# ANTIBODIES AND METHODS OF MAKING AND USING SAME

## REFERENCE TO RELATED APPLICATIONS

**[0001]** This application claims priority to U.S. Provisional Patent Application No. 63/676,069, filed July 26, 2024, the disclosure of which is hereby incorporated by reference in its entirety.

## REFERENCE TO SEQUENCE LISTING

**[0002]** The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled TLIX006WOSEQLISTING.xml, created on July 24, 2025, which is 29,053 bytes in size. The information in the electronic Sequence Listing is hereby incorporated by reference in its entirety.

## BACKGROUND

Field

**[0003]** The present disclosure generally relates to enhanced biodistribution and/or pharmacokinetics of antibodies (including minibodies and cys-diabodies), and compositions therefor.

Description of the Related Art

**[0004]** The present disclosure generally relates to antibodies, including antigen binding fragments thereof, such as minibodies and cys-diabodies. Antibodies can be used to bind targets for therapeutic or diagnostic uses.

## SUMMARY

**[0005]** Provided herein is an antibody that varies from an original antibody by comprising a negatively charged organic moiety associated therewith, wherein the negatively charged moiety confers a surface-exposed negative charge to the antibody, wherein the antibody has at least about 10% more total surface-exposed negative charge conferred by the negatively charged organic moiety relative to the original antibody.

**[0006]** Also provided is an antibody that varies from an original antibody by comprising a negatively charged organic moiety associated therewith, wherein the antibody is a minibody or cys-diabody, wherein the negatively charged organic moiety confers a surface-exposed negative charge to the antibody, wherein the antibody has at least about 10% more total surface-exposed negative charge conferred by the negatively charged organic moiety relative to the original antibody, and wherein the antibody has a negatively charged organic moiety-to-antibody ratio of at least 1.2.

**[0007]** Further provided herein is a composition comprising: any one of the antibody of the present disclosure; and a pharmaceutically acceptable carrier.

**[0008]** Also provided is a composition comprising: an antibody; and a negatively charged organic moiety or precursor thereof configured to be conjugated to the antibody, wherein the negatively charged organic moiety or precursor thereof is present in an amount sufficient to disrupt or conceal a positive patch on a surface of the antibody, or to confer an additional negative charge to the antibody, when conjugated thereto.

**[0009]** Provided herein is a kit for radiolabeling an antibody, comprising: a first chelating ligand for radiolabeling an antibody; and a negatively charged organic moiety configured to be conjugated to the antibody.

**[0010]** Also provided herein is a method of enhancing biodistribution and/or pharmacokinetics of a radiolabeled antibody, comprising: selecting a first radiolabeled antibody comprising: a first antibody; and a radionuclide, wherein the first antibody is labeled with the radionuclide via a first chelating ligand; and providing a second radiolabeled antibody comprising: the first antibody; the radionuclide, wherein the first antibody is labeled with the radionuclide via the first chelating ligand; and a negatively charged organic moiety conjugated thereto, by conjugating the negatively charged organic moiety to the first antibody in an amount sufficient to provide the second radiolabeled antibody having at least 10% more surface-exposed negative charges conferred by the conjugated negatively charged organic moiety compared to the first radiolabeled antibody, thereby generating a second radiolabeled antibody having enhanced biodistribution and/or pharmacokinetics compared to the first radiolabeled antibody.

**[0011]** Provided herein is a method of making a radiolabeled antibody, comprising: providing an antibody; labeling the antibody with a radionuclide via a first chelating ligand;

and conjugating a negatively charged organic moiety to the antibody to obtain a negatively charged organic moiety-to-antibody ratio of at least about 1.2, thereby making a radiolabeled antibody.

**[0012]** Also provided is a method of making an antibody conjugate, comprising: providing an antibody; and conjugating a negatively charged organic moiety to the antibody to disrupt or conceal at least one positive patch on a surface of the antibody, or to confer an additional negative charge to the antibody, to thereby generate an antibody conjugate.

**[0013]** Provided herein is a method of enhancing biodistribution and/or pharmacokinetics of a radiolabeled minibody or cys-diabody, comprising: selecting a first radiolabeled antibody comprising: a first antibody, wherein the first antibody is a minibody or a cys-diabody; and a radionuclide, wherein the first antibody is labeled with the radionuclide via a first chelating ligand; and providing a second radiolabeled antibody comprising: the first antibody; the radionuclide, wherein the first antibody is labeled with the radionuclide via the first chelating ligand; and a negatively charged organic moiety conjugated thereto, by conjugating the negatively charged organic moiety to the first antibodyto obtain a negatively charged organic moiety-to-antibody ratio of at least about 1.2.

**[0014]** Also provided is the antibody, minibody, or cys-diabody made by any one of the methods of the present disclosure.

**[0015]** Further provided is a method of treating a subject, comprising: identifying a subject in need of treatment with any one of the antibody, minibody, or cys-diabody of the present disclosure; and administering to the subject a therapeutically effective amount of the antibody, minibody, or cys-diabody, or the composition of the present disclosure.

**[0016]** Provided herein is a method of treating a subject for a cancer, comprising: identifying a subject in need of treatment for a cancer; and administering to the subject a therapeutically effective amount of the antibody, minibody, or cys-diabody of any one of arrangements 1-22, or the composition of arrangement 23, to thereby treat the cancer.

**[0017]** Also provided is a method of radiotherapy, comprising: identifying a subject in need of radiotherapy; and administering to the subject a therapeutically effective amount of any one of the antibody, minibody, or cys-diabody of the present disclosure, or the composition of the present disclosure, wherein the antibody, minibody, or cys-diabody comprises a radionuclide.

**[0018]** Provided herein is a method of imaging a subject, comprising: administering to a subject a composition comprising an effective amount of any one of the antibody, minibody, or cys-diabody of the present disclosure, or the composition of the present disclosure, wherein the antibody, minibody, or cys-diabody is detectably labeled; and imaging the subject to detect the labeled antibody, minibody, or cys-diabody in the subject.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0019]** FIG. 1A is a block diagram showing a non-limiting example of a method of enhancing biodistribution and/or pharmacokinetics of an antibody.

**[0020]** FIG. 1B is a block diagram showing a non-limiting example of a method of enhancing biodistribution and/or pharmacokinetics of a minibody or cys-diabody.

**[0021]** FIG. 2 is a block diagram showing a non-limiting example of a method of making an antibody.

**[0022]** FIG. 3 is a block diagram showing a non-limiting example of a method of making an antibody conjugate.

**[0023]** FIG. 4 is a block diagram showing a non-limiting example of a method of treating a subject.

**[0024]** FIG. 5 is a graph comparing tissue distribution of minibodies with and without overconjugation, according some non-limiting embodiments of the present disclosure.

**[0025]** FIG. 6 is a graph comparing tissue distribution of minibodies with and without overconjugation, according some non-limiting embodiments of the present disclosure.

**[0026]** FIG. 7 is a graph comparing tissue distribution of minibodies with and without overconjugation, according some non-limiting embodiments of the present disclosure.

**[0027]** FIG. 8 is a graph comparing tissue distribution of cys-diabodies with and without overconjugation, according some non-limiting embodiments of the present disclosure.

**[0028]** FIG. 9 is a graph comparing tissue distribution of minibodies with and without overconjugation, according some non-limiting embodiments of the present disclosure.

**[0029]** FIG. 10 is a schematic diagram of some non-limiting structural features of a minibody.

**[0030]** FIG. 11 is a schematic diagram of some non-limiting structural features of a cys-diabody.

**[0031]** FIGs 12A-12D are a collection of non-limiting examples of amino acid sequences of minibodies or cys-diabodies.

**[0032]** FIG. 13 is a collection of non-limiting examples of $C_H3$ amino acid sequences.

## DETAILED DESCRIPTION

**[0033]** The biodistribution and pharmacokinetics of antibodies, minibodies and cys-diabodies, when administered to a subject, is an important determinant of their safety and efficacy. Improved biodistribution and pharmacokinetics can affect the dosing and schedule of administration of antibody therapeutics, such as during radioimmunotherapy or when used as antibody-drug conjugates. It can also affect the dosing and imaging time point for radiodiagnostic imaging with antibodies, minibodies and cys-diabodies. The biodistribution and pharmacokinetics of antibodies can vary depending on a number of factors, including some properties of surface-exposed electrostatic charges of the antibody.

**[0034]** Provided herein are antibodies, including minibodies, cys-diabodies, and conjugates thereof, having enhanced and/or altered biodistribution and/or pharmacokinetics and methods for enhancing and/or altering the biodistribution and/or pharmacokinetics of antibodies. The antibodies of the present disclosure generally are generated by hyper- or over-conjugation of an original antibody with a negatively charged organic moiety, which can enhance and/or alter the biodistribution and/or pharmacokinetics compared to the original antibody without the hyper- or overconjugation. A "negatively charged organic moiety" is sometimes referred to herein as a "negative adduct". As used herein, "hyper-conjugation" or "overconjugation" refers to conjugation with a negatively charged organic moiety such that the formulation when administered to a subject has an negative adduct-to-antibody ratio of 1.2 or greater.

**[0035]** Without being bound by theory, it is thought that some antibodies have a cluster of positively charged amino acids that form a patch of positive electrostatic charges as described by an isopotential surface over the structure of the antibody, and this localized charge concentration can contribute to accumulation of the antibody in radiosensitive or drug sensitive tissues, such as the kidneys, when administered to a subject. Undesired antibody accumulation in the kidneys can be due to increased glomerular capture and retention (and/or increased

reabsorption and retention from glomerular filtrate) relative to an antibody (e.g., antibody conjugate) of the present disclosure.

**[0036]** Chemical modifications of minibodies and cys-diabodies that includes conjugating these constructs with negatively charged organic moieties can drastically redirect the clearance from the renal route to the hepatic route. Without limitation, some examples of modifications include conjugation with negatively charged organic dyes, such as Licor dyes such as IRDye®800. In some embodiments, conjugation of minibodies and cys-diabodies at a high negative adduct to antibody ratio, which in certain cases may be described as a high Chelator-to-Minibody/cys-diabody Ratio (CMR >= 5) with metal chelators such as, but not limited to, diethylenetriaminepentaacetic acid (DTPA), 2,2′,2”,2”’-(1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayl)tetraacetic acid (DOTA), and 2,2′,2”-(10-(4-((2-aminoethyl)amino)-1-carboxy-4-oxobutyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (DOTAGA), also triggers the same effect. Without being bound by theory, it is thought that conjugation of the negatively charged organic moieties can disrupt, conceal, or otherwise provide an additional negative charge to the antibody to change the biodistribution. Further, while conjugation of a neutral organic moiety, such as a neutral chelating ligand, may remove some surface-exposed positive charges when conjugation is through lysine residues, simply removing positive charges may be less effective at changing biodistribution compared to adding negative charges through conjugation of the negatively charged organic moieties through the lysine residues.

**[0037]** Conjugation of the negatively charged organic moiety to the antibody can reduce, eliminate or conceal one or more of the positive patches, or provide a net negative charge so as to redirect clearance of the antibody from the kidney route to the liver route. In some embodiments, conjugation of the negatively charged organic moiety reduces the accumulation of the antibody in the kidney by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or more, or by a percentage in a range defined by any two of the preceding values (e.g., 10-80%, 10-50%, 30-80%, 40-70%, etc.) In some embodiments, conjugation of the negatively charged organic moiety increases accumulation of the antibody in the liver. In some embodiments, the change in biodistribution due to conjugation of the negatively charged organic moiety is readily observed for an antibody construct having a molecular weight (as a dimer) of ~50-80 kDa, such as a minibody or a cys-diabody. In general, modification of the

original antibody by overconjugation at least maintains the same binding specificity and binding affinity of the original antibody, while enhancing the biodistribution and/or pharmacokinetics.

**[0038]** In some embodiments, the biodistribution of the antibody can be controlled by modifying the electrostatic charge associated with the cluster. As noted above, in some embodiments, the cluster of positively charged amino acids can promote accumulation in the kidneys, and reducing the positive charge, or increasing the negative charge associated with the cluster can promote accumulation away from the kidneys, e.g., promote accumulation in the liver. Thus, in some embodiments, biodistribution of a toxin or radionuclide conjugated to the antibody can be biased toward the kidneys by increasing the positive charges associated with the cluster, and biodistribution of a toxin or radionuclide conjugated to the antibody can be biased toward the liver by reducing the positive charges, or increasing the negative charges associated with the cluster. In some embodiments, distribution of the antibody to the liver is promoted where a liver disorder (e.g., liver cancer) is treated by administering the antibody.

**[0039]** In the radioimmunotherapy context, the accumulation of the antibody in the kidney can lead to renal toxicity. Thus, the antibodies of the present disclosure having reduced accumulation in the kidney can allow for administering a greater dose of a radiolabeled antibody (e.g., greater specific activity, greater frequency and/or number of administration) while reducing renal toxicity. In therapeutic contexts, it allows for drugs to be delivered to a subject having reduced accumulation of the antibody-conjugate in the kidneys as well. This can be especially useful for antibody fragments, such as minibodies, diabodies (such as cys-diabodies) and nanobodies, as well as other constructs of similar size (~30-80 kDa).

Terms

**[0040]** The term "antibody" includes all varieties of antibodies, including antigen binding fragments thereof. Further included are constructs that include 1, 2, 3, 4, 5, and/or 6 CDRs. In some embodiments, tandem scFvs can be provided, which can provide two arms with bivalent binding. In some embodiments, these CDRs can be distributed between their appropriate framework regions in a traditional antibody. In some embodiments, the CDRs can be contained within a heavy and/or light chain variable region. In some embodiments, the CDRs can be within a heavy chain and/or a light chain. In some embodiments, the CDRs can be within a single peptide chain. Unless otherwise denoted herein, the antibodies described

herein bind to the noted target molecule. The term "target" or "target molecule" denotes the protein to which the antigen binding construct binds.

**[0041]** The term "antibody" includes, but is not limited to, genetically engineered or otherwise modified forms of immunoglobulins, such as intrabodies, chimeric antibodies, fully human antibodies, humanized antibodies, antibody fragments, scFv, and heteroconjugate antibodies (for example, bispecific antibodies, diabodies, triabodies, tetrabodies, etc.). Also, the term "antibody" includes camelid derived immunoglobulins like single heavy-chain antibodies and nanobodies. The term "antibody" includes scFv and minibodies. Thus, each and every embodiment provided herein in regard to "antibodies" is also envisioned as scFv and/or minibody embodiments, unless explicitly denoted otherwise. The term "antibody" includes a polypeptide of the immunoglobulin family or a polypeptide comprising fragments of an immunoglobulin that is capable of noncovalently, reversibly, and in a specific manner binding a corresponding antigen. An exemplary antibody structural unit comprises a tetramer. In some embodiments, a full-length antibody can be composed of two identical pairs of polypeptide chains, each pair having one "light" and one "heavy" chain (connected through a disulfide bond). The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, hinge, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. For full length chains, the light chains are classified as either kappa or lambda. For full length chains, the heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD, and IgE, respectively. The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (VL) and variable heavy chain (VH) refer to these regions of light and heavy chains respectively. As used in this application, an "antibody" encompasses all variations of antibody and fragments thereof. Thus, within the scope of this concept are full length antibodies, chimeric antibodies, humanized antibodies, single chain antibodies (scFv), Fab, Fab', and multimeric versions of these fragments (for example, F(ab')2) with the same binding specificity. In some embodiments, the antibody binds specifically to a desired target.

**[0042]** Antibodies further include one or more immunoglobulin chains that are chemically conjugated to, or expressed as, fusion proteins with other proteins. It also includes

bispecific antibodies. A bispecific or bifunctional antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites.

**[0043]** Other antigen-binding fragments or antibody portions of the present disclosure include, bispecific scFv antibodies where the antibody molecule recognizes two different epitopes, camelid single binding domains (sdAb or nanobodies), minibodies, and cys-diabodies.

**[0044]** The term "antibody fragment" includes, but is not limited to one or more antigen binding fragments of antibodies alone or in combination with other molecules, including, but not limited to Fab', $F(ab')_2$, Fab, Fv, rIgG (reduced IgG), scFv fragments, single domain fragments (nanobodies), peptibodies, minibodies, single variable domain on a heavy chain (VHH), diabodies and cys-diabodies. The term "scFv" refers to a single chain Fv ("fragment variable") antibody in which the variable domains of the heavy chain and of the light chain of a traditional two chain antibody have been joined to form one chain.

**[0045]** A minibody is an antibody format that has a smaller molecular weight than the full-length antibody while maintaining the bivalent binding property against an antigen. Because of its smaller size (~80 kDa), absence of $C_H2$ domain that binds Fc-gamma and FcRn receptors, and absence of glycosylation, the minibody has a faster clearance from the blood system and potentially enhanced penetration when targeting tumor tissue. With the ability for strong targeting combined with rapid clearance, the minibody is advantageous for diagnostic imaging and delivery of radioactive payloads for which prolonged circulation times may result in adverse patient dosing or dosimetry. In some embodiments, it can also be advantageous for delivery of a cytotoxic payload due to the above-mentioned features such as tumor penetration and faster clearance. A "minibody" as described herein, encompasses a homodimer, wherein each monomer is a single-chain variable fragment (scFv) linked to a human IgG $C_H3$ domain by a hinge sequence. A non-limiting, schematic diagram of a minibody is shown in FIG. 10. In some embodiments, a minibody is a bivalent or bispecific, covalently bound heterodimer of ~80 kDa. In some embodiments, each monomer (half-molecule) is comprised of a variable heavy ($V_H$) domain linked to the corresponding variable light ($V_L$) domain by an approximate 15-18 amino acid Gly-Ser-rich linker sequence. In some embodiments, each single-chain variable fragment (scFv) is linked to a human IgG1, IgG2, IgG3 or IgG4 $C_H3$ domain by a hinge sequence.

