

METHODS OF SOLID PHASE ANTIBODY CONJUGATION

[0001] The benefit under 35 U.S.C. §119(e) of U.S. Provisional Application No. 63/532,286 filed August 11, 2023, is hereby claimed, and the disclosure thereof is hereby incorporated by reference herein.

FIELD OF THE INVENTION

[0002] The present disclosure relates to methods of reducing, oxidizing, and/or conjugating cys-mAbs on a solid support.

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0003] Incorporated by reference in its entirety herein is a nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: One 50,042 kilobyte XML document named "10476-WO01-SEC_Seqlisting.XML," created on August 7, 2024.

BACKGROUND OF THE INVENTION

[0004] Conjugated biomolecules are a diverse array of substances that comprise multiple precursor molecules, at least one of which is derived from a biological system. Most often, the biologically derived component(s) are produced using recombinