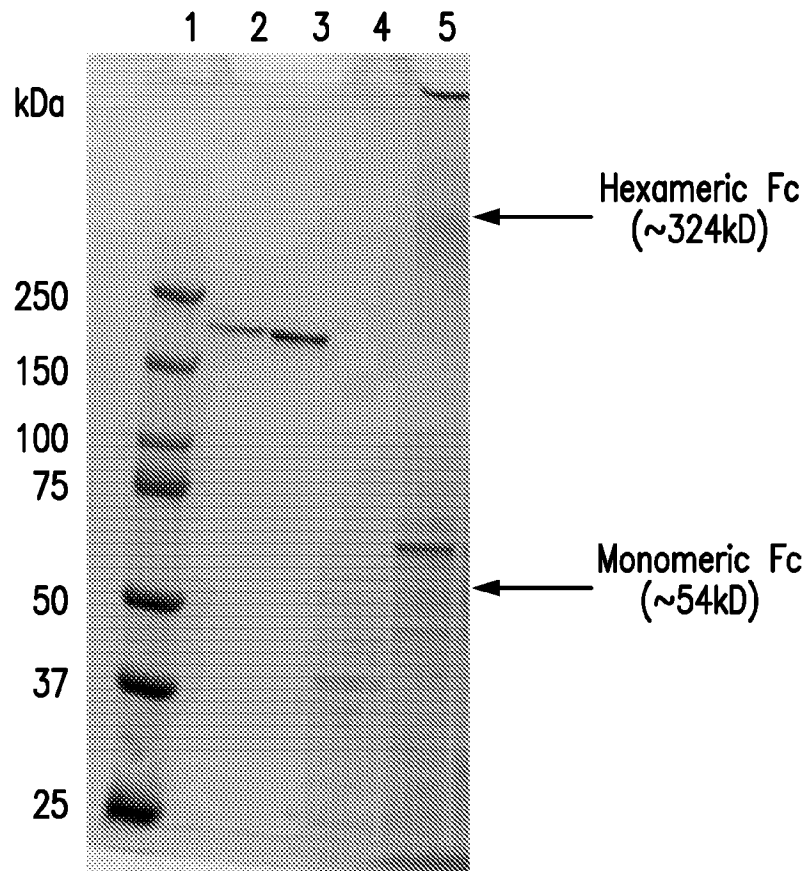


FIG. 1C

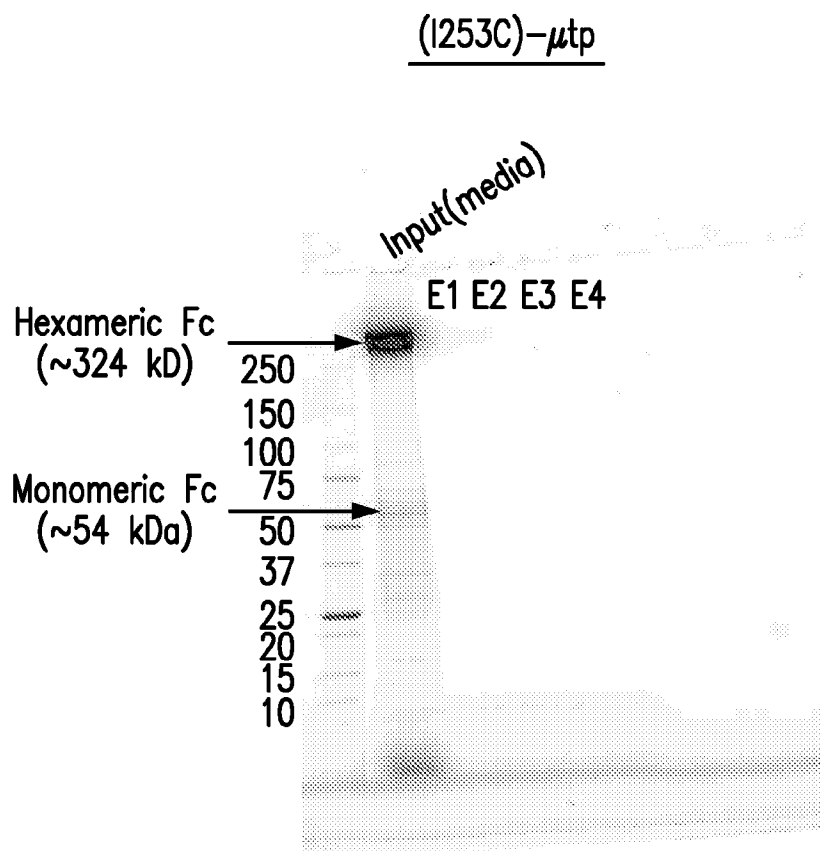


FIG. 2A

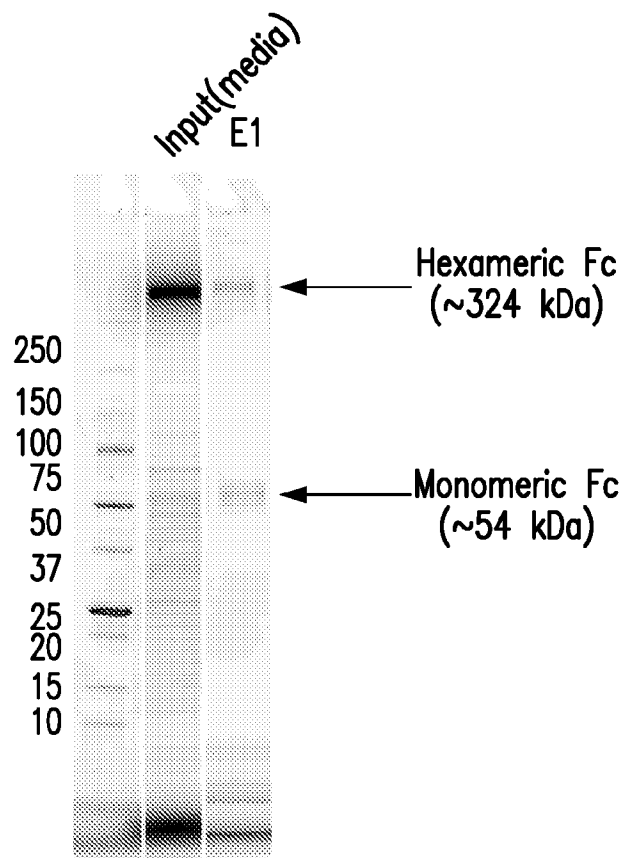