**[0046]** The term "hinge" denotes at least a part of a hinge region for an antigen binding construct, such as an antibody or a minibody. A hinge region can include a combination of the upper hinge, core (or middle) hinge and lower hinge regions. In some embodiments, the hinge is defined according to any of the antibody hinge definitions. Native IgG1, IgG2, and IgG4 antibodies have hinge regions having of 12-15 amino acids. IgG3 has an extended hinge region, having 62 amino acids, including 21 prolines and 11 cysteines. The functional hinge region of naturally occurring antibodies, deduced from crystallographic studies, extends from amino acid residues 216-237 of the IgG1 H chain (EU numbering) and includes a small segment of the N terminus of the $C_H2$ domain in the lower hinge, with the lower hinge being the N terminus of $C_H2$ domain. The hinge can be divided into three regions; the "upper hinge," the "core," and the "lower hinge".

**[0047]** The term "upper hinge" denotes the first part of the hinge that starts at the end of the scFv. The upper hinge includes the amino acids from the end of the scFv up to, but not including the first cysteine residue in the core hinge. The term "effective upper hinge" denotes that enough of the sequence is present to allow the section to function as an upper hinge; the term encompasses functional variants and fragments of the designated hinge section.

**[0048]** The term "core hinge" denotes the second part of the hinge region that is C-terminal to the upper hinge. The core hinge can contain the inter-chain disulfide bridges and a high content of prolines.

**[0049]** The term "lower hinge" denotes the third part of the hinge region that is C-terminal to the core hinge. In the context of a minibody or antibody fragment, the lower hinge connects to the $C_H3$ domain Mb. As above, the term "effective lower hinge" denotes that enough of the sequence is present to allow the section to function as a lower hinge; the term encompasses functional variants and fragments of the designated hinge section. The term "lower hinge" as used herein can encompass various amino acid sequences including naturally occurring IgG lower hinge sequences and artificial extension sequences in place of one another or a combination thereof provided herein. In some embodiments, the various extensions can be considered to be a lower hinge region in its entirety or a replacement.

**[0050]** In some embodiments a lower hinge can be a native IgG1, 2, 3 or 4 lower hinge, an/or $(G_3)S_n$ or $(G_4)S_n$ (n can be any number of S's; in some embodiments it is 1 or 2)

and/or no lower hinge and/or any combination of amino acids (doesn't have to be G's and S's). In some embodiments, the lower hinge can comprise GGGSSGGGSG (SEQ ID NO: 1).

**[0051]** The term "diabody" denotes a dimer that comprises heavy chain ($V_H$) domains and light-chain variable ($V_L$) domains. Each heavy chain domain is connected to a light chain domain through a linker, forming a monomer. Two monomers are covalently linked through a bridging moiety to form the diabody. A "cys-diabody" denotes a diabody whose monomer chains are covalently linked by a disulfide bond. A non-limiting, schematic diagram of a cys-diabody is shown in FIG. 11. A cys-diabody can have a molecular weight of ~50 kDa.

**[0052]** The term "extension sequence" denotes a region that connects a first VH domain to a second VH domain or a first VL to a second VL domain, in for example, a diabody. Extension sequences can connect the domains through the C-terminus of each domain. In some embodiments, extension sequences connect the domains through covalent bonds. In some embodiments, the extension sequence will include one or more cysteine, allowing for one or more disulfide bonds to be formed between two such extension sequences. An example of a pair of extension sequences is shown in the schematic diagrams on the right side of FIG. 11, as the line with two cysteines connecting either the two heavy chain domains or the two light chain domains. A non-limiting example of an extension sequence of -(Gly)2-(Cys) is shown in the schematic diagrams on the left side of Fig. 11. In some embodiments, the extension sequence includes 1, 2, 3, or more cysteines per monomer chain. While the extension sequence will be towards the C-terminus of the constructs in FIG. 11, it need not be the absolute last amino acid in the variable domain. That is, the linker can be positioned slightly N-terminal to the C-terminus. For example, the extension sequence can be placed within the 10 amino acids at the C-terminus. Similarly, additional sequence can be placed between the native C- terminus and where the extension sequence starts. The extension sequence can connect VH to VH or VL to VL through a disulfide bond.

**[0053]** In some embodiments, a linker can be any suitable linker for the antibody (e.g., minibody, cys-diabody). In some embodiments, a linker sequence can include a motif that is $(G_3)S_n$ or $(G_4)S_n$ (n can be any integer; in some embodiments it is 1 or 2). In some embodiments, the linker can comprise GSTSGGGSGGGSGGGGSS (SEQ ID NO: 2).

**[0054]** The term "treating" or "treatment" of a condition can refer to preventing the condition, slowing the onset and/or rate of development of the condition, reducing the risk of

developing the condition, preventing and/or delaying the development of symptoms associated with the condition, reducing or ending symptoms associated with the condition, generating a complete or partial regression of the condition, or some combination thereof. The term "prevent" does not require the absolute prohibition of the disorder or disease.

**[0055]** A "therapeutically effective amount" or a "therapeutically effective dose" is an amount that produces a desired therapeutic effect in a subject, such as preventing, treating a target condition, delaying the onset of the disorder and/or symptoms, and/or alleviating symptoms associated with the condition. This amount will vary depending upon a variety of factors, including but not limited to the characteristics of the therapeutic compound (including activity, pharmacokinetics, pharmacodynamics, and bioavailability), the physiological condition of the subject (including age, sex, disease type and stage, general physical condition, responsiveness to a given dosage, and type of medication), the nature of the pharmaceutically acceptable carrier or carriers in the formulation, and/or the route of administration. One skilled in the clinical and pharmacological arts will be able to determine a therapeutically effective amount through routine experimentation, for example by monitoring a subject's response to administration of a compound and adjusting the dosage accordingly, given the present disclosure. For additional guidance, *see* Remington: The Science and Practice of Pharmacy 21st Edition, Univ. of Sciences in Philadelphia (USIP), Lippincott Williams & Wilkins, Philadelphia, PA, 2005.

**[0056]** The term "complementarity-determining domains" or "complementarity-determining regions ("CDRs") interchangeably refer to the hypervariable regions of $V_L$ and $V_H$. The CDRs are the target molecule-binding site of the antibody chains that harbors specificity for such target molecule. In some embodiments, there are three CDRs (CDR1-3, numbered sequentially from the N-terminus) in each $V_L$ and/or $V_H$, constituting about 15-20% of the variable domains. The CDRs are structurally complementary to the epitope of the target molecule and are thus directly responsible for the binding specificity. The remaining stretches of the $V_L$ or $V_H$, the so-called framework regions (FRs), exhibit less variation in amino acid sequence (Kuby, Immunology, 4th ed., Chapter 4. W.H. Freeman & Co., New York, 2000).

**[0057]** The positions of the CDRs and framework regions can be determined using various well-known definitions in the art, e.g., Kabat *(Wu, T. T., E. A. Kabat.* 1970. *An analysis of the sequences of the variable regions of Bence Jones proteins and myeloma light chains and*

*their implications for antibody complementarity.* J. Exp. Med. 132*:* 211-*250*; Kabat, E. A., Wu, T. T., Perry, H., Gottesman, K., and Foeller, C. (1991) Sequences of Proteins of Immunological Interest, 5th ed., NIH Publication No. 91–3242, Bethesda, MD); Chothia (Chothia and Lesk, J. Mol. Biol., 196:901-917 (1987); Chothia et al., Nature, 342:877-883 (1989); Chothia et al., J. Mol. Biol., 227:799-817 (1992); Al-Lazikani et al., J. Mol. Biol., 273:927-748 (1997)); ImMunoGeneTics database (IMGT) (on the worldwide web at imgt.org/) Giudicelli, V., Duroux, P., Ginestoux, C., Folch, G., Jabado-Michaloud, J., Chaume, D. and Lefranc, M.-P. IMGT/LIGM-DB, the IMGT® comprehensive database of immunoglobulin and T cell receptor nucleotide sequences Nucl. Acids Res., 34, D781-D784 (2006), PMID: 16381979; Lefranc, M.-P., Pommié, C., Ruiz, M., Giudicelli, V., Foulquier, E., Truong, L., Thouvenin-Contet, V. and Lefranc, G., IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains Dev. Comp. Immunol., 27, 55-77 (2003). PMID: 12477501; Brochet, X., Lefranc, M.-P. and Giudicelli, V. IMGT/V-QUEST: the highly customized and integrated system for IG and TR standardized V-J and V-D-J sequence analysis Nucl. Acids Res, 36, W503-508 (2008); AbM (Martin et al., Proc. Natl. Acad. Sci. USA, 86:9268-9272 (1989); North (North B., Lehmann A., Dunbrack R.L., A new clustering of antibody CDR loop conformations, J. Mol. Biol. (2011) 406(2): 228–256); AHo (Honegger A., Pluckthun, Yet another numbering scheme for immunoglobulin variable domains: an automatic modeling and analysis tool, J. Mol. Biol. (2001) 309, 657-670); the contact definition (MacCallum et al., J. Mol. Biol., 262:732-745 (1996)), and/or the automatic modeling and analysis tool Honegger A, Plückthun A. (world wide web at bioc dot uzh dot ch/antibody/Numbering/index dot html).

**[0058]** An "antibody variable light chain" or an "antibody variable heavy chain" as used herein refers to a polypeptide comprising the VL or VH, respectively. The endogenous VL is encoded by the gene segments V (variable) and J (junctional), and the endogenous VH by V, D (diversity), and J. Each of VL or VH includes the CDRs as well as the framework regions. In this application, antibody variable light chains and/or antibody variable heavy chains may, from time to time, be collectively referred to as "antibody chains." These terms encompass antibody chains containing mutations that do not disrupt the basic structure of VL or VH, as one skilled in the art will readily recognize. In some embodiments, full length heavy

and/or light chains are contemplated. In some embodiments, only the variable region of the heavy and/or light chains are contemplated as being present.

**[0059]** Antibodies can exist as intact immunoglobulins or as a number of fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce F(ab)'2, a dimer of Fab' which itself is a light chain (VL-CL) joined to VH-CH1 by a disulfide bond. The F(ab)'2 may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the F(ab)'2 dimer into an Fab' monomer. The Fab' monomer is a Fab with part of the hinge region. (Paul, W. E., "Fundamental Immunology," 3d Ed., New York: Raven Press, 1993). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized *de novo* either chemically or by using recombinant DNA methodology. Thus, the term "antibody," as used herein, also includes antibody fragments either produced by the modification of whole antibodies, or those synthesized *de novo* using recombinant DNA methodologies (for example, single chain Fv) or those identified using phage display libraries (*see*, for example, McCafferty, J. et al., "Phage antibodies: filamentous phage displaying antibody variable domains," Nature, Vol. 348, No. 66301, pp. 552-554, 1990).

**[0060]** For preparation of monoclonal or polyclonal antibodies, any technique known in the art can be used (*see*, for example, Kohler, G. et al., "Continuous cultures of fused cells secreting antibody of predefined specificity," Nature, Vol. 256, No. 5517, pp. 495-497, 1975; Kozbor, D. et al., "The production of monoclonal antibodies from human lymphocytes," Immunology Today, Vol. 4, No. 3, pp. 72-79, 1983; Cole, et al., "Monoclonal Antibodies and Cancer Therapy," Alan R. Liss, Inc., pp. 77-96, 1985; Wang, S., "Advances in the production of human monoclonal antibodies," Antibody Technology Journal, Vol. 1, pp. 1-4, 2011; Sharon, J. et al., "Recombinant polyclonal antibodies for cancer therapy," J. Cell Biochem., Vol. 96, No. 2, pp. 305-313, 2005; Haurum, J. S., "Recombinant polyclonal antibodies: the next generation of antibody therapeutics?," Drug Discov. Today, Vol. 11, No. 13-14, pp. 655-660, 2006). Techniques for the production of single chain antibodies (U.S. Pat. No. 4,946,778) can be adapted to produce antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express fully human monoclonal antibodies. Alternatively, phage display technology can be used to identify high affinity

binders to selected antigens (*see*, for example, McCafferty et al., supra; Marks, J. D. et al., "By-passing immunization: building high affinity human antibodies by chain shuffling," Biotechnology (N. Y.), Vol. 10, No. 7, pp. 779-783, 1992).

**[0061]** Methods for humanizing or primatizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which 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. In some embodiments, the terms "donor" and "acceptor" sequences can be employed. Humanization can be essentially performed following the method of Winter and co-workers (*see*, for example, Jones, P. T. et al., "Replacing the complementarity-determining regions in a human antibody with those from a mouse," Nature, Vol. 321, No. 6069, pp. 522-525, 1986; Riechmann, L. et al., "Reshaping human antibodies for therapy," Nature, Vol. 332, No. 6162, pp. 323-327, 1988; Verhoeyen, M. et al., "Reshaping human antibodies: grafting an antilysozyme activity," Science, Vol. 239, No. 4847, pp. 1534-1536, 1988; Presta, L. G., "Antibody engineering,", Curr. Op. Struct. Biol., Vol. 2, No. 4, pp. 593-596, 1992), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such humanized antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some complementarity determining region ("CDR") residues and possibly some framework ("FR") residues are substituted by residues from analogous sites in rodent antibodies.

**[0062]** A "chimeric antibody" is an antibody molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, for example, an enzyme, toxin, hormone, growth factor, and drug; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity.

**[0063]** A pharmaceutically acceptable carrier may be a pharmaceutically acceptable material, composition, or vehicle that is involved in carrying or transporting a

compound of interest from one tissue, organ, or portion of the body to another tissue, organ, or portion of the body. For example, the carrier may be a liquid or solid filler, diluent, excipient, solvent, or encapsulating material, or some combination thereof. Each component of the carrier is "pharmaceutically acceptable" in that it is be compatible with the other ingredients of the formulation. It also must be suitable for contact with any tissue, organ, or portion of the body that it may encounter, meaning that it must not carry a risk of toxicity, irritation, allergic response, immunogenicity, or any other complication that excessively outweighs its therapeutic benefits. The pharmaceutical compositions described herein may be administered by any suitable route of administration. A route of administration may refer to any administration pathway known in the art, including but not limited to aerosol, enteral, nasal, ophthalmic, oral, parenteral, rectal, transdermal (for example, topical cream or ointment, patch), or vaginal. "Transdermal" administration may be accomplished using a topical cream or ointment or by means of a transdermal patch. "Parenteral" refers to a route of administration that is generally associated with injection, including infraorbital, infusion, intraarterial, intracapsular, intracardiac, intradermal, intramuscular, intraperitoneal, intrapulmonary, intraspinal, intrasternal, intrathecal, intrauterine, intravenous, subarachnoid, subcapsular, subcutaneous, transmucosal, or transtracheal. In some embodiments, the antigen binding construct can be delivered intraoperatively as a local administration during an intervention or resection.

**[0064]** The phrase "specifically (or selectively) bind," when used in the context of describing the interaction between an antigen, for example, a protein, to an antibody or antibody-derived binding agent, refers to a binding reaction that is determinative of the presence of the antigen in a heterogeneous population of proteins and other biologics, for example, in a biological sample, for example, a blood, serum, plasma or tissue sample. Thus, under designated immunoassay conditions, in some embodiments, the antibodies or binding agents with a particular binding specificity bind to a particular antigen at least two times the background and do not substantially bind in a significant amount to other antigens present in the sample. Specific binding to an antibody or binding agent under such conditions may require the antibody or agent to have been selected for its specificity for a particular protein. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select

antibodies specifically immunoreactive with a protein (*see*, for example, Harlow, E. & Lane D., "Using Antibodies, A Laboratory Manual," Cold Spring Harbor Laboratory Press, 1998, for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity). Typically a specific or selective binding reaction will produce a signal at least twice over the background signal and more typically at least than 10 to 100 times over the background.

**[0065]** The term "equilibrium dissociation constant (KD, M)" refers to the dissociation rate constant ($k_d$, $time^{-1}$) divided by the association rate constant (ka, $time^{-1}$ $M^{-1}$). Equilibrium dissociation constants can be measured using any known method in the art. The antibodies of the present invention generally will have an equilibrium dissociation constant of less than about $10^{-7}$ or $10^{-8}$ M, for example, less than about $10^{-9}$ M or $10^{-10}$ M, in some embodiments, less than about $10^{-11}$ M, $10^{-12}$ M, or $10^{-13}$ M.

**[0066]** The term "isolated," when applied to a nucleic acid or protein, denotes that the nucleic acid or protein is essentially free of other cellular components with which it is associated in the natural state. In some embodiments, it can be in either a dry or aqueous solution. Purity and homogeneity can be determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high-performance liquid chromatography. A protein that is the predominant species present in a preparation is substantially purified. In particular, an isolated gene is separated from open reading frames that flank the gene and encode a protein other than the gene of interest. The term "purified" denotes that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel or size exclusion chromatography analysis. In some embodiments, this can denote that the nucleic acid or protein is at least 85% pure, more preferably at least 95% pure, and most preferably at least 99% pure of molecules that are present under *in vivo* conditions.

**[0067]** The term "nucleic acid" or "polynucleotide" refers to deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (for example, degenerate codon substitutions), alleles,

orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer, M. A. et al., "Enhanced evolutionary PCR using oligonucleotides with inosine at the 3'-terminus," Nucleic Acid Res., Vol. 19, No. 18, pp. 5081, 1991; Ohtsuka, E. et al., "An alternative approach to deoxyoligonucleotides as hybridization probes by insertion of deoxyinosine at ambiguous codon positions," J. Biol. Chem., Vol. 260, No. 5, pp. 2605-2608, 1985; Rossolini, G. M. et al., "Use of deoxyinosine-containing primers vs degenerate primers for polymerase chain reaction based on ambiguous sequence information," Mol. Cell. Probes, Vol. 8, No. 2, pp. 91-98, 1994).