FIG. 2B

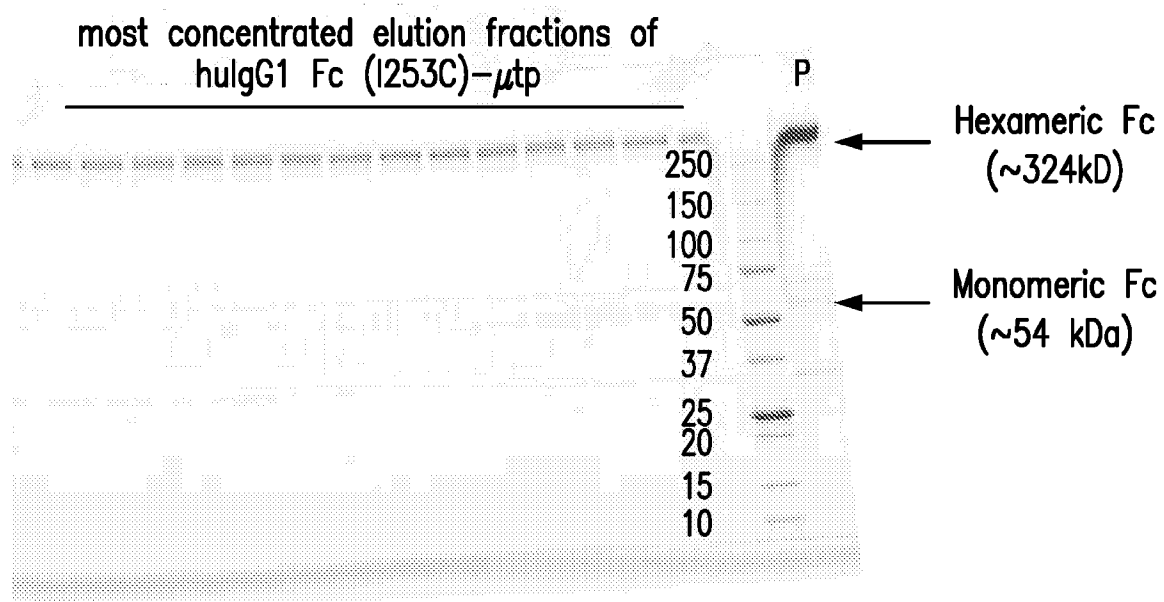


FIG. 2C

20210115-PSC0823-56.d Deconvoluted (Isotope Width=10.5)