**[0068]** The terms "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer.

**[0069]** The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, for example, hydroxyproline, gamma-carboxyglutamate, and O-phosphoserine. Amino acid analogs refer to compounds that have the same basic chemical structure as a naturally occurring amino acid, for example, an alpha-carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, for example, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (for example, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

**[0070]** The term "conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refer to those nucleic acids which encode identical or essentially identical

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

**[0071]** As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

**[0072]** The following eight groups each contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (*see*, for example, Creighton, T. E., "Proteins - Structures and Molecular Properties," W. H. Freeman & Co. Ltd., 1984).

**[0073]** The term "percentage of sequence identity" can be determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (for example, a polypeptide of the invention),

which does not comprise additions or deletions, for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

**[0074]** The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same sequences. Two sequences are "substantially identical" if two sequences have a specified percentage of amino acid residues or nucleotides that are the same (for example, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity over a specified region, or, when not specified, over the entire sequence of a reference sequence), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Some embodiments provided herein provide polypeptides or polynucleotides that are substantially identical to the polypeptides or polynucleotides, respectively, exemplified herein. Optionally, the identity exists over a region that is at least about 15, 25 or 50 nucleotides in length, or more preferably over a region that is 100 to 500 or 1000 or more nucleotides in length, or over the full length of the reference sequence. With respect to amino acid sequences, identity or substantial identity can exist over a region that is at least 5, 10, 15 or 20 amino acids in length, optionally at least about 25, 30, 35, 40, 50, 75 or 100 amino acids in length, optionally at least about 150, 200 or 250 amino acids in length, or over the full length of the reference sequence. With respect to shorter amino acid sequences, for example, amino acid sequences of 20 or fewer amino acids, in some embodiments, substantial identity exists when one or two amino acid residues are conservatively substituted, according to the conservative substitutions defined herein.

**[0075]** In some embodiments, the percent identity is over the hinge regions noted herein (the hinge region and/or its subparts of upper, core, and lower hinge regions). In such situations, the percent identity of the hinge region or its subpart can be identified separately from the rest of the protein or nucleic acid sequence. Thus, two hinge regions (or upper, core, and/or lower regions) can have a specified percentage of amino acid residues or nucleotides

that are the same (for example, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity over a specified region, or, when not specified, over the entire sequence of a reference sequence), while allowing for the remainder of the protein to either stay 100% identical to the comparison protein, our while also allowing the remainder of the protein to also have variation by a specified percent identity.

**[0076]** For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

**[0077]** A "comparison window", as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison can be conducted, for example, by the local homology algorithm of Smith and Waterman (1970) Adv. Appl. Math. 2:482c, by the homology alignment algorithm of Needleman, S. B. et al., "A general method applicable to the search for similarities in the amino acid sequence of two proteins," J. Mol. Biol., Vol. 48, No. 3, pp. 443-453, 1970, by the search for similarity method of Pearson, W. R. et al., "Improved tools for biological sequence comparison," Proc. Natl. Acad. Sci. U.S.A., Vol. 85, No. 8, pp. 2444-2448, 1988, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by manual alignment and visual inspection (*see*, for example, Ausubel, F. M. et al., Current Protocols in Molecular Biology, Supplement, 1995).

**[0078]** Two examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul, S. F. et al., "Gapped BLAST and PSI-BLAST: a new generation of

protein database search programs," Nucleic Acids Res., Vol. 25, No. 17, pp. 3389-3402, 1977, and Altschul, S. F. et al., "Basic local alignment search tool," J. Mol. Biol., Vol. 215, No. 3, pp. 403-410, 1990, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul, S. F. et al., supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) or 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (*see*, Henikoff, S. et al., "Amino acid substitution matrices from protein blocks," Proc. Natl. Acad. Sci. U.S.A., Vol. 89, No. 22, pp. 10915-10919, 1992) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

**[0079]** The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (*see*, for example, Karlin, S. et al., "Applications and statistics for multiple high-scoring segments in molecular sequences," Proc. Natl. Acad. Sci. U.S.A., Vol. 90, No. 12, pp. 5873-5787, 1993). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the

smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

**[0080]** An indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the antibodies raised against the polypeptide encoded by the second nucleic acid, as described below. Thus, in some embodiments, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules or their complements hybridize to each other under stringent conditions, as described below. Yet another indication that two nucleic acid sequences are substantially identical is that the same primers can be used to amplify the sequence.

**[0081]** The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. The term is intended to include non-radioactive isotopes (ADC), radioactive isotopes (e.g., Lu177, Ac225, Cu67, Th227, At.211, I131, I125, Y90, Re186, Re188, Sm153, Bi212, 213Bi, P32, Tb161, Pb212 and radioactive isotopes of Lu), chemotherapeutic agents (as defined elsewhere herein). Other cytotoxic agents are described below. A tumoricidal agent causes destruction of tumor cells.

**[0082]** A "toxin" is any substance capable of having a detrimental effect on the growth or proliferation of a cell.

**[0083]** A "therapeutic ion" refers to an electrically charged particle that that is useful in the treatment of a disorder related to a target molecule. Examples of therapeutic ions include $^{18}F$, $^{18}F$-FAC, $^{32}P$, $^{33}P$, $^{45}Ti$, $^{47}Sc$, $^{52}Fe$, $^{59}Fe$, $^{62}Cu$, $^{64}Cu$, $^{67}Cu$, $^{67}Ga$, $^{68}Ga$, $^{75}Sc$, $^{77}As$, $^{86}Y$, $^{90}Y$, $^{89}Sr$, $^{89}Zr$, $^{94}Tc$, $^{94}Tc$, $^{99}mTc$, $^{99}Mo$, $^{105}Pd$, $^{105}Rh$, $^{111}Ag$, $^{111}In$, $^{123}I$, $^{124}I$, $^{125}I$, $^{131}I$, $^{142}Pr$, $^{143}Pr$, $^{149}Pm$, $^{149}Tb$, $^{153}Sm$, $^{154\text{-}158}Gd$, $^{161}Tb$, $^{166}Dy$, $^{166}Ho$, $^{169}Er$, $^{175}Lu$, $^{177}Lu$, $^{186}Re$, $^{188}Re$, $^{189}Re$, $^{194}Ir$, $^{198}Au$, $^{199}Au$, $^{211}At$, $^{211}Pb$, $^{212}Bi$, $^{212}Pb$, $^{213}Bi$, $^{223}Ra$, $^{227}Th$ and $^{225}Ac$. These are also options of therapeutic agents.

**[0084]** A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and CYTOXANTM cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and

piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOLTM); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTINTM), CPT-11 (irinotecan, CAMPTOSARTM), acetylcamptothecin, scopolectin, and 9-aminocamptothecin); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (e. g., calicheamicin, especially calicheamicin gammall and calicheamicin omegall (see, e.g., Agnew, Chem Intl. Ed. Engl., 33: 183-186 (1994)); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCINTM doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; metabolic inhibitor such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate,

epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK.RTM. polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINETM, FILDESINTM); dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); thiotepa; taxoids, e.g., TAXOL.RTM. paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANETM Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, Ill.), and TAXOTERETM docetaxel (Rhone-Poulenc Rorer, Antony, France); chloranbucil; gemcitabine (GEMZARTM); 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine (VELBANTM); platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine (ONCOVINTM); oxaliplatin; leucovovin; vinorelbine (NAVELBINETM); novantrone; edatrexate; daunomycin; aminopterin; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine (XELODATM); pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone, and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATINTM) combined with 5-FU and leucovovin. These are also options of therapeutic agents.

**[0085]** The terms "subject," "patient," and "individual" interchangeably refer to an entity that is being examined and/or treated. This can include, for example, a mammal, for example, a human or a non-human primate mammal. The mammal can also be a laboratory mammal, for example, mouse, rat, rabbit, hamster. In some embodiments, the mammal can be

an agricultural mammal (for example, equine, ovine, bovine, porcine, camelid) or domestic mammal (for example, canine, feline).

**[0086]** The term "and/or" shall be taken to provide explicit support for both meanings or for either meaning.

Antibodies

**[0087]** As discussed herein, antibodies having enhanced biodistribution and/or pharmacokinetics obtained through hyper- or overconjugation of the antibody with a negatively charged organic moiety are provided. Provided herein is an antibody (e.g., overconjugated antibody) that varies from an original antibody at least by having a negatively charged organic moiety associated therewith, wherein the negatively charged moiety confers a surface-exposed negative charge to the antibody, wherein the antibody (e.g., overconjugated antibody) has at least about 10% more total surface-exposed negative charge conferred by the negatively charged organic moiety relative to the original antibody. Conjugation of the negatively charged organic moiety to the original antibody can lead to overconjugation. In some embodiments, the antibody is any antibody construct. In some embodiments, overconjugation is especially useful for constructs about the same size as or smaller than a minibody or cys-diabody. In some embodiments, the overconjugation is especially useful in constructs that are less than 90 kDa in size.

**[0088]** In some embodiments, the antibody has at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200% or greater, or a percentage in a range defined by any two of the preceding values (e.g., 10-200%, 10-50%, 20-100%, 20-80%, 30-90%, 50-150%, etc.) more total surface-exposed negative charge conferred by the negatively charged organic moiety relative to the original antibody. In some embodiments, the antibody has at least about 30% more total surface-exposed negative charge conferred by the negatively charged organic moiety relative to the original antibody. In some embodiments, an increase in the total surface-exposed negative charge conferred by the negatively charged organic moiety relative to the original antibody includes a reduction in surface-exposed positive charge. In some embodiments, an increase in the total surface-exposed negative charge conferred by the negatively charged organic moiety relative to the original antibody includes a sum of an

increase in the surface-exposed negative charge and a reduction in surface-exposed positive charge.

**[0089]** The surface-exposed negative charge of the antibody can be determined using any suitable option. In some embodiments, isopotential surfaces of the antibody are determined using the Adaptive Poisson-Boltzmann Solver, the Van der Waals radii, Solvent Accessible Area, or a combination thereof.

**[0090]** The antibody can include any suitable negatively charged organic moiety. In some embodiments, the negatively charged organic moiety has a molecular weight of at least about 400 Da, e.g., at least about 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000 Da or more, or a molecular weight in a range defined by any two of the preceding values (e.g., 500-2000 Da, 700-1500 Da, 1000-1200 Da, , etc.). In some embodiments, the negative adducts used are IRDye800 (1166.20 Da), DTPA (704.0 Da); DOTA (551.6 Da); and/or DOTAGA (458.46 Da). The neutral adduct DFO is 752.9 Da.

**[0091]** In general, the negatively charged organic moiety has at least a net negative charge under physiological conditions (e.g., physiological pH, or pH 7.2-7.5, or pH 7.3-7.4). In some embodiments, the negatively charged organic moiety includes a negative charge of -3 or more negative, e.g., -4 or more negative, or -5 or more negative, -6 or more negative, -7 or more negative, -8 or more negative, -9 or more negative, -10 or more negative, or a charge in a range defined by any two of the preceding values (e.g., -6 to -3, or -6 to -4) under physiological conditions (e.g., physiological pH). In some embodiments, the negatively charged organic moiety includes a negative charge of -4 to -6 under physiological conditions (e.g., physiological pH). Suitable, non-limiting examples of negative adducts carry the following negative charges at physiological pH: DTPA: -5; DOTA: -4; DOTAGA: -4; IRDye®800: -4.

**[0092]** In some embodiments, the negatively charged organic moiety includes a negatively charged chelating ligand or organic dye. As used herein, "chelating ligand" has its customary and ordinary meaning as understood by one of ordinary skill in the art in view of the present disclosure. A chelating ligand denotes a molecule that can form multiple coordinate bonds with a metal ion (e.g., a radionuclide) to form a metal-chelating ligand complex. Any suitable negatively charged chelating ligand can be used. Suitable negatively charged chelating ligands include, without limitation, ethylenediaminetetraacetic acid (EDTA),

diethylenetriaminepentaacetic acid (DTPA), 2,2′,2″,2‴-(1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayl)tetraacetic acid (DOTA), 2,2′,2″-(10-(4-((2-aminoethyl)amino)-1-carboxy-4-oxobutyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (DOTAGA), 2,2′,2″-(1,4,7-triazacyclononane-1,4,7-triyl)triacetic acid (NOTA), 2,2′-(7-(1-carboxy-4-((2,5-dioxopyrrolidin-1-yl)oxy)-4-oxobutyl)-1,4,7-triazonane-1,4-diyl)diacetic acid (NODAGA), 4-[2-(bis-carboxymethyl-amino)-ethyl]-7-carboxymethyl-[1,4,7]triazonan-1-yl-acetic acid (NETA), porphyrins, polyamines, crown ethers, bis-thiosemicarbazones, and polyoximes. In some embodiments, the negatively charged chelating ligand is DTPA, DOTA or DOTAGA. Any suitable negatively charged organic dye can be used as the negatively charged organic moiety. In some embodiments, the negatively charged organic dye is IRDye®800.

**[0093]** In some embodiments, the antibody (e.g., overconjugated antibody) has a negatively charged organic moiety-to-antibody ratio (sometimes called "negative adduct to antibody ratio" or "chelator to minibody ratio" (CMR)) of at least about 1.2, e.g., at least about 1.5, about 2, about 2.5, about 3, about 3.5, about 4, about 4.5, about 5, about 5.5, about 6, about 7 or greater, or at least a ratio value in a range defined by any two of the preceding values (e.g., 1.2-6, 1.2-5, 1.5-5, 2-6, 3-5, 4-6, etc.). In some embodiments, the negatively charged organic moiety-to-antibody ratio of at least about 1.2 applies to negatively charged organic moieties having a charge of -6 or a more positive charge (e.g., a negative charge of -6 to -1, -6 to -2, -6 to -3, or -6 to -4).

**[0094]** The negatively charged organic moiety-to-antibody ratio or negative adduct to antibody ratio or chelator to minibody ratio (CMR) denotes the average number of organic moiety (e.g., chelating ligand) conjugated to the antibody (e.g., minibody or cys-diabody) per unit number of antibody (as a dimer). In some embodiments, the antibody (e.g., overconjugated antibody) has a negatively charged organic moiety-to-antibody ratio of at least about 3. In some embodiments, the antibody (e.g., overconjugated antibody) has a negatively charged organic moiety-to-antibody ratio of at least about 5.0. The negatively charged organic moiety-to-antibody ratio can be determined using any suitable option, e.g., UV-Vis spectroscopy or mass spectrometry. The negatively charged organic moiety-to-antibody ratio is determined as an average value for a composition containing the conjugated antibody. In general, the negatively charged organic moiety-to-antibody ratio "of an antibody" refers to the average value of the

number of organic moiety conjugated to the antibody per unit number of antibody when the ratio is measured in a composition of the conjugated antibody.

**[0095]** In some embodiments, the negatively charged organic moiety-to-antibody ratio is lower than 1.2 where the charge on the organic moiety is more negative. For example, an organic moiety having a negative charge of -8 may require fewer (e.g., about half the number) of moieties than an organic moiety having a negative charge of -4 to achieve a comparable change in biodistribution when the negatively charged organic moiety is conjugated to the antibody, as described herein. In some embodiments, the negatively charged organic moiety-to-antibody ratio is 1.2 or less, e.g., 1.0 or less, 0.8 or less, or 0.5 or less, or a number in a range defined by any two of the preceding values (e.g., 0.5-1.2, 0.5-1.0, 0.8-1.2, etc.) where the organic moiety as a negative charge of -6 or more negative, e.g., -7, -8, -9, or more negative. Thus, in some embodiments, the effective charge provided by the conjugation approach can be adjusted based on the amount of negative charge provided by the moiety (e.g., one may want more of a moiety with a single negative charge or could use less of a moiety with multiple negative charges). In such embodiments, the ratio can be reduced beneath 1.2 or even 1 (e.g., to 0.5).

**[0096]** In some embodiments, the antibody is a radiolabeled antibody. In some embodiments, the antibody is radiolabeled via a chelating ligand. In some embodiments, a chelating ligand is conjugated to the antibody and is configured to function as a chelator for one or more radionuclides. In some embodiments, the antibody is radiolabeled by having the chelating ligand conjugated thereto, and further radiolabeling with one or more radionuclides. Any suitable chelating ligand for radiolabeling an antibody can be used. In some embodiments, the chelating ligand includes one or more of ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), DOTA, DOTAGA, NOTA, NODAGA, NETA, deferoxamine (Df or DfO), porphyrins, polyamines, crown ethers, bis- thiosemicarbazones, and polyoximes. In some embodiments, the chelating ligand includes deferoxamine (Df).

**[0097]** In some embodiments, the antibody is radiolabeled via the negatively charged chelating ligand, e.g., DOTA, DOTAGA or DTPA. Thus, in some embodiments, the negatively charged organic moiety serves as a chelating ligand for radiolabeling, and for providing overconjugation of the antibody (e.g., to increase the surface-exposed negative charge). In some embodiments, the negatively charged organic moiety is a negatively charged

chelating ligand, and wherein the overconjugated antibody is radiolabeled via the negatively charged chelating ligand.

**[0098]** In some embodiments, the antibody is further conjugated to a therapeutic agent, as described herein.

**[0099]** In some embodiments, the antibody for which biodistribution is to be modified according to the present disclosure has one or more patches of surface-exposed charges (e.g., negative patches or positive patches) that bias biodistribution. In some embodiments, the antibody before conjugation of the negatively charged organic moiety thereto, has one or more positive patches that promote accumulation of the antibody in the kidneys. In some embodiments, the antibody includes at least one cluster of surface-exposed positively charged amino acids, the cluster having at least two surface-exposed, positively charged amino acids within about 30 angstroms of each other, e.g., within about 25, 20, 15, 14, 13, 12, 11, 10, or about 5 angstroms or less of each other, or within a distance in a range defined by any two of the preceding values (e.g., 30-5 angstroms, 30-10 angstroms, 20-5 angstroms, 15-10 angstroms, 12-10 angstroms). In some embodiments, the cluster includes 2, 3, 4, 5, 6 or more surface-exposed, positively charged amino acids within about 30 angstroms of each other. In some embodiments, a cluster of surface-exposed positively charged amino acids is within a polypeptide of the antibody. In some embodiments, the cluster of surface-exposed positively charged amino acids is in a framework region (FR) or a hinge region of the antibody.

**[0100]** In some embodiments, the surface-exposed positively charged amino acids of the cluster are outside of any CDR of the antibody (e.g., the original antibody). In some embodiments, the cluster is in a light chain variable region (VL) of the antibody (e.g., in a framework region of VL). In some embodiments, the cluster is in a VL FR2 of the antibody. In some embodiments, the cluster is in a hinge region, e.g., the upper hinge region, of the antibody.

**[0101]** In some embodiments, at least two of the surface-exposed positively charged amino acids of the cluster in the original antibody are within a contiguous stretch of 15 or fewer (e.g., 15, 12, 10, 8, 7, 6, 5, 4, 3, or 2) residues in a polypeptide of the antibody. In some embodiments, at least two of the surface-exposed positively charged amino acids of the cluster in the antibody are at least 20, at least 25, at least 30, at least 40, at least 50, at least 60,

least 75, at least 100, at least 150, at least 200, or more residues apart, or are apart by a number of residues in a range defined by any two of the preceding values (e.g., 20-200, 20-50, 40-75, 30-100, 50-150, 100-200, etc.), in a polypeptide of the antibody.

**[0102]** In some embodiments, the antibody has a molecular weight (as a dimer of polypeptides, e.g., excluding any non-polypeptide features) of about 10, 20, 30, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, or about 100 kDa, or a molecular weight (as a dimer of polypeptides, e.g., excluding any non-polypeptide features) in a range defined by any two of the preceding values. In some embodiments, the antibody has a molecular weight (as a dimer of polypeptides, e.g., excluding any non-polypeptide features) of about 10-100 kDa, e.g., about 10-90 kDa, about 30-100 kDa, about 40-90 kDa, or about 30-80 kDa. In some embodiments, the antibody has a molecular weight (as a dimer of polypeptides, e.g., excluding any non- polypeptide features) of about 40-60 kDa, about 70-90 kDa, or about 45-85 kDa. In some embodiments, the antibody has a molecular weight (as a dimer of polypeptides, e.g., excluding any non-polypeptide features) in a range of about 40 kDa to about 90 kDa. In some embodiments, the antibody has a molecular weight (as a dimer of polypeptides, e.g., excluding any non-polypeptide features) of about 100 kDa or less, e.g., about 90 kDa or less, or about 80 kDa or less.

**[0103]** In some embodiments, where the antibody is derived from an original antibody, the polypeptide portions of the antibody conjugate (e.g., the overconjugated antibody) or radiolabeled antibody is substantially identical to the corresponding polypeptide portions of the original antibody, other than the bonding pattern of the negatively charged organic moiety. In some embodiments, the variable regions of the antibody conjugate or radiolabeled antibody (e.g., $V^L$ and $V_H$) are substantially identical to the corresponding variable regions of the original antibody, other than any potential bonding pattern of the negatively charged organic moiety.

**[0104]** In some embodiments, the antibody includes an antigen-binding fragment, such as, without limitation, a scFv fragment. In some embodiments, the antibody is a minibody or a cys-diabody. In some embodiments, the antibody is a minibody. In some embodiments, the antibody is a cys-diabody.

**[0105]** Also provided is an antibody (e.g., overconjugated antibody) that varies from an original antibody by having a negatively charged organic moiety associated therewith,

wherein the antibody is a minibody or cys-diabody, wherein the negatively charged organic moiety confers a surface-exposed negative charge to the antibody, wherein the antibody has at least about 10% more total surface-exposed negative charge conferred by the negatively charged organic moiety relative to the original antibody, and wherein the antibody has a negatively charged organic moiety-to-antibody ratio of at least about 1.2 (e.g., at least about 2, 3, 3.5, 4, 4.5, 5, 5.5, 6, 7, or more).

**[0106]** In any of the antibodies, minibodies, or cys-diabodies (e.g., conjugates thereof) of the present disclosure, in some embodiments, the average number of the negatively charged organic moiety per antibody, minibody or cys-diabody is about 1.2 or more, e.g., about 1.5 or more, about 2 or more, about 2.5 or more, about 3 or more, about 3.5 or more, about 4 or more, about 4.5 or more, about 5 or more, about 5.5 or more, about 6 or more, about 6.5 or more, about 7 or more, about 7.5 or more, about 8 or more, or a number in range defined by any two of the preceding values (e.g., 1.2-8, 1.2-7, 1.5-6, 2-6, 3-7, 5-8, 5-6, etc.). In some embodiments, the average number of the negatively charged organic moiety per antibody, minibody or cys-diabody is 1.2 or more. In some embodiments, the average number of the negatively charged organic moiety per antibody, minibody or cys-diabody is 3 or more. In some embodiments, the average number of the negatively charged organic moiety per antibody, minibody or cys-diabody is 5 or more.

**[0107]** In any of the antibodies, minibodies, or cys-diabodies (e.g., conjugates thereof) of the present disclosure, in some embodiments, the antibody, minibody or cys-diabody binds specifically to any suitable antigen target. In some embodiments, the antibody, minibody, or cys-diabody specifically binds to Delta-like ligand 3 (DLL3), Fibroblast activation protein (FAP), CD8, CD4, CD3, interferon γ (IFNγ), integrin αVβ6, folate receptor α (FOLRα), or prostate-specific membrane antigen (PSMA). In some embodiments, the antibody, minibody, or cys-diabody includes a light chain variable region having 3 LCDR sequences (e.g., LCDR1, LCDR2, and LCDR3) of the 3 corresponding LCDRs in any one of the amino acid sequences shown in FIGs. 12A-12D, and a heavy chain having 3 HCDR sequences (e.g., HCDR1, HCDR2, HCDR3) of the 3 corresponding HCDRs in any one of the amino acid sequences shown in FIGs. 12A-12D.

**[0108]** The antibody, minibody, or cys-diabody can have monovalent, bivalent, or multivalent antigen binding specificity. In some embodiments, the antibody, minibody, or cys-

diabody is monovalent. In some embodiments, the antibody, minibody, or cys-diabody is bivalent. A bispecific antibody, minibody, or cys-diabody can have two different heavy/light chain pairs and/or it can recognize two different epitopes. In some embodiments, the antibody, minibody, or cys-diabody is multivalent.

Compositions

**[0109]** Also provided is a composition, e.g., a pharmaceutical composition, that includes any of the antibody, minibody or cys-diabody of the present disclosure; and a pharmaceutically acceptable carrier. The composition can be a therapeutic composition, e.g., for use in treating a subject in need of treating a disease or condition. In some embodiments, the therapeutic composition is for use in treating cancer. In some embodiments, the pharmaceutically acceptable carrier includes a pharmaceutically acceptable buffer.

**[0110]** Also provided is a composition, e.g., a conjugation reaction composition, that includes an antibody (e.g., a minibody, a cys-diabody); and a negatively charged organic moiety or precursor thereof configured to be conjugated to the antibody, wherein the negatively charged organic moiety or precursor thereof is present in an amount sufficient to disrupt or conceal a positive patch on a surface of the antibody, or to confer an additional negative charge to the antibody, when conjugated thereto. In some embodiments, the negatively charged organic moiety includes a negatively charged chelating ligand or organic dye, as described herein. In some embodiments, a precursor of the negatively charged organic moiety includes a reactive group (such as, without limitation, a maleimide, N-hydroxysuccinimide, isothiocyanate) that allows the negatively charged organic moiety to conjugate to the antibody (e.g., form a covalent bond with a side chain group and/or backbone of a polypeptide of the antibody). In some embodiments, the antibody includes one or more positive patches (e.g., as described by an isopotential surface formed by a cluster of surface-exposed positively charged amino acids), and the composition includes the negatively charged organic moiety or precursor thereof configured to conjugate to the antibody and disrupt or conceal the positive patch, or confer an additional negative charge to the antibody. In some embodiments, the positive patch is outside of any CDR of the antibody. In some embodiments, the positive patch is in a framework region (FR) or hinge region of the antibody. In some embodiments, the positive patch is in a $V_L$ FR2 of the antibody. In some embodiments, the positive patch is in an upper hinge region of the antibody.

**[0111]** In some embodiments, the antibody includes a cluster of surface-exposed positively charged amino acids, the cluster comprising at least two surface-exposed, positively charged amino acids within about 30 angstroms of each other, e.g., within about 25, 20, 15, 14, 13, 12, 11, 10, or about 5 angstroms or less of each other, or within a distance in a range defined by any two of the preceding values (e.g., 30-5 angstroms, 30-10 angstroms, 20-5 angstroms, 15-10 angstroms, 12-10 angstroms). In some embodiments, the cluster includes 2, 3, 4, 5, 6 or more surface-exposed, positively charged amino acids within about 30 angstroms of each other.

**[0112]** In some embodiments, the negatively charged organic moiety is present in the composition in an amount sufficient to provide an antibody conjugate (e.g., an overconjugated antibody) having a negatively charged organic moiety-to-antibody ratio of at least about 1.2, e.g., at least about 1.5, about 2, about 2.5, about 3, about 3.5, about 4, about 4.5, about 5, about 5.5, about 6, about 7, or greater, or at least a ratio value in a range defined by any two of the preceding values (e.g., 1.2-6, 1.2-5, 1.5-5, 2-6, 3-5, 4-6, etc.). In some embodiments, the antibody is a radiolabeled antibody. In some embodiments, the antibody is radiolabeled via a second chelating ligand, such as, without limitation, deferoxamine (Df). In some embodiments, the antibody is further conjugated to a therapeutic agent.

**[0113]** The composition can include any suitable negatively charged organic moiety. In some embodiments, the composition includes any one of the negatively charged chelating ligands described herein. In some embodiments, the composition includes any one of the negatively charged organic dyes described herein.

Use As Imaging Agents

**[0114]** Any suitable radionuclide can be used to radiolabel an antibody or conjugate thereof of the present disclosure. In some embodiments, the radionuclide is one or more of: $^{18}F$, $^{18}F$-FAC, $^{32}P$, $^{33}P$, $^{45}Ti$, $^{47}Sc$, $^{52}Fe$, $^{59}Fe$, $^{62}Cu$, $^{64}Cu$, $^{67}Cu$, $^{67}Ga$, $^{68}Ga$, $^{75}Sc$, $^{77}As$, $^{86}Y$, $^{90}Y$, $^{89}Sr$, $^{89}Zr$, $^{94}Tc$, $^{94}Tc$, $^{99}mTc$, $^{99}Mo$, $^{105}Pd$, $^{105}Rh$, $^{111}Ag$, $^{111}In$, $^{123}I$, $^{124}I$, $^{125}I$, $^{131}I$, $^{142}Pr$, $^{143}Pr$, $^{149}Pm$, $^{153}Sm$, $^{154\text{-}158}Gd$, $^{161}Tb$, $^{166}Dy$, $^{166}Ho$, $^{169}Er$, $^{175}Lu$, $^{177}Lu$, $^{186}Re$, $^{188}Re$, $^{189}Re$, $^{194}Ir$, $^{198}Au$, $^{199}Au$, $^{211}At$, $^{211}Pb$, $^{212}Bi$, $^{212}Pb$, $^{213}Bi$, $^{223}Ra$, $^{225}Ac$, and $^{227}Th$. In some embodiments, exemplary paramagnetic ions substances that can be used as detectable markers include, but are not limited to, ions of transition and lanthanide metals (e.g. metals having atomic numbers of 6 to 9, 21-29, 42, 43, 44, or 57-71). These metals include ions of Cr, V, Mn, Fe, Co, Ni, Cu,

La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu. In some embodiments, the detectable label is a radionuclide such as Yttrium-90, Lutetium-177, or Actinium-225. Additional embodiments of a radionuclide include Copper-67, Astatine-211, Lead-212/Bismuth-212, Actinium-225/ Bismuth-213, and Thorium-227. In some embodiments, treatment of a target cell with these radionuclides can result in cell damage and death to a target tissue.

Use As Therapeutic Agents

**[0115]** Any of the antibodies, minibodies, or cys-diabodies (e.g., conjugate thereof) of the present disclosure, in some embodiments, can be comprised in a therapeutic composition or a pharmaceutical composition, e.g., for use in treating a subject in need of treating a disease or condition. In some embodiments, the therapeutic composition is for use in treating cancer. In some embodiments, a pharmaceutical composition comprises a pharmaceutically acceptable carrier such as a pharmaceutically acceptable buffer.

**[0116]** In some embodiments, an antibody, minibody, or cys-diabody (e.g., conjugate thereof) described herein is further conjugated to a therapeutic agent. A "therapeutic agent" as used herein is an atom, molecule, or compound that is useful in the treatment of a disorder related to a target molecule. Examples of therapeutic agents include, but are not limited to, drugs, small molecule drugs, chemotherapeutic agents, therapeutic antibodies and antibody fragments, toxins, radioisotopes, enzymes (for example, enzymes to cleave prodrugs to a cytotoxic agent at the site of the antigen binding construct binding), nucleases, hormones, immunomodulators, antisense oligonucleotides, chelators, boron compounds, photoactive agents and dyes, elastin-like polypeptides such as PLGA, and nanoparticles. Examples of disorders include those related to one or more target molecules.

**[0117]** In some embodiments, antibodies, minibodies, or cys-diabodies (e.g., conjugate thereof) described herein are further conjugated to a therapeutic agent. While minibodies, cys-diabodies, or other antigen binding fragments can have a shorter circulation half-life compared to a full-length antibody, in some embodiments, these formats can exhibit superior tumor penetration based on their smaller size and be therapeutically effective when appropriately armed with a cytotoxic drug or radioisotope. In some embodiments, an antibody, minibody, or cys-diabody, drug-conjugate approach can be employed. In some embodiments, a therapeutic approach includes radioimmunotherapy by attaching an appropriate radiolabel

such as, Iodine-131, a beta-emitter or alpha-emitter, such as, Yttrium-90, Lutetium-177, Copper-67, Astatine-211, Lead-212, Bismuth-212, Actinium-225, Bismuth-213, and Thorium-227, which can deliver cell damage and death to a target tissue. In some embodiments, treatment with these fragments armed with a cytotoxic drug or radionuclide result in less nonspecific toxicity as they will be cleared from the body more rapidly.

**[0118]** In some embodiments, the label and/or therapeutic agent comprises $^{18}F$, $^{18}F$-FAC, $^{32}P$, $^{33}P$, $^{45}Ti$, $^{47}Sc$, $^{52}Fe$, $^{59}Fe$, $^{62}Cu$, $^{64}Cu$, $^{67}Cu$, $^{67}Ga$, $^{68}Ga$, $^{75}Sc$, $^{77}As$, $^{86}Y$, $^{90}Y$, $^{89}Sr$, $^{89}Zr$, $^{94}Tc$, $^{94}Tc$, $^{99}mTc$, $^{99}Mo$, $^{105}Pd$, $^{105}Rh$, $^{111}Ag$, $^{111}In$, $^{123}I$, $^{124}I$, $^{125}I$, $^{131}I$, $^{142}Pr$, $^{143}Pr$, $^{149}Pm$, $^{149}Tb$, $^{153}Sm$, $^{154\text{-}158}Gd$, $^{161}Tb$, $^{166}Dy$, $^{166}Ho$, $^{169}Er$, $^{175}Lu$, $^{177}Lu$, $^{186}Re$, $^{188}Re$, $^{189}Re$, $^{194}Ir$, $^{198}Au$, $^{199}Au$, $^{211}At$, $^{211}Pb$, $^{212}Bi$, $^{212}Pb$, $^{213}Bi$, $^{223}Ra$, $^{227}Th$ and $^{225}Ac$, or any combination thereof.

**[0119]** In some embodiments, the antibody, minibody, and/or cys-diabody (e.g., conjugate thereof) described herein are further conjugated to a therapeutic agent such as a chemotherapeutic agent. Chemotherapeutic agents are often cytotoxic or cytostatic in nature and may include alkylating agents, antimetabolites, anti-tumor antibiotics, topoisomerase inhibitors, mitotic inhibitors hormone therapy, targeted therapeutics and immunotherapeutics. In some embodiments the chemotherapeutic agents that may be used as detectable markers in accordance with the embodiments of the disclosure include, but are not limited to, 13-cis-Retinoic Acid, 2-Chlorodeoxyadenosine, 5-Azacitidine, 5-Fluorouracil, 6-Mercaptopurine, 6-Thioguanine, actinomycin-D, adriamycin, aldesleukin, alemtuzumab, alitretinoin, all-transretinoic acid, alpha interferon, altretamine, amethopterin, amifostine, anagrelide, anastrozole, arabinosylcytosine, arsenic trioxide, amsacrine, aminocamptothecin, aminoglutethimide, asparaginase, azacytidine, bacillus calmette-guerin (BCG), bendamustine, bevacizumab, bexarotene, bicalutamide, bortezomib, bleomycin, busulfan, calcium leucovorin, citrovorum factor, capecitabine, canertinib, carboplatin, carmustine, cetuximab, chlorambucil, cisplatin, cladribine, cortisone, cyclophosphamide, cytarabine, darbepoetin alfa, dasatinib, daunomycin, decitabine, denileukin diftitox, dexamethasone, dexasone, dexrazoxane, dactinomycin, daunorubicin, decarbazine, docetaxel, doxorubicin, doxifluridine, eniluracil, epirubicin, epoetin alfa, erlotinib, everolimus, exemestane, estramustine, etoposide, filgrastim, fluoxymesterone, fulvestrant, flavopiridol, floxuridine, fludarabine, fluorouracil, flutamide, gefitinib, gemcitabine, gemtuzumab ozogamicin, goserelin, granulocyte - colony stimulating factor, granulocyte macrophage-colony stimulating factor, hexamethylmelamine,

hydrocortisone hydroxyurea, ibritumomab, interferon alpha, interleukin – 2, interleukin-11, isotretinoin, ixabepilone, idarubicin, imatinib mesylate, ifosfamide, irinotecan, lapatinib, lenalidomide, letrozole, leucovorin, leuprolide, liposomal Ara-C, lomustine, mechlorethamine, megestrol, melphalan, mercaptopurine, mesna, methotrexate, methylprednisolone, mitomycin C, mitotane, mitoxantrone, nelarabine, nilutamide, octreotide, oprelvekin, oxaliplatin, paclitaxel, pamidronate, pemetrexed, panitumumab, PEG Interferon, pegaspargase, pegfilgrastim, PEG-L-asparaginase, pentostatin, plicamycin, prednisolone, prednisone, procarbazine, raloxifene, rituximab, romiplostim, ralitrexed, sapacitabine, sargramostim, satraplatin, sorafenib, sunitinib, semustine, streptozocin, tamoxifen, tegafur, tegafur-uracil, temsirolimus, temozolamide, teniposide, thalidomide, thioguanine, thiotepa, topotecan, toremifene, tositumomab, trastuzumab, tretinoin, trimitrexate, alrubicin, vincristine, vinblastine, vindestine, vinorelbine, vorinostat, or zoledronic acid.