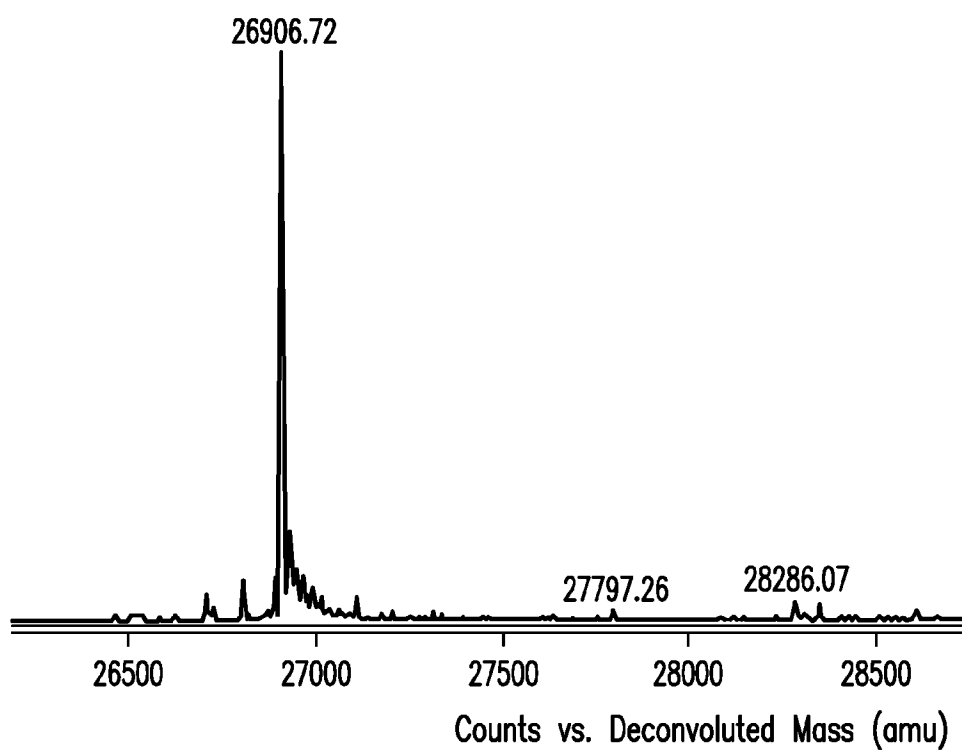


FIG. 3A

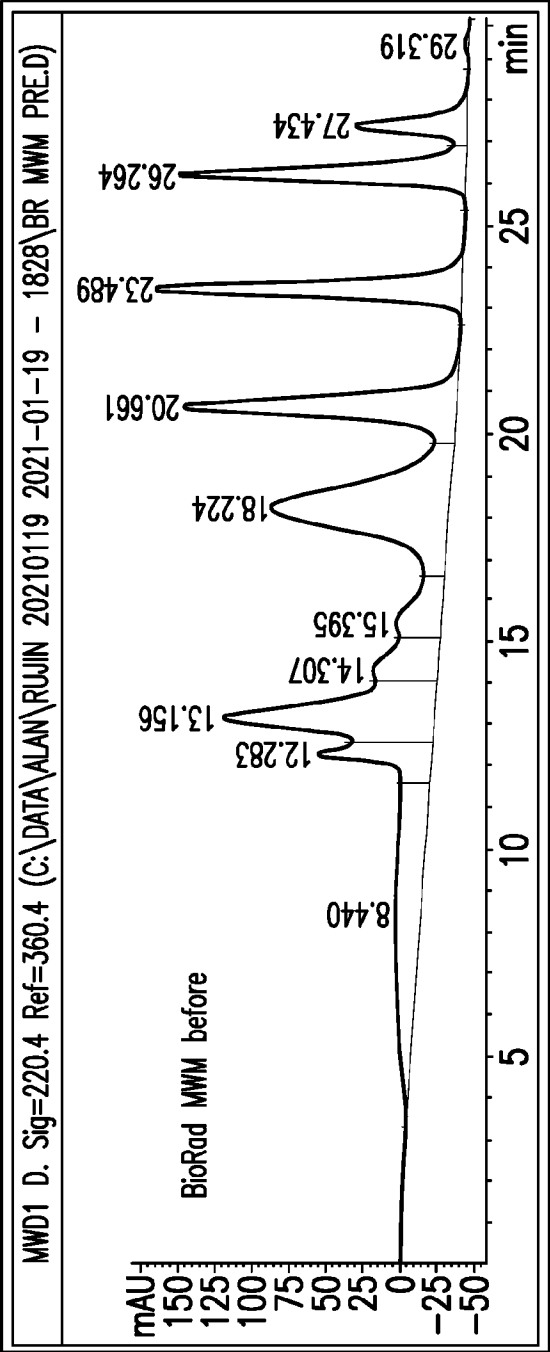


FIG. 3B

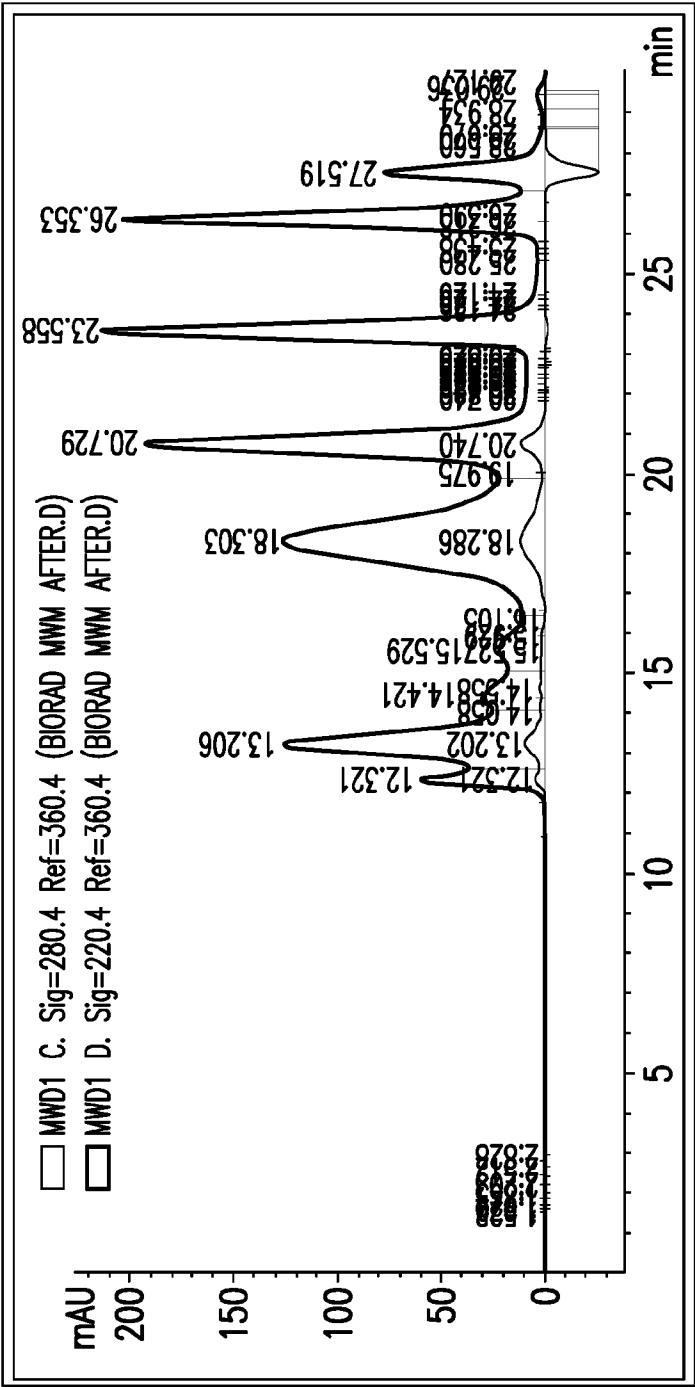


FIG. 3B Continued

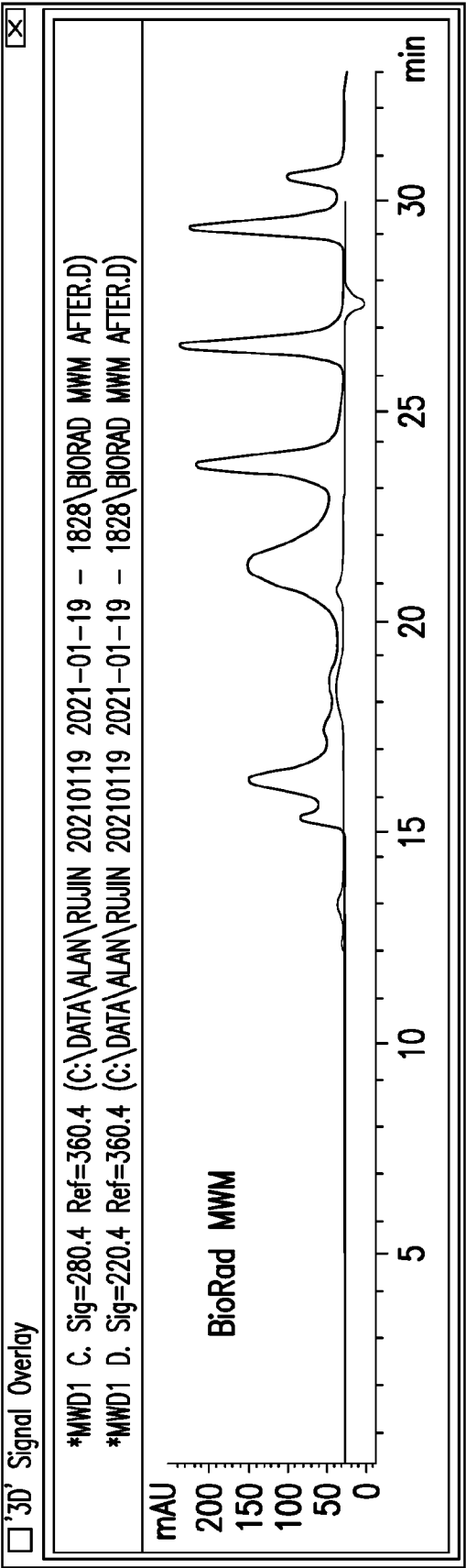


FIG. 3B Continued

Purified hulG1 Fc (I253C)-μtp

13 min: higher oligomer
15 min: hexamer (89%)
19 min: Monomer