**[0120]** In some embodiments, the antibody, minibody, and/or cys-diabody (e.g., conjugate thereof) described herein are further conjugated to a cytotoxic agent (e.g., a toxin). A cytotoxic agent or toxin that may be used in accordance with the embodiments of the disclosure include, but are not limited to, Auristatin E, Auristatin F, Dolastatin 10, Dolastatin 15, combretastatin and their analogs, maytansinoid, calicheamicin, alpha-amanitin, pyrrolobenzodiazepine dimers, epothilones, duocarmycin and their analogs, tubulysin D, basillistatins, ricin, abrin, ribonuclease (RNase), DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtheria toxin, Pseudomonas exotoxin, and Pseudomonas endotoxin.

**[0121]** Any of the antibodies, minibodies, or cys-diabodies (e.g., conjugate thereof) described herein may be further conjugated with one or more additional therapeutic agents, detectable markers, nanoparticles, carriers or a combination thereof. For example, an antigen binding construct may be radiolabeled with Iodine-131 and conjugated to a lipid carrier, such that the anti-target molecule-lipid conjugate forms a micelle. The micelle can incorporate one or more therapeutic or detectable markers.

**[0122]** The pharmaceutical, or therapeutic, compositions described herein can be administered by any suitable route of administration. A route of administration can refer to any administration pathway known in the art, including but not limited to aerosol, enteral, nasal, ophthalmic, oral, parenteral, rectal, transdermal (e.g., topical cream or ointment, patch), or

vaginal. "Transdermal" administration can be accomplished using a topical cream or ointment or by means of a transdermal patch. "Parenteral" refers to a route of administration that is generally associated with injection, including infraorbital, infusion, intraarterial, intracapsular, intracardiac, intradermal, intramuscular, intraperitoneal, intrapulmonary, intraspinal, intrasternal, intrathecal, intraventricular, intrauterine, intravenous, subarachnoid, subcapsular, subcutaneous, transmucosal, or transtracheal. In some embodiments, the antigen binding construct can be delivered intraoperatively as a local administration during an intervention or resection.

Kits

**[0123]** Also provided is a kit for radiolabeling an antibody (e.g., minibody or cys-diabody) that includes a first chelating ligand for radiolabeling an antibody (e.g., minibody or cys-diabody); and a negatively charged organic moiety configured to be conjugated to the antibody. The kit can include any one of the antibodies (e.g., before conjugation to a negatively charged organic moiety) of the present disclosure. The first chelating ligand can be any suitable chelating ligand for radiolabeling the antibody, e.g., those described herein. Any suitable negatively charged organic moiety can be included in the kit, e.g., those described herein. In some embodiments, the kit includes a radionuclide. In some embodiments, the kit includes buffers. In some embodiments, the kit includes packaging. In some embodiments, the kit includes instructions.

Methods

**[0124]** Provided herein are methods of enhancing and/or making antibodies (e.g., minibodies, cys-diabodies) by overconjugation. With reference to Fig. 1A, a method of enhancing biodistribution and/or pharmacokinetics of a radiolabeled antibody is provided. The method **100A** can include, at block **110A**, selecting a first radiolabeled antibody that includes a first antibody and a radionuclide, wherein the first antibody is labeled with the radionuclide via a first chelating ligand. The method can include, at block **120A**, providing a second radiolabeled antibody that includes: the first antibody; the radionuclide, wherein the first antibody is labeled with the radionuclide via the first chelating ligand; and a negatively charged organic moiety conjugated thereto. The second radiolabeled antibody can be provided by conjugating the negatively charged organic moiety to the first antibody in an amount sufficient to provide the second radiolabeled antibody having at least 10% more surface-exposed

negative charge conferred by the conjugated negatively charged organic moiety compared to the first radiolabeled antibody, thereby generating a second radiolabeled antibody having enhanced biodistribution and/or pharmacokinetics compared to the first radiolabeled antibody. In some embodiments, the antibody is any antibody construct.

**[0125]** The first antibody can be radiolabeled and conjugated with the negatively charged organic moiety in any suitable order. In some embodiments, the first antibody is radiolabeled before conjugating the negatively charged organic moiety. In some embodiments, the first antibody is radiolabeled after conjugating the negatively charged organic moiety. In some embodiments, the first antibody is radiolabeled concurrently with conjugating the negatively charged organic moiety. In some embodiments, the first antibody is conjugated with the first chelating ligand before conjugating the negatively charged organic moiety. In some embodiments, the first antibody is conjugated with the first chelating ligand after conjugating the negatively charged organic moiety. In some embodiments, the first antibody is conjugated with the first chelating ligand concurrently with conjugating the negatively charged organic moiety.

**[0126]** In some embodiments, the first antibody is associated with an amount of surface-exposed positive charge. The surface-exposed positive charge associated with the first antibody can be determined using any suitable option, such as, but not limited to an Adaptive Poisson-Boltzmann Solver.

**[0127]** In some embodiments, the amount of negatively charged organic moiety used to conjugate the negatively charged organic moiety to the first antibody is sufficient to produce an antibody conjugate that has at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200% or greater, or a percentage in a range defined by any two of the preceding values (e.g., 10-200%, 10-50%, 20-100%, 20-80%, 30-90%, 50-150%, etc.) more surface-exposed negative charge conferred by the negatively charged organic moiety relative to the first radiolabeled antibody. In some embodiments, the antibody conjugate has at least about 30% more surface-exposed negative charge conferred by the negatively charged organic moiety relative to the original antibody.

**[0128]** In some embodiments, conjugating the negatively charged organic moiety to the first antibody includes obtaining a negatively charged organic moiety-to-antibody ratio of at least about 0.5, e.g., at least about 1, about 1.5, about 2, about 2.5, about 3, about 3.5,

about 4, about 4.5, about 5, about 5.5 about 6, or greater, or at least a ratio value in a range defined by any two of the preceding values (e.g., 0.5-6, 0.5-5, 1-5, 2-6, 3-5, 4-6, etc.). In some embodiments, conjugating the negatively charged organic moiety to the first antibody includes obtaining a negatively charged organic moiety-to-antibody ratio of at least about 3. In some embodiments, conjugating the negatively charged organic moiety to the first antibody includes obtaining a negatively charged organic moiety-to-antibody ratio of at least about 5.

**[0129]** With reference to Fig. 1B, a method of enhancing biodistribution and/or pharmacokinetics of a radiolabeled minibody or cys-diabody is provided. The method **100B** can include, at block **110B**, selecting a first radiolabeled antibody that includes: a first antibody, wherein the first antibody is a minibody or a cys-diabody; and a radionuclide, wherein the first antibody is labeled with the radionuclide via a first chelating ligand. The method can further include, at block **120B**, providing a second radiolabeled antibody that includes: the first antibody; the radionuclide, wherein the first antibody is labeled with the radionuclide via the first chelating ligand; and a negatively charged organic moiety conjugated thereto, by conjugating the negatively charged organic moiety to the first antibody so as to provide a negatively charged organic moiety-to-antibody ratio of at least about 1.2, e.g., at least about 1.5, about 2, about 2.5, about 3, about 3.5, about 4, about 4.5, about 5, about 5.5, about 6, about 7 or greater, or at least a ratio value in a range defined by any two of the preceding values (e.g., 1.2-6, 1.2-5, 1.5-5, 2-6, 3-5, 4-6, etc.). As noted above, when the amount of negative charge on the moiety is increased (to more negative charges), then the ratio can be adjusted from the above so as to require fewer individual moieties (as each moiety will carry multiple negative charges).

**[0130]** With reference to Fig. 2, a method of making a radiolabeled antibody is provided. The method **200** can includes, at block **210**, providing an antibody (e.g., a minibody, a cys-diabody); and at block **220**, labeling the antibody with a radionuclide via a first chelating ligand. The method can further include, at block **230**, conjugating a negatively charged organic moiety to the antibody, wherein the conjugating includes using a negatively charged organic moiety-to-antibody ratio of at least about 1.2. The antibody can be any suitable antibody, such as, without limitation, a minibody or cys-diabody. Radiolabeling the antibody and conjugating the negatively charged organic moiety to the antibody can be done in any suitable order. Thus, in Fig. 2, block **220** can be performed before block **230**, or block **230** can be performed before

block **220**. In some embodiments, block **220** and block **230** is performed concurrently. Alternatively, the method can be a method of making a therapeutic agent-antibody conjugate, where instead of the radionuclide, the antibody at block **220** is conjugated to a therapeutic agent.

**[0131]** In some embodiments, conjugating the negatively charged organic moiety to the antibody includes using a negatively charged organic moiety-to-antibody ratio of at least about 1.2, e.g., at least about 1.5, about 2, about 2.5, about 3, about 3.5, about 4, about 4.5, about 5, about 5.5, about 6, about 7 or greater, or at least a ratio value in a range defined by any two of the preceding values (e.g., 0.5-6, 0.5-5, 1-5, 2-6, 3-5, 4-6, etc.). In some embodiments, conjugating the negatively charged organic moiety to the antibody includes using a negatively charged organic moiety-to-antibody ratio of at least about 3. In some embodiments, conjugating the negatively charged organic moiety to the antibody includes using a negatively charged organic moiety-to-antibody ratio of at least about 5.

**[0132]** With reference to Fig. 3, a method of making an antibody conjugate is provided. The method **300** can include, at block **310**, providing an antibody (e.g., a minibody, a cys-diabody). The method can also include, at block **320**, conjugating a negatively charged organic moiety to the antibody to disrupt or conceal at least one positive patch on a surface of the antibody, or to confer an additional negative charge to the antibody, wherein the negatively charged organic moiety is a negatively charged chelating ligand or organic dye. In some embodiments, conjugating the negatively charged organic moiety to the first antibody includes obtaining a negatively charged organic moiety-to-antibody ratio of at least about 1.2, as disclosed herein.

**[0133]** In some embodiments, the antibody is radiolabeled, e.g., via a second chelating ligand. In some embodiments, the method includes labeling the antibody conjugate with a radionuclide. In some embodiments, the method includes contacting the antibody conjugate with a radionuclide.

**[0134]** In any method disclosed herein, in some embodiments the first or second chelating ligand includes deferoxamine (Df). In some embodiments, the first or second chelating ligand includes the negatively charged organic moiety (e.g., a negatively charged chelating ligand, as described herein).

**[0135]** In any method disclosed herein, in some embodiments, the antibody is further conjugated to a therapeutic agent, as described herein.

**[0136]** In any method disclosed herein, in some embodiments, the negatively charged organic moiety includes a negatively charged chelating ligand or organic dye. Any suitable negatively charged chelating ligand or organic dye, such as but not limited to those described herein, can be used. In some embodiments, the negatively charged chelating ligand includes DTPA, DOTA or DOTAGA. In some embodiments, the negatively charged organic dye includes is IRDye®800.

**[0137]** In any method of the present disclosure, in some embodiments, the antibody has a molecular weight (as a dimer of polypeptides, e.g., excluding any non-polypeptide features) of about 10, 20, 30, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, or about 100 kDa, or a molecular weight (as a dimer of polypeptides, e.g., excluding any non-polypeptide features) in a range defined by any two of the preceding values. In some embodiments, the antibody has a molecular weight (as a dimer of polypeptides, e.g., excluding any non-polypeptide features) of about 10-100 kDa, e.g., about 10-90 kDa, about 30-100 kDa, about 40-90 kDa, or about 30-80 kDa. In some embodiments, the antibody has a molecular weight (as a dimer of polypeptides, e.g., excluding any non-polypeptide features) of about 40-60 kDa, about 70-90 kDa, or about 45-85 kDa. In some embodiments, the antibody has a molecular weight (as a dimer of polypeptides, e.g., excluding any non-polypeptide features) in a range of about 40 kDa to about 90 kDa.

**[0138]** In any method, in some embodiments, the antibody includes an antigen-binding fragment, such as, without limitation, a scFv fragment. In some embodiments, the antibody is a minibody or a cys-diabody. In some embodiments, the antibody is a minibody. In some embodiments, the minibody includes a $C_H3$ having any one of the amino acid sequences shown in FIG. 13. In some embodiments, the minibody includes a $C_H3$ having at least 80%, 85%, 90%, 95%, 97%, or about 100% identity, or percent identity in a range defined by any two of the preceding values (e.g., 80-100%, 85-97%, 85-95%, 90-100%, etc.), to any one of the amino acid sequences shown in FIG. 13. In some embodiments, the antibody is a cys-diabody.

**[0139]** In some embodiments, the $V_L$ FR sequences (FR1, FR2, FR3, and FR4) of the antibody are each from the corresponding $V_L$ FR sequences (FR1, FR2, FR3, and FR4) of

any one of the sequences shown in FIGs. 12A-12D. In some embodiments, the $V_L$ FR sequences (FR1, FR2, FR3, FR4) are each at least 80%, 85%, 90%, 95%, 97%, or about 100% identical, or identical by a percentage in a range defined by any two of the preceding values (e.g., 80-100%, 85-97%, 85-95%, 90-100%, etc.), to the corresponding $V_L$ FR sequences (FR1, FR2, FR3, and FR4) of any one of the sequences shown in FIGs. 12A-12D. In some embodiments, the antibody includes heavy chain variable region ($V_H$) framework sequences (FR1, FR2, FR3, and FR4) of any one of the sequences shown in FIGs. 12A-12D. In some embodiments, the $V_H$ FR1, FR2, FR3, and FR4 are each at least 80%, 85%, 90%, 95%, 97%, or about 100% identical, or identical by a percentage in a range defined by any two of the preceding values (e.g., 80-100%, 85-97%, 85-95%, 90-100%, etc.), to the corresponding $V_H$ FR1, FR2, FR3, and FR4 of any one of the sequences shown in FIGs. 12A-12D. In some embodiments, the $V_L$ and $V_H$ FR sequences of the antibodies are paired according to any one of the constructs shown in FIGs. 12A-12D.

**[0140]** In any method, in some embodiments, the antibody includes a cluster of surface-exposed positively charged amino acids, the cluster comprising at least two surface-exposed, positively charged amino acids within about 30 angstroms of each other, e.g., within about 25, 20, 15, 14, 13, 12, 11, 10, or about 5 angstroms or less of each other, or within a distance in a range defined by any two of the preceding values (e.g., 30-5 angstroms, 30-10 angstroms, 20-5 angstroms, 15-10 angstroms, 12-10 angstroms). In some embodiments, the cluster includes 2, 3, 4, 5, 6 or more surface-exposed, positively charged amino acids within about 30 angstroms of each other.

**[0141]** In any of the methods of the present disclosure, in some embodiments, the antibody, minibody or cys-diabody binds specifically to any suitable antigen target. In some embodiments, the antibody, minibody, or cys-diabody specifically binds to DLL3, FAP, CD8, CD4, CD3, IFNγ, αVβ6, FOLRα, or PSMA. In some embodiments, the antibody, minibody, or cys-diabody includes a light chain variable region having 3 LCDR sequences (e.g., LCDR1, LCDR2, and LCDR3) of the 3 corresponding LCDRs in any one of the amino acid sequences shown in FIGs. 12A-12D, and a heavy chain having 3 HCDR sequences (e.g., HCDR1, HCDR2, HCDR3) of the 3 corresponding HCDRs in any one of the amino acid sequences shown in FIGs. 12A-12D.

**[0142]** The antibodies (e.g., minibodies, cys-diabodies) of the present disclosure can be produced using any suitable option. In some embodiments, any method of enhancing an antibody of the present disclosure includes producing the antibody. In some embodiments, the antibodies (e.g., minibodies, cys-diabodies) are produced by expressing a nucleic acid encoding the antibody (e.g., encoding monomer chains of the antibody) in a suitable host cell expression system (e.g., mammalian cell line, bacteria, insect cell line, yeast, etc.).

**[0143]** Conjugating a negatively charged organic moiety to an antibody can be done using any suitable option. In some embodiments, conjugating includes contacting the antibody with a reactive precursor of the negatively charged organic moiety under suitable conditions. In some embodiments, the reactive precursor of the negatively charged organic moiety can include one or more reactive groups that can form a covalent bond with a polypeptide of the antibody. In some embodiments, the reactive group includes, without limitation, maleimide, N-hydroxysuccinimide, and isothiocyanate. The ratio of negatively charged organic moiety to antibody can be adjusted using any suitable option, e.g., by adjusting the concentration of the negatively charged organic moiety (or precursor thereof), adjusting the relative concentrations of the negatively charged organic moiety (or precursor thereof) and the antibody, adjusting the length of time for the conjugation reaction, etc.

**[0144]** Also provided herein is a method of treating a subject using an antibody, minibody, or cys-diabody (e.g., the conjugates thereof) of the present disclosure. With reference to Fig. 4, a non-limiting method of treating a subject us provided. The method **400** can include, at block **410**, identifying a subject in need of treatment, for example, for cancer, with the antibody, minibody, or cys-diabody (e.g., the conjugates thereof) of the present disclosure, and at block **420**, administering a therapeutically effective amount of the antibody, minibody, or cys-diabody (e.g., the conjugates thereof) to the subject. In some embodiments, the method includes administering to the subject a therapeutically effective amount of a composition, e.g., a pharmaceutical composition, containing the antibody, minibody, or cys-diabody (e.g., the conjugates thereof) of the present disclosure. In some embodiments, the method is a radiotherapy method, where the subject in need of radiotherapy (e.g., a subject having cancer) is identified, and the antibody, minibody, or cys-diabody (e.g., the conjugates thereof) administered to the subject is labeled with a radionuclide. In some embodiments, the subject has cancer. In some embodiments, the subject has cancer or other condition associated

with the antigen to which the antibody, minibody, or cys-diabody specifically binds. In some embodiments, a greater dose of radiation can be administered using the antibody, minibody, or cys-diabody (e.g., the conjugates thereof) of the present disclosure, compared to the original antibody, minibody, or cys-diabody (e.g., the original construct without the negatively charged organic moiety conjugated thereto).

**[0145]** Also provided is a method of radioimmunotherapy. The method can include identifying a subject in need of radioimmunotherapy, and administering to the subject a therapeutically effective amount of the antibody, minibody, or cys-diabody (e.g., the conjugates thereof) of the present disclosure.

**[0146]** Also provided is a method treating a subject for a cancer. The method can include identifying a subject in need of treatment for a cancer; and administering to the subject a therapeutically effective amount of any of the antibody, minibody, or cys-diabody (e.g., the conjugates thereof) of the present disclosure, to thereby treat the cancer. In some embodiments, the cancer is non-small cell lung cancer (NSCLC), Small Cell Lung Cancer (SCLC), Thymic Carcinoma, Lymphoma, Myxoid/Round Cell Liposarcoma, Liposarcoma, Synovial Sarcoma, Recurrent Adult Soft Tissue Sarcoma, Gliosarcoma, Astrocytoma, Acute Myelogenous Leukemia (AML), Malignant Solitary Fibrous Tumor of the Pleura (MSFT), Penile Cancer, Diffuse Intrinsic Pontine Glioma (DIPG), Thyroid Carcinoma, Head and neck Squamous Carcinoma (SCCHN), Adenocarcinoma of the Lung, Vulvar Cancer (squamous cell carcinoma), Bladder Cancer, Cervical Squamous Cell Carcinoma, Germ Cell Tumors, Testicular Cancer, Pancreatic Ductal Adenocarcinoma, Pancreatic Adenocarcinoma, Non-Melanoma Skin Cancers, Retroperitoneal and Peritoneal Carcinoma, Melanoma, Unresectable or Metastatic Melanoma, Mucosal Melanoma of the Head and Neck, Uveal Melanoma, Non-Cutaneous Melanoma, Cutaneous T-Cell Lymphoma, Occult Primary tumors, Biliary Cancer, Gastrointestinal Stromal Tumors (GIST), Mesothelioma, Biphasic Mesothelioma, Malignant Pleural Mesothelioma, Kidney cancer, Myelodysplastic syndrome, Liver Hepatocellular Carcinoma, Esophageal and Esophagogastric Junction Carcinoma, Extrahepatic Bile Duct Adenocarcinoma, Small Intestinal Malignancies, Gastric Adenocarcinoma, Cholangiocarcinoma, Intrahepatic ad extrahepatic Cholangiocarcinomas, Ovarian Surface Epithelial Carcinomas, Non-epithelial and epithelial Ovarian cancers, Breast Carcinoma, Triple Negative Breast Cancer, Endometrial carcinoma, Uterine sarcoma, Bone Cancers,

Colorectal Adenocarcinoma, Prostatic Adenocarcinoma, Hormone-Resistant Prostate Cancer, Neuroendocrine tumors, Solid tumors, Follicular Lymphoma, Kaposi Sarcoma, Carcinoma of the Genitourinary Tract, Fallopian Tube Cancer, Malignant Glioma, Waldenstrom Macroglobulinemia, Richter Syndrome, Refractory Splenic Marginal Zone Lymphoma, Refractory Small Lymphocytic Lymphoma, Refractory Nodal Marginal Zone Lymphoma, Refractory Lymphoplasmacytic Lymphoma, Refractory Extranodal Marginal Zone Lymphoma of the Mucosa-Associated Lymphoid Tissue, Refractory Chronic Lymphocytic Leukemia, Multiple Myeloma, Hodgkin's Lymphoma, Non-Hodgkin's Lymphoma, Diffuse Large B-Cell Lymphoma, Nasopharyngeal Carcinoma, Gastroesophageal Junction Adenocarcinoma, renal cell carcinomas, colon carcinomas, Transitional cell carcinoma (TCC), urothelial carcinoma (UCC), glioblastoma multiforme (GBM), Gallbladder cancers, and Merkel Cell Carcinoma.

**[0147]** In some embodiments, the cancer is: Prostate cancer, Lung cancers, Melanoma, Breast malignancies, CNS and brain Malignancies, Skin malignancies, Occult Primary tumors, Kidney cancers, Gastrointestinal malignancies, Ovarian Neoplasms, Renal Cancers, Biliary Cancer, Bladder cancer, Esophageal Neoplasms, Cervical cancers, Solid tumors, Head and neck cancers, Urogenital Neoplasms, Germ Cell Tumors, Testicular Cancer, Pancreatic cancers, Glioma, Liver cancers, Malignant Neoplasms of the Bone, Colorectal cancers, Thyroid Cancer, Thoracic and respiratory tumors, Lymphomas, Male and female genitourinary Malignancies, Bile duct cancers, Hematological Malignancies, Multiple Myeloma, Gallbladder cancers, endocrine tumors, ocular cancers, and tumors of the hematopoietic and lymphoid tissues.

**[0148]** In some embodiments, the antibody, minibody, or cys-diabody of the present disclosure is conjugated to a cytotoxic agent, and the method includes targeting a cancer or tumor with the cytotoxic agent.

**[0149]** In some embodiments, the method is a method of imaging a subject, where the antibody, minibody, or cys-diabody (e.g., the conjugates thereof) is detectably labeled, and the method further includes imaging the subject to detect the labeled antibody, minibody, or cys-diabody (e.g., the conjugates thereof) in the subject. The subject can be imaged using any suitable option for the detecting the detectable label. In some embodiments, the imaging includes positron emission tomography (PET), computed tomography (CT), single-photon

emission computed tomography (SPECT), magnetic resonance imaging (NMR), or detection of fluorescence emissions. In some embodiments, detection can be via near-infrared (NIR) and/or Cerenkov.

**[0150]** In some embodiments, the antibody, minibody, or cys-diabody (e.g., the conjugates thereof) specifically binds to an antigen target, such as, but not limited to those targets disclosed herein.

**[0151]** Additional non-limiting embodiments of the present disclosure are provided in the following numbered arrangements.

1. An antibody that varies from an original antibody by comprising a negatively charged organic moiety associated therewith, wherein the negatively charged moiety confers a surface- exposed negative charge to the antibody, wherein the antibody has at least about 10% more total surface-exposed negative charge conferred by the negatively charged organic moiety relative to the original antibody.

2. The antibody of arrangement 1, wherein the antibody has at least about 30% more total negative charge conferred by the negatively charged organic moiety relative to the original antibody.

3. The antibody of arrangement 1 or 2, wherein the antibody has a negatively charged organic moiety-to-antibody ratio of at least about 1.2.

4. The antibody of arrangement 3, wherein the antibody has a negatively charged organic moiety-to-antibody ratio of at least about 3.0.

5. The antibody of arrangement 3, wherein the antibody has a negatively charged organic moiety-to-antibody ratio of at least about 5.0.

6. The antibody of any one of the preceding arrangements, wherein the negatively charged organic moiety comprises a negatively charged chelating ligand or a negatively charged organic dye.

7. The antibody of arrangement 6, wherein the negatively charged chelating ligand is selected from the group consisting of: ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), DOTA, DOTAGA, NOTA, NODAGA, NETA, porphyrins, polyamines, crown ethers, bis-thiosemicarbazones, and polyoximes.

8. The antibody of arrangement 7, wherein the negatively charged chelating ligand is DTPA, DOTA or DOTAGA.

9. The antibody of arrangement 6, wherein the negatively charged organic dye is IRDye®800.

10. The antibody of any one of the preceding arrangements, wherein the antibody is a radiolabeled antibody.

11. The antibody of arrangement 10, wherein the antibody is radiolabeled via a second chelating ligand.

12. The antibody of arrangement 11, wherein the second chelating ligand comprises deferoxamine (Df).

13. The antibody of any one of arrangements 1-11, wherein the antibody is radiolabeled via the negatively charged chelating ligand.

14. The antibody of any one of arrangements 1-9, wherein the antibody is further conjugated to a therapeutic agent.

15. The antibody of arrangement 14, wherein the therapeutic agent is selected from among drugs, small molecule drugs, chemotherapeutic agents, therapeutic antibodies and antibody fragments, toxins, radioisotopes, enzymes (for example, enzymes to cleave prodrugs to a cytotoxic agent at the site of the antigen binding construct binding), nucleases, hormones, immunomodulators, antisense oligonucleotides, chelators, boron compounds, photoactive agents and dyes, elastin-like polypeptides such as PLGA, and nanoparticles.

16. The antibody of any one of the preceding arrangements, wherein the molecular weight of the antibody is in a range of about 10 kDa to about 100 kDa.

17. The antibody of any one of the preceding arrangements, wherein the antibody comprises an antigen-binding fragment.

18. The antibody of any one of the preceding arrangements, wherein the antibody is a minibody or a cys-diabody.

19. An antibody that varies from an original antibody by comprising a negatively charged organic moiety associated therewith, wherein the antibody is a minibody or cys-diabody, wherein the negatively charged organic moiety confers a surface-exposed negative charge to the antibody, wherein the antibody has at least about 10% more total surface-exposed negative charge conferred by the negatively charged organic moiety relative to the original antibody, and wherein the antibody has a negatively charged organic moiety-to-antibody ratio of at least 1.2.

20. The antibody of any one of the preceding arrangements, comprising at least one cluster of surface-exposed positively charged amino acids, the cluster comprising at least two surface- exposed, positively charged amino acids within about 30 angstroms of each other.

21. The antibody of any one of the preceding arrangements, wherein the antibody specifically binds to DLL3, FAP, CD8, CD4, CD3, IFNγ, Integrin αVβ6, FOLRα, or PSMA.

22. The antibody of any one of the preceding arrangements, wherein the antibody comprises a light chain variable region comprising 3 LCDR sequences in any one of the amino acid sequences shown in FIGs. 12A-12D, and a heavy chain variable region comprising the 3 HCDR sequences of 3 HCDRs in any one of the amino acid sequences shown in FIGs. 12A-12D.

23. A composition comprising: the antibody of any one of the preceding arrangements; and a pharmaceutically acceptable carrier.

24. A composition comprising: an antibody; and a negatively charged organic moiety or precursor thereof configured to be conjugated to the antibody, wherein the negatively charged organic moiety or precursor thereof is present in an amount sufficient to disrupt or conceal a positive patch on a surface of the antibody, or to confer an additional negative charge to the antibody, when conjugated thereto.

25. The composition of arrangement 24, wherein the negatively charged organic moiety is present in an amount sufficient to provide an antibody conjugate having a negatively charged organic moiety-to-antibody ratio of at least about 1.2.

26. The composition of arrangement 24 or 25, wherein the antibody is a radiolabeled antibody.

27. The composition of arrangement 26, wherein the antibody is radiolabeled via a second chelating ligand.

28. The composition of arrangement 27, wherein the second chelating ligand comprises deferoxamine (Df).

29. The antibody of arrangement 24 or 25, wherein the antibody is further conjugated to a therapeutic agent.

30. The antibody of arrangement 29, wherein the therapeutic agent is selected from among drugs, small molecule drugs, chemotherapeutic agents, therapeutic antibodies and antibody fragments, toxins, radioisotopes, enzymes (for example, enzymes to cleave prodrugs

to a cytotoxic agent at the site of the antigen binding construct binding), nucleases, hormones, immunomodulators, antisense oligonucleotides, chelators, boron compounds, photoactive agents and dyes, elastin-like polypeptides such as PLGA, and nanoparticles.

31. The composition of any one of arrangements 24-30, wherein the antibody comprises at least one cluster of surface-exposed positively charged amino acids, the cluster comprising at least two surface-exposed, positively charged amino acids within about 30 angstroms of each other.

32. The composition of any one of arrangements 24-31, wherein the negatively charged organic moiety comprises a negatively charged chelating ligand or organic dye.

33. The composition of arrangement 32, wherein the negatively charged chelating ligand is selected from the group consisting of: ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), DOTA, DOTAGA, NOTA, NODAGA, NETA, porphyrins, polyamines, crown ethers, bis-thiosemicarbazones, and polyoximes.

34. The composition of arrangement 32, wherein the negatively charged organic dye is IRDye®800.

35. A kit for radiolabeling an antibody, comprising: a first chelating ligand for radiolabeling an antibody; and a negatively charged organic moiety configured to be conjugated to the antibody.

36. The kit of arrangement 35, wherein the negatively charged organic moiety is a negatively charged chelating ligand or organic dye.

37. The kit of arrangement 35 or 36, further comprising a radionuclide.

38. A method of enhancing biodistribution and/or pharmacokinetics of a radiolabeled antibody, comprising: selecting a first radiolabeled antibody comprising: a first antibody; and a radionuclide, wherein the first antibody is labeled with the radionuclide via a first chelating ligand; and providing a second radiolabeled antibody comprising: the first antibody; the radionuclide, wherein the first antibody is labeled with the radionuclide via the first chelating ligand; and a negatively charged organic moiety conjugated thereto, by conjugating the negatively charged organic moiety to the first antibody in an amount sufficient to provide the second radiolabeled antibody having at least 10% more surface-exposed negative charges conferred by the conjugated negatively charged organic moiety compared to

the first radiolabeled antibody, thereby generating a second radiolabeled antibody having enhanced biodistribution and/or pharmacokinetics compared to the first radiolabeled antibody.

39. The method of arrangement 38, comprising conjugating the negatively charged organic moiety to obtain a negatively charged organic moiety-to-antibody ratio of at least about 1.2.

40. A method of making a radiolabeled antibody, comprising: providing an antibody; labeling the antibody with a radionuclide via a first chelating ligand; and conjugating a negatively charged organic moiety to the antibody to obtain a negatively charged organic moiety-to-antibody ratio of at least about 1.2, thereby making a radiolabeled antibody.

41. The method of arrangement 40, wherein the conjugating comprises obtaining a negatively charged organic moiety-to-antibody ratio of at least about 3.0.

42. The method of any one of arrangements 38-41, wherein the first chelating ligand comprises deferoxamine (Df).

43. The method of any one of arrangements 38-41, wherein the first chelating ligand comprises the negatively charged organic moiety.

44. A method of making an antibody conjugate, comprising: providing an antibody; and conjugating a negatively charged organic moiety to the antibody to disrupt or conceal at least one positive patch on a surface of the antibody, or to confer an additional negative charge to the antibody, to thereby generate an antibody conjugate.

45. The method of arrangement 44, wherein the negatively charged organic moiety is a negatively charged chelating ligand or organic dye.

46. The method of arrangement 44 or 45, wherein the antibody is radiolabeled.

47. The method of arrangement 46, wherein the antibody is radiolabeled via a second chelating ligand.

48. The method of arrangement 47, wherein the second chelating ligand comprises deferoxamine (Df).

49. The method of any one of arrangements 44-48, further comprising labeling the antibody conjugate with a radionuclide.

50. The method of any one of arrangements 44-48, further comprising contacting the antibody conjugate with a radionuclide.

51. The method of arrangement 44, wherein the antibody is further conjugated to a therapeutic agent.

52. The antibody of arrangement 51, wherein the therapeutic agent is selected from among drugs, small molecule drugs, chemotherapeutic agents, therapeutic antibodies and antibody fragments, toxins, radioisotopes, enzymes (for example, enzymes to cleave prodrugs to a cytotoxic agent at the site of the antigen binding construct binding), nucleases, hormones, immunomodulators, antisense oligonucleotides, chelators, boron compounds, photoactive agents and dyes, elastin-like polypeptides such as PLGA, and nanoparticles.

53. The method of any one of arrangements 44-52, wherein the at least one positive patch is outside of any CDR of the antibody.

54. The method of any one of arrangements 44-53, wherein the at least one positive patch is in a framework region (FR) or hinge region of the antibody.

55. The method of any one of arrangements 44-54, wherein the at least one positive patch is in a $V_L$ FR2 of the antibody.

56. The method of any one of arrangements 44-55, wherein the at least one positive patch is in an upper hinge region of the antibody.

57. The method of any one of arrangements 38-56, wherein the molecular weight of the antibody is in a range of about 10 kDa to about 100 kDa.

58. The method of any one of arrangements 38-57, wherein the antibody comprises an antigen-binding fragment.

59. The method of any one of arrangements 38-58, wherein the antibody is a minibody or a cys-diabody.

60. A method of enhancing biodistribution and/or pharmacokinetics of a radiolabeled minibody or cys-diabody, comprising: selecting a first radiolabeled antibody comprising: a first antibody, wherein the first antibody is a minibody or a cys-diabody; and a radionuclide, wherein the first antibody is labeled with the radionuclide via a first chelating ligand; and providing a second radiolabeled antibody comprising: the first antibody; the radionuclide, wherein the first antibody is labeled with the radionuclide via the first chelating ligand; and a negatively charged organic moiety conjugated thereto, by conjugating the negatively charged organic moiety to the first antibodyto obtain a negatively charged organic moiety-to-antibody ratio of at least about 1.2.

61. The method of arrangement 60, wherein the conjugating comprises obtaining a negatively charged organic moiety-to-antibody ratio of at least about 3.0.