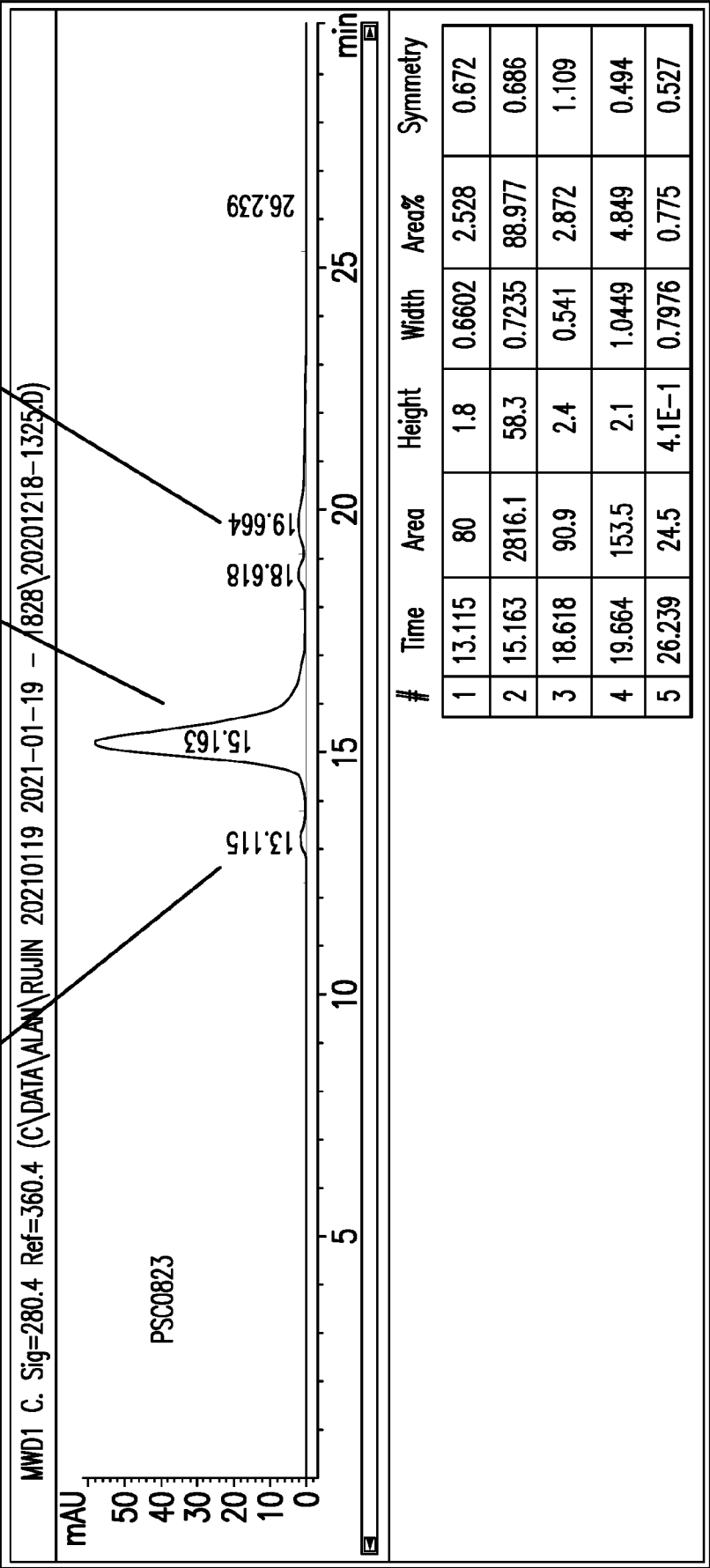


FIG. 3C

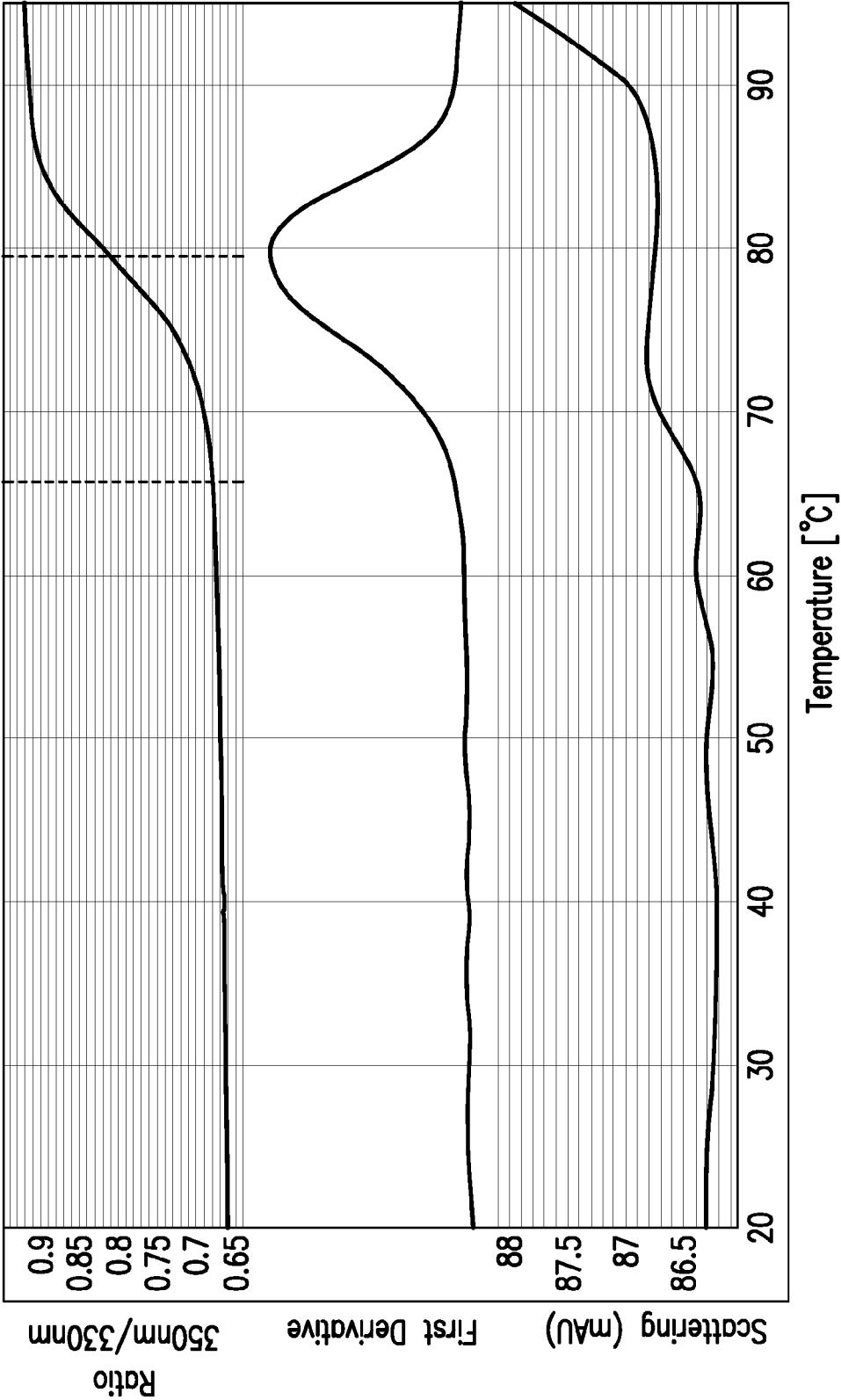


FIG. 3D

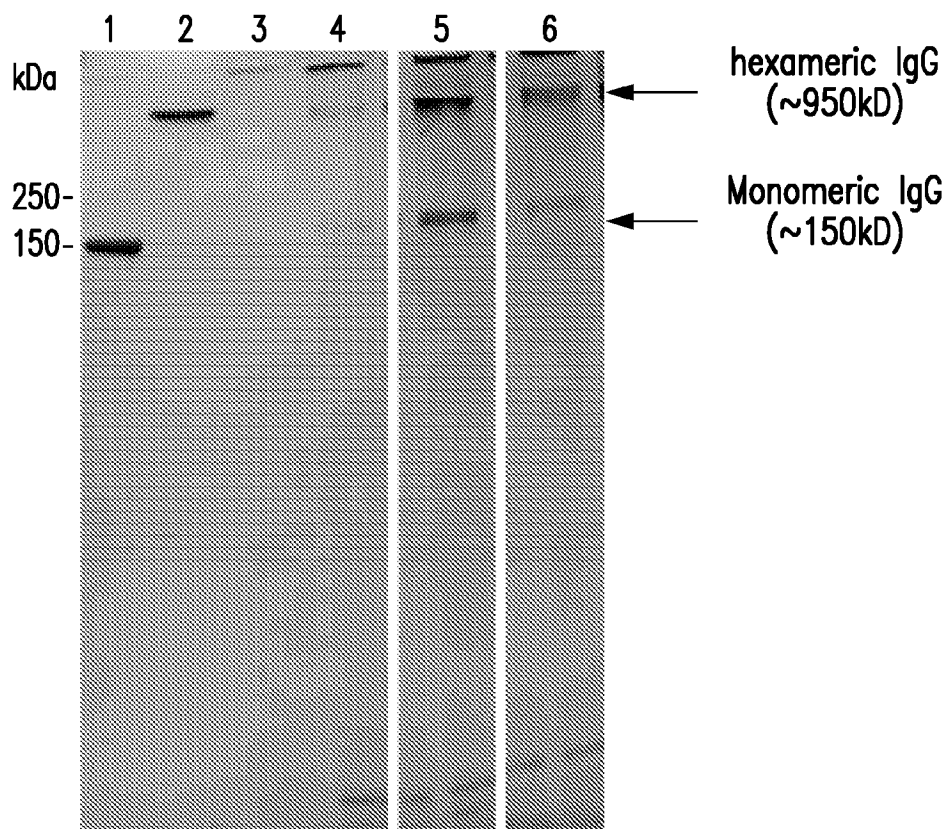


FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 22/26505

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C07K 16/00, A61P 37/02 (2022.01)

CPC - C07K 16/461, C07K 2317/53, C07K 2317/52, C07K 2317/524, C07K 2317/526, C07K 2317/71

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2018/0169255 A1 (MEDIMMUNE, LLC) 21 June 2018 (21.06.2018) abstract, [0050], [0066], [0113], [0122], [0148]	1-3, 18-20, 43
A	US 2015/0232557 A1 (EMERGENT PRODUCT DEVELOPMENT SEATTLE LLC) 20 August 2015 (20.08.2015) para [0061], [0064], [0174]	1-3, 18-20, 43

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

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

"D" document cited by the applicant in the international application

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

29 June 2022

Date of mailing of the international search report

JUL 27 2022

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Kari Rodriguez

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 22/26505

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4-17, 21-42 and 44
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.