62. The method of arrangement 60 or 61, wherein the first chelating ligand comprises deferoxamine (Df).

63. The method of arrangement 60 or 61, wherein the first chelating ligand comprises the negatively charged organic moiety.

64. The method of any one of arrangements 38-63, wherein the negatively charged organic moiety comprises a negatively charged chelating ligand or organic dye.

65. The method of arrangement 64, wherein the negatively charged chelating ligand is selected from the group consisting of: ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), DOTA, DOTAGA, NOTA, NODAGA, NETA, porphyrins, polyamines, crown ethers, bis-thiosemicarbazones, and polyoximes

66. The method of arrangement 64, wherein the negatively charged organic dye is IRDye®800.

67. The method of any one of arrangements 38-66, wherein the antibody comprises at least one cluster of surface-exposed positively charged amino acids, the cluster comprising at least two surface-exposed, positively charged amino acids within about 30 angstroms of each other.

68. The method of any one of arrangements 38-67, wherein the antibody specifically binds to DLL3, FAP, CD8, CD4, CD3, IFNγ, Integrin αVβ6, FOLRα, or PSMA.

69. The antibody of any one of arrangements 38-68, wherein the antibody comprises a light chain variable region comprising 3 LCDR sequences of the 3 LCDRs in any one of the amino acid sequences shown in FIGs. 12A-12D, and a heavy chain variable region comprising the 3 HCDR sequences in any one of the amino acid sequences shown in FIGs. 12A-12D.

70. An antibody, minibody, or cys-diabody made by the method of any one of arrangements 38-69.

71. A method of treating a subject, comprising: identifying a subject in need of treatment with the antibody, minibody, or cys-diabody of any one of arrangements 1-22; and administering to the subject a therapeutically effective amount of the antibody, minibody, or cys-diabody, or the composition of arrangement 23.

72. A method of treating a subject for a cancer, comprising: identifying a subject in need of treatment for a cancer; and administering to the subject a therapeutically effective amount of the antibody, minibody, or cys-diabody of any one of arrangements 1-22, or the composition of arrangement 23, to thereby treat the cancer.

73. A method of radiotherapy, comprising: identifying a subject in need of radiotherapy; and administering to the subject a therapeutically effective amount of the antibody, minibody, or cys-diabody of any one of arrangements 1-22, or the composition of arrangement 23, wherein the antibody, minibody, or cys-diabody comprises a radionuclide.

74. A method of imaging a subject, comprising: administering to a subject a composition comprising an effective amount of the antibody, minibody, or cys-diabody of any one of arrangements 1-22, or the composition of arrangement 23, wherein the antibody, minibody, or cys-diabody is detectably labeled; and imaging the subject to detect the labeled antibody, minibody, or cys-diabody in the subject.

75. The antibody, composition, or the method of any one of the preceding arrangements, wherein the antibody is an antigen binding fragment having molecular of more than 10 kDa and less than 90 kDa.

76. The antibody, composition, or the method of any one of the preceding arrangements, wherein the antibody is a nanobody.

77. Use of the antibody, minibody, or cys-diabody of any one of arrangements 1-22, or the composition of arrangement 23, for treatment of cancer in a subject in need thereof.

78. Use of the antibody, minibody, or cys-diabody of any one of arrangements 1-22, or the composition of arrangement 23, for preparation of a medicament for treatment of cancer in a subject in need thereof.

## EXAMPLES

Example 1

**[0152]** This non-limiting example shows changing the biodistribution of a minibody by conjugating a negatively charged organic dye to the minibody.

**[0153]** AB16M2-37 is an anti-human-FAP minibody, which was engineered through humanization of an antibody from a mouse hybridoma clone. The amino acid sequence of (the monomer chain of) IAB16M2-37 is shown in Fig. 12A. The minibody was labeled with $^{89}$Zr through a chelating ligand (Df) (CMR = 1 to 3). The radiolabeled minibody, $^{89}$Zr-Df-

IAB16M2-37, was administered to mice and the amount of radiation remaining in the liver, kidneys, and spleen at 24 hours was measured and expressed as a percentage of the injected dose per gram of tissue (%ID/g). The radiolabeled minibody showed a greater accumulation in the kidneys than in the liver or spleen (FIG. 5; bar on left for each tissue).

**[0154]** The radiolabeled minibody was further modified to conjugate the negatively charged organic infra-red dye, Licor IRDye800. A preferred CMR is >1 to 3. The radiolabeled, infra-red dye-labeled minibody, $^{89}$Zr-Df-LicorIR800-IAB16M2-37 (FIG. 5; bar on right for each tissue), when administered to mice, exhibited reduced accumulation in the kidneys compared to the radiolabeled minibody without the dye (FIG. 5). Further, accumulation of the radiolabeled, infra-red dye-labeled minibody in the liver increased compared to the radiolabeled minibody without the dye. In addition, the radiolabeled, infra- red dye-labeled minibody showed a greater accumulation in the liver compared to the kidney (FIG. 5; bar on right for each tissue). The accumulation in the spleen did not change significantly. Similar results were obtained with another negatively charged organic dye.

**[0155]** These results show that conjugating a negatively charged organic moiety, such as a negatively charged organic dye, to a minibody drastically redirects its *in vivo* clearance from the renal route to the hepatic route.

Example 2

**[0156]** This non-limiting example shows changing the biodistribution of a minibody by conjugating a negatively charged organic dye to the minibody, and by changing the chelator-to-minibody ratio of a negatively charged chelating ligand.

**[0157]** IAB16M2-56 is an anti-human-FAP minibody, which was engineered through humanization of an antibody from a mouse hybridoma clone. The amino acid sequence of (the monomer chain of) IAB16M2-56 is shown in Fig. 12B. Analysis of isopotential surfaces of the minibody using an Adaptive Poisson-Boltzmann Solver revealed clusters of surface-exposed positively charged amino acids (e.g., lysine or arginine) within 6 residues of each other, for example, in a light chain variable region (VL) framework region 2 (FR2) sequence (WYQQKPGKAPKLLIY (SEQ ID NO: 3)), and an upper hinge region sequence (EPKSSDKTHT (SEQ ID NO: 4)). These clusters are highlighted in bold in FIG. 12B.

**[0158]** The minibody was labeled with $^{89}$Zr through a chelating ligand (Df). The Df was present at a CMR of 1 to 3. The radiolabeled minibody, $^{89}$Zr-Df-IAB16M2-56, was

administered to mice and the amount of radiation remaining in the liver, kidneys, and spleen at 24 hours was measured and expressed as a percentage of the injected dose per gram of tissue (%ID/g). The radiolabeled minibody showed a greater accumulation in the kidneys and spleen than in the liver (FIG. 6; center bar for each tissue).

**[0159]** The radiolabeled minibody was further modified to conjugate the negatively charged organic infra-red dye, Licor IRDye800. The radiolabeled, infra-red dye-labeled minibody, $^{89}$Zr-Df-LicorIR800-IAB16M2-56 (FIG. 6; bar on right for each tissue), when administered to mice, exhibited reduced accumulation in the kidneys compared to the radiolabeled minibody without the dye (FIG. 6). Further, accumulation of the radiolabeled, infra-red dye-labeled minibody in the liver increased compared to the radiolabeled minibody without the dye. In addition, the radiolabeled, infra-red dye-labeled minibody showed a greater accumulation in the liver compared to the kidney (FIG. 6; bar on right for each tissue). The accumulation in the spleen was also reduced by conjugation of the negatively charged organic infra-red dye.

**[0160]** In addition, the radiolabeled minibody was further modified to conjugate a negatively charged chelating ligand, DTPA, in addition to the Df for $^{89}$Zr labeling, at a chelator-to-minibody ratio (CMR) of at least 5. DTPA was conjugated at a CMR of 5.39. Df was conjugated at a CMR of 2.46. The radiolabeled, overconjugated minibody (FIG. 6; bar on left for each tissue), when administered to mice, also exhibited reduced accumulation in the kidneys compared to the radiolabeled minibody without the additional conjugation of the negatively charged chelating ligand (FIG. 6). Further, accumulation of the radiolabeled, overconjugated minibody in the liver increased compared to the radiolabeled minibody without the additional conjugation of the negatively charged chelating ligand. In addition, the radiolabeled, overconjugated minibody showed a greater accumulation in the liver compared to the kidney (FIG. 6; bar on left for each tissue). The accumulation in the spleen was also reduced by conjugation of the negatively charged chelating ligand.

**[0161]** These results show that conjugating an excess amount (e.g., CMR $\geq$ 1.2) of a negatively charged organic moiety, such as a chelating ligand, to a minibody drastically redirects its *in vivo* clearance from the renal route to the hepatic route. Further, the effects of the negatively charged chelating ligand on minibody biodistribution is similar to the effect of conjugating a negatively charged organic dye on minibody biodistribution.

Example 3

**[0162]** This non-limiting example shows the relationship between CMR and biodistribution.

**[0163]** The relationship between CMR and biodistribution of the minibody was studied using the radiolabeled minibody of Example 2. The radiolabeled minibody was further modified by conjugation of DTPA at different CMRs.

**[0164]** As shown in Fig. 7, conjugation of Df (which is not a negatively charged moiety when conjugated to the minibody) at different CMR (1.31 or 1.97) did not significantly affect accumulation in the kidneys (FIG. 7; two left-most bars for each tissue, where the first bar from left is CMR (Df) 1.31, and the second bar form left is CMR (Df) 1.97). Even at higher CMR (CMR = 5, 6, or 7), conjugation of Df alone had at most a modest effect on tissue distribution (FIG. 7; see first three bars from right for each tissue). There was a slight increase in accumulation in the liver and a greater increase in accumulation in the spleen at CMR = 1.97 compared to CMR of 1.31. CMR 5-7 showed only a modest increase in liver accumulation, and little change in spleen accumulation.

**[0165]** Further modification of the radiolabeled minibody (CMR (Df) = 2) with conjugation of DTPA at CMR 0.8 caused a marked reduction in kidney accumulation of the overconjugated minibody (FIG. 7; "CMR=0.8", third bar from left for each tissue). The reduction in kidney accumulation was accompanied by an increase in liver accumulation. This is in contrast to the results with Df, which required a much higher CMR to achieve a comparable change in distribution. This shows that addition of the negatively charged DTPA to the minibody provides a more salient effect on tissue distribution than simply removing positive charges by attaching a neutral chelating ligand such as Df to (surface-exposed) lysine residues in the minibody. Consistent with this, the minibody does not appear to have a patch of surface exposed negative charges that could promote liver uptake, and removing the positive charges by conjugating Df does not appear sufficient to promote liver uptake.

**[0166]** The CMR for DTPA conjugation was generally correlated with the extent of kidney and liver accumulation. In particular, increasing CMR for DTPA conjugation from 0.8 to 7.5 resulted in a general, progressive decrease in kidney accumulation (FIG. 7; compare "DTPA 0.8", "DTPA 2.2", "DTPA 3.4", "DTPA 4.1", "DTPA 5.4", "DTPA 7.5", starting from the third bar from left and ending at the fourth bar from the right for each tissue). Conversely,

accumulation in the liver generally increased as CMR for DTPA conjugation increased. Accumulation in the spleen was relatively low and did not show a clear pattern of change with the change in CMR for DTPA conjugation.

**[0167]** These results show that the tissue distribution of a minibody can be modified by changing the conjugation of a negatively charged chelating ligand, where increased conjugation reduces kidney accumulation and increases liver accumulation.

Example 4

**[0168]** This non-limiting example shows changing the biodistribution of a cys-diabody by overconjugation of a negatively charged chelating ligand.

**[0169]** IAB16C3-26 is an anti-human-FAP cys-diabody, which was engineered through humanization of an antibody from a mouse hybridoma clone. The amino acid sequence of (the monomer chain of) IAB16C3-26 is shown in Fig. 12C. The cys-diabody was labeled with $^{89}$Zr through a chelating ligand, Df (CMR (Df) 2.03). The radiolabeled cys- diabody, $^{89}$Zr-Df-IAB16C3-26, was administered to mice and the amount of radiation remaining in the liver, kidneys, and spleen at 24 hours was measured and expressed as a percentage of the injected dose per gram of tissue (%ID/g). The radiolabeled cys-diabody showed a greater accumulation in the kidneys than in the liver or spleen (FIG. 8; bar on left for each tissue).

**[0170]** The radiolabeled cys-diabody was further modified to conjugate a negatively charged chelating ligand, DTPA, in addition to the Df for $^{89}$Zr labeling, at higher chelator-to-cys-diabody ratio (CMR). DTPA was conjugated at a CMR of 3.42, and Df was conjugated at a CMR of 0.86. The radiolabeled, overconjugated cys-diabody (FIG. 8; bar on right for each tissue), when administered to mice, exhibited reduced accumulation in the kidneys compared to the radiolabeled cys-diabody without the additional conjugation of the negatively charged chelating ligand (FIG. 8). Further, accumulation of the radiolabeled, overconjugated cys-diabody in the liver increased compared to the radiolabeled cys-diabody without the additional conjugation of the negatively charged chelating ligand. In addition, the radiolabeled, overconjugated cys-diabody showed a greater accumulation in the liver compared to the kidney (FIG. 8; bar on right for each tissue). The accumulation in the spleen did not change as much as the other tissues.

**[0171]** These results show that conjugating an excess amount of a negatively charged chelating ligand, to a cys-diabody drastically redirects its *in vivo* clearance from the

renal route to the hepatic route. Further, the effects of the negatively charged chelating ligand on cys-diabody biodistribution is similar to the effect on minibody biodistribution.

Example 5

**[0172]** This non-limiting example shows changing the biodistribution of a minibody by overconjugation of a negatively charged chelating ligand.

**[0173]** IAB57M1-3 is an anti-human-DLL3 minibody, which was engineered through humanization of an antibody from a mouse hybridoma clone. The amino acid sequence of (the monomer chain of) IAB57M1-3 is shown in Fig. 12D (with the signal peptide underlined). Analysis of isopotential surfaces of the minibody using an Adaptive Poisson-Boltzmann Solver revealed clusters of surface-exposed positively charged amino acids (e.g., lysine or arginine) within 6 residues of each other, for example, in a light chain variable region (VL) framework region 2 (FR2) sequence (WYQQKPGQAPRLLIY (SEQ ID NO: 3)), and an upper hinge region sequence (EPKSSDKTHT (SEQ ID NO: 4)). These clusters are highlighted in bold in FIG. 12D.

**[0174]** The minibody was labeled with $^{89}$Zr through a chelating ligand, Df at a CMR of 1 to 3. The radiolabeled minibody, $^{89}$Zr-Df-IAB57M1-3 (or $^{89}$Zr-DFO-IAB57M1-3), was administered to mice and the amount of radiation remaining in the liver, kidneys, and spleen at 24 hours was measured and expressed as a percentage of the injected dose per gram of tissue (%ID/g). The radiolabeled minibody showed a greater accumulation in the kidneys than in the liver and spleen (FIG. 9; bar on far right for each tissue).

**[0175]** The radiolabeled minibody was further modified to conjugate a negatively charged chelating ligand, DTPA or DOTA, in addition to the Df for $^{89}$Zr labeling, at a higher chelator-to-minibody ratio (CMR). DTPA was conjugated at a CMR of 6.3 and Df was conjugated at a CMR of 2.3, while DOTA was conjugated at a CMR of 4.6 and Df was conjugated at a CMR of 2.6. Each of the radiolabeled, overconjugated minibodies, $^{89}$Zr-Df-DTPA-IAB57M1-3 (FIG. 9; bar on far left for each tissue) and $^{89}$Zr-Df-DOTA-IAB57M1-3 (FIG. 10; second bar from left for each tissue), when administered to mice, exhibited reduced accumulation in the kidneys compared to the radiolabeled minibody without the additional conjugation of the negatively charged chelating ligands (FIG. 9). Further, accumulation of each of the radiolabeled, overconjugated minibodies in the liver increased compared to the radiolabeled minibody without the additional conjugation of the negatively charged chelating

ligand. In addition, the radiolabeled, overconjugated minibodies each showed a greater accumulation in the liver compared to the kidney (FIG. 9; two bars on left for each tissue). The accumulation in the spleen was not altered significantly.

**[0176]** These results show that modifying the tissue distribution of a minibody by overconjugating a negatively charged chelating ligand can be achieved by different negatively charged chelating ligands. The results also show that a neutral adduct, and specifically a neutral chelating ligand such as Df, when overconjugated, does not by itself affect tissue distribution away from the kidney and towards the liver.

**[0177]** The minibody was alternatively labeled with $^{177}$Lu using DTPA as the chelating ligand. The DTPA was conjugated with a CMR of 5.51. A CMR for a typical radiolabeling process is around 1-3. The $^{177}$Lu radiolabeled, overconjugated minibody (FIG. 9; second bar from right for each tissue), when administered to mice, exhibited reduced accumulation in the kidneys compared to the $^{89}$Zr radiolabeled minibody without the additional conjugation of the negatively charged chelating ligand (FIG. 9). Further, accumulation of the $^{177}$Lu radiolabeled, overconjugated minibody in the liver increased compared to the $^{89}$Zr radiolabeled minibody without the additional conjugation of the negatively charged chelating ligand. In addition, the $^{177}$Lu radiolabeled, overconjugated minibody showed a greater accumulation in the liver compared to the kidney (FIG. 9; second bar from right for each tissue).

**[0178]** These results show that modifying the tissue distribution of a minibody by overconjugating a negatively charged chelating ligand can be achieved by with different radionuclides.

Example 6

**[0179]** This non-limiting example shows radioimmunotherapy treatment of a subject using an antibody of the present disclosure.

**[0180]** A subject in need of treating a cancer is identified. A minibody conjugate that specifically binds a target in the tumor and is overconjugated with a negatively charged chelating ligand is labeled with a radionuclide. The radiolabeled, overconjugated minibody is administered to the subject. The radiation dose delivered to the subject is greater than the dose that would have been delivered using a minibody that is not overconjugated with the negatively charged chelating ligand, while renal toxicity remains comparable.

Example 7

**[0181]** This non-limiting example shows treatment of a subject using an antibody of the present disclosure.

**[0182]** A subject in need of treating a liver cancer is identified. A minibody conjugate that specifically binds a target in the tumor and is overconjugated with a negatively charged chelating ligand is further conjugated with a cytotoxic agent. The cytotoxic agent-conjugated, overconjugated minibody is administered to the subject to treat the liver cancer.

**[0183]** With respect to the use of substantially any plural and/or singular terms herein, those having skill in the art can translate from the plural to the singular and/or from the singular to plural as is appropriate to the context and/or application. The various singular/plural permutations can be expressly set forth herein for sake of clarity.

**[0184]** It will be understood by those within the art that, in general, terms used herein, and especially in the appended claims (for example, bodies of the appended claims) are generally intended as "open" terms (for example, the term "including" should be interpreted as "including but not limited to," the term "having" should be interpreted as "having at least," the term "includes" should be interpreted as "includes but is not limited to," etc.). It will be further understood by those within the art that if a specific number of an introduced claim recitation is intended, such an intent will be explicitly recited in the claim, and in the absence of such recitation no such intent is present. For example, as an aid to understanding, the following appended claims can contain usage of the introductory phrases "at least one" and "one or more" to introduce claim recitations. However, the use of such phrases should not be construed to imply that the introduction of a claim recitation by the indefinite articles "a" or "an" limits any particular claim containing such introduced claim recitation to embodiments containing only one such recitation, even when the same claim includes the introductory phrases "one or more" or "at least one" and indefinite articles such as "a" or "an" (for example, "a" and/or "an" should be interpreted to mean "at least one" or "one or more"); the same holds true for the use of definite articles used to introduce claim recitations. In addition, even if a specific number of an introduced claim recitation is explicitly recited, those skilled in the art will recognize that such recitation should be interpreted to mean at least the recited number (for example, the bare recitation of "two recitations," without other modifiers, means at least two recitations, or two

or more recitations). Furthermore, in those instances where a convention analogous to "at least one of A, B, and C, etc." is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (for example, "a system having at least one of A, B, and C" would include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B, and C together, etc.). In those instances where a convention analogous to "at least one of A, B, or C, etc." is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (for example, "a kit having at least one of A, B, or C" would include but not be limited to kits that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B, and C together, etc.). It will be further understood by those within the art that virtually any disjunctive word and/ or phrase presenting two or more alternative terms, whether in the description, claims, or drawings, should be understood to contemplate the possibilities of including one of the terms, either of the terms, or both terms. For example, the phrase "A or B" will be understood to include the possibilities of "A" or "B" or "A and B."

**[0185]** In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

**[0186]** As will be understood by one skilled in the art, for any and all purposes, such as in terms of providing a written description, all ranges disclosed herein also encompass any and all possible sub-ranges and combinations of sub-ranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, etc. As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, etc. As will also be understood by one skilled in the art all language such as "up to," "at least," "greater than," "less than," and the like include the number recited and refer to ranges which can be subsequently broken down into sub-ranges as discussed above. Finally, as will be understood by one skilled in the art, a range includes each individual member. Thus, for example, a group having 1-3 articles refers to groups having 1, 2, or 3 articles. Similarly, a group having 1-5 articles refers to groups having 1, 2, 3, 4, or 5 articles, and so forth.

**[0187]** Wherever a method of using a compound (e.g., a method comprising administering an antibody) is disclosed herein, the corresponding compound for use is also expressly contemplated. For example, for the disclosure of a method of treating a subject, comprising administering a therapeutically effective amount of an antibody, the corresponding antibody for use in treating the subject is also contemplated.

**[0188]** While various aspects and embodiments have been disclosed herein, other aspects and embodiments will be apparent to those skilled in the art. The various aspects and embodiments disclosed herein are for purposes of illustration and are not intended to be limiting, with the true scope and spirit being indicated by the following claims.

WHAT IS CLAIMED IS:

1. An antibody that varies from an original antibody by comprising a negatively charged organic moiety associated therewith, wherein the negatively charged moiety confers a surface-exposed negative charge to the antibody, wherein the antibody has at least about 10% more total surface-exposed negative charge conferred by the negatively charged organic moiety relative to the original antibody.

2. The antibody of claim 1, wherein the antibody has at least about 30% more total negative charge conferred by the negatively charged organic moiety relative to the original antibody.

3. The antibody of claim 1 or 2, wherein the antibody has a negatively charged organic moiety-to-antibody ratio of at least about 1.2.

4. The antibody of claim 3, wherein the antibody has a negatively charged organic moiety-to-antibody ratio of at least about 3.0.

5. The antibody of claim 3, wherein the antibody has a negatively charged organic moiety-to-antibody ratio of at least about 5.0.

6. The antibody of any one of the preceding claims, wherein the negatively charged organic moiety comprises a negatively charged chelating ligand or a negatively charged organic dye.

7. The antibody of claim 6, wherein the negatively charged chelating ligand is selected from the group consisting of:

ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), DOTA, DOTAGA, NOTA, NODAGA, NETA, porphyrins, polyamines, crown ethers, bis-thiosemicarbazones, and polyoximes.

8. The antibody of claim 7, wherein the negatively charged chelating ligand is DTPA, DOTA or DOTAGA.

9. The antibody of claim 6, wherein the negatively charged organic dye is IRDye®800.

10. The antibody of any one of the preceding claims, wherein the antibody is a radiolabeled antibody.

11. The antibody of claim 10, wherein the antibody is radiolabeled via a second chelating ligand.

12. The antibody of claim 11, wherein the second chelating ligand comprises deferoxamine (Df).

13. The antibody of any one of claims 1-11, wherein the antibody is radiolabeled via the negatively charged chelating ligand.

14. The antibody of any one of claims 1-9, wherein the antibody is further conjugated to a therapeutic agent.

15. The antibody of claim 14, wherein the therapeutic agent is selected from among drugs, small molecule drugs, chemotherapeutic agents, therapeutic antibodies and antibody fragments, toxins, radioisotopes, enzymes (for example, enzymes to cleave prodrugs to a cytotoxic agent at the site of the antigen binding construct binding), nucleases, hormones, immunomodulators, antisense oligonucleotides, chelators, boron compounds, photoactive agents and dyes, elastin-like polypeptides such as PLGA, and nanoparticles.

16. The antibody of any one of the preceding claims, wherein the molecular weight of the antibody is in a range of about 10 kDa to about 100 kDa.

17. The antibody of any one of the preceding claims, wherein the antibody comprises an antigen-binding fragment.

18. The antibody of any one of the preceding claims, wherein the antibody is a minibody or a cys-diabody.

19. An antibody that varies from an original antibody by comprising a negatively charged organic moiety associated therewith, wherein the antibody is a minibody or cys-diabody, wherein the negatively charged organic moiety confers a surface-exposed negative charge to the antibody, wherein the antibody has at least about 10% more total surface-exposed negative charge conferred by the negatively charged organic moiety relative to the original antibody, and

wherein the antibody has a negatively charged organic moiety-to-antibody ratio of at least 1.2.

20. The antibody of any one of the preceding claims, comprising at least one cluster of surface-exposed positively charged amino acids, the cluster comprising at least two surface-exposed, positively charged amino acids within about 30 angstroms of each other.

21. The antibody of any one of the preceding claims, wherein the antibody specifically binds to DLL3, FAP, CD8, CD4, CD3, IFNγ, Integrin αVβ6, FOLRα, or PSMA.

22. The antibody of any one of the preceding claims, wherein the antibody comprises a light chain variable region comprising 3 LCDR sequences of the 3 LCDRs in any one of the amino acid sequences shown in FIGs. 12A-12D, and a heavy chain variable region comprising the 3 HCDR sequences of 3 HCDRs in any one of the amino acid sequences shown in FIGs. 12A-12D.

23. A composition comprising:

the antibody of any one of the preceding claims; and

a pharmaceutically acceptable carrier.

24. A composition comprising:

an antibody; and

a negatively charged organic moiety or precursor thereof configured to be conjugated to the antibody, wherein the negatively charged organic moiety or precursor thereof is present in an amount sufficient to disrupt or conceal a positive patch on a surface of the antibody, or to confer an additional negative charge to the antibody, when conjugated thereto.

25. The composition of claim 24, wherein the negatively charged organic moiety is present in an amount sufficient to provide an antibody conjugate having a negatively charged organic moiety-to-antibody ratio of at least about 1.2.

26. The composition of claim 24 or 25, wherein the antibody is a radiolabeled antibody.

27. The composition of claim 26, wherein the antibody is radiolabeled via a second chelating ligand.

28. The composition of claim 27, wherein the second chelating ligand comprises deferoxamine (Df).

29. The antibody of claim 24 or 25, wherein the antibody is further conjugated to a therapeutic agent.

30. The antibody of claim 29, wherein the therapeutic agent is selected from among drugs, small molecule drugs, chemotherapeutic agents, therapeutic antibodies and antibody fragments, toxins, radioisotopes, enzymes (for example, enzymes to cleave prodrugs to a cytotoxic agent at the site of the antigen binding construct binding), nucleases, hormones, immunomodulators, antisense oligonucleotides, chelators, boron compounds, photoactive agents and dyes, elastin-like polypeptides such as PLGA, and nanoparticles.

31. The composition of any one of claims 24-30, wherein the antibody comprises at least one cluster of surface-exposed positively charged amino acids, the cluster comprising at least two surface-exposed, positively charged amino acids within about 30 angstroms of each other.

32. The composition of any one of claims 24-31, wherein the negatively charged organic moiety comprises a negatively charged chelating ligand or organic dye.

33. The composition of claim 32, wherein the negatively charged chelating ligand is selected from the group consisting of: ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), DOTA, DOTAGA, NOTA, NODAGA, NETA, porphyrins, polyamines, crown ethers, bis-thiosemicarbazones, and polyoximes.

34. The composition of claim 32, wherein the negatively charged organic dye is IRDye®800.

35. A kit for radiolabeling an antibody, comprising:

a first chelating ligand for radiolabeling an antibody; and

a negatively charged organic moiety configured to be conjugated to the antibody.

36. The kit of claim 35, wherein the negatively charged organic moiety is a negatively charged chelating ligand or organic dye.

37. The kit of claim 35 or 36, further comprising a radionuclide.

38. A method of enhancing biodistribution and/or pharmacokinetics of a radiolabeled antibody, comprising:

selecting a first radiolabeled antibody comprising:

a first antibody; and

a radionuclide, wherein the first antibody is labeled with the radionuclide via a first chelating ligand; and

providing a second radiolabeled antibody comprising:

the first antibody;

the radionuclide, wherein the first antibody is labeled with the radionuclide via the first chelating ligand; and

a negatively charged organic moiety conjugated thereto,

by conjugating the negatively charged organic moiety to the first antibody in an amount sufficient to provide the second radiolabeled antibody having at least 10% more surface-exposed negative charges conferred by the conjugated negatively charged organic moiety compared to the first radiolabeled antibody, thereby generating a second radiolabeled antibody having enhanced biodistribution and/or pharmacokinetics compared to the first radiolabeled antibody.

39. The method of claim 38, comprising conjugating the negatively charged organic moiety to obtain a negatively charged organic moiety-to-antibody ratio of at least about 1.2.

40. A method of making a radiolabeled antibody, comprising:

providing an antibody;

labeling the antibody with a radionuclide via a first chelating ligand; and

conjugating a negatively charged organic moiety to the antibody to obtain a negatively charged organic moiety-to-antibody ratio of at least about 1.2, thereby making a radiolabeled antibody.

41. The method of claim 40, wherein the conjugating comprises obtaining a negatively charged organic moiety-to-antibody ratio of at least about 3.0.

42. The method of any one of claims 38-41, wherein the first chelating ligand comprises deferoxamine (Df).

43. The method of any one of claims 38-41, wherein the first chelating ligand comprises the negatively charged organic moiety.

44. A method of making an antibody conjugate, comprising:

providing an antibody; and

conjugating a negatively charged organic moiety to the antibody to disrupt or conceal at least one positive patch on a surface of the antibody, or to confer an additional negative charge to the antibody, to thereby generate an antibody conjugate.

45. The method of claim 44, wherein the negatively charged organic moiety is a negatively charged chelating ligand or organic dye.

46. The method of claim 44 or 45, wherein the antibody is radiolabeled.

47. The method of claim 46, wherein the antibody is radiolabeled via a second chelating ligand.

48. The method of claim 47, wherein the second chelating ligand comprises deferoxamine (Df).

49. The method of any one of claims 44-48, further comprising labeling the antibody conjugate with a radionuclide.

50. The method of any one of claims 44-48, further comprising contacting the antibody conjugate with a radionuclide.

51. The method of claim 44, wherein the antibody is further conjugated to a therapeutic agent.

52. The antibody of claim 51, wherein the therapeutic agent is selected from among drugs, small molecule drugs, chemotherapeutic agents, therapeutic antibodies and antibody fragments, toxins, radioisotopes, enzymes (for example, enzymes to cleave prodrugs to a cytotoxic agent at the site of the antigen binding construct binding), nucleases, hormones, immunomodulators, antisense oligonucleotides, chelators, boron compounds, photoactive agents and dyes, elastin-like polypeptides such as PLGA, and nanoparticles.

53. The method of any one of claims 44-52, wherein the at least one positive patch is outside of any CDR of the antibody.

54. The method of any one of claims 44-53, wherein the at least one positive patch is in a framework region (FR) or hinge region of the antibody.

55. The method of any one of claims 44-54, wherein the at least one positive patch is in a VL FR2 of the antibody.

56. The method of any one of claims 44-55, wherein the at least one positive patch is in an upper hinge region of the antibody.

57. The method of any one of claims 38-56, wherein the molecular weight of the antibody is in a range of about 10 kDa to about 100 kDa.

58. The method of any one of claims 38-57, wherein the antibody comprises an antigen-binding fragment.

59. The method of any one of claims 38-58, wherein the antibody is a minibody or a cys-diabody.

60. A method of enhancing biodistribution and/or pharmacokinetics of a radiolabeled minibody or cys-diabody, comprising:

selecting a first radiolabeled antibody comprising:

a first antibody, wherein the first antibody is a minibody or a cys-diabody; and a radionuclide, wherein the first antibody is labeled with the radionuclide via a first chelating ligand; and

providing a second radiolabeled antibody comprising: the first antibody; the radionuclide, wherein the first antibody is labeled with the radionuclide via the first chelating ligand; and

a negatively charged organic moiety conjugated thereto,

by conjugating the negatively charged organic moiety to the first antibody to obtain a negatively charged organic moiety-to-antibody ratio of at least about 1.2.

61. The method of claim 60, wherein the conjugating comprises obtaining a negatively charged organic moiety-to-antibody ratio of at least about 3.0.

62. The method of claim 60 or 61, wherein the first chelating ligand comprises deferoxamine (Df).

63. The method of claim 60 or 61, wherein the first chelating ligand comprises the negatively charged organic moiety.

64. The method of any one of claims 38-63, wherein the negatively charged organic moiety comprises a negatively charged chelating ligand or organic dye.

65. The method of claim 64, wherein the negatively charged chelating ligand is selected from the group consisting of:

ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), DOTA, DOTAGA, NOTA, NODAGA, NETA, porphyrins, polyamines, crown ethers, bis-thiosemicarbazones, and polyoximes

66. The method of claim 64, wherein the negatively charged organic dye is IRDye®800.

67. The method of any one of claims 38-66, wherein the antibody comprises at least one cluster of surface-exposed positively charged amino acids, the cluster comprising at least two surface-exposed, positively charged amino acids within about 30 angstroms of each other.

68. The method of any one of claims 38-67, wherein the antibody specifically binds to DLL3, FAP, CD8, CD4, CD3, IFNγ, Integrin αVβ6, FOLRα, or PSMA.

69. The antibody of any one of claims 38-68, wherein the antibody comprises a light chain variable region comprising 3 LCDR sequences of the 3 LCDRs in any one of the amino

acid sequences shown in FIGs. 12A-12D, and a heavy chain variable region comprising the 3 HCDR sequences of 3 HCDRs in any one of the amino acid sequences shown in FIGs. 12A-12D.

70. An antibody, minibody, or cys-diabody made by the method of any one of claims 38-69.

71. A method of treating a subject, comprising:

identifying a subject in need of treatment with the antibody, minibody, or cys-diabody of any one of claims 1-22; and

administering to the subject a therapeutically effective amount of the antibody, minibody, or cys-diabody, or the composition of claim 23.

72. A method of treating a subject for a cancer, comprising:

identifying a subject in need of treatment for a cancer; and

administering to the subject a therapeutically effective amount of the antibody, minibody, or cys-diabody of any one of claims 1-22, or the composition of claim 23, to thereby treat the cancer.

73. A method of radiotherapy, comprising:

identifying a subject in need of radiotherapy; and

administering to the subject a therapeutically effective amount of the antibody, minibody, or cys-diabody of any one of claims 1-22, or the composition of claim 23, wherein the antibody, minibody, or cys-diabody comprises a radionuclide.

74. A method of imaging a subject, comprising:

administering to a subject a composition comprising an effective amount of the antibody, minibody, or cys-diabody of any one of claims 1-22, or the composition of claim 23, wherein the antibody, minibody, or cys-diabody is detectably labeled; and

imaging the subject to detect the labeled antibody, minibody, or cys-diabody in the subject.

75. The antibody, composition, or the method of any one of the preceding claims, wherein the antibody is an antigen binding fragment having molecular of more than 10 kDa and less than 90 kDa.

76. The antibody, composition, or the method of any one of the preceding claims, wherein the antibody is a nanobody.

77. Use of the antibody, minibody, or cys-diabody of any one of claims 1-22, or the composition of claim 23, for treatment of cancer in a subject in need thereof.

78. Use of the antibody, minibody, or cys-diabody of any one of claims 1-22, or the composition of claim 23, for preparation of a medicament for treatment of cancer in a subject in need thereof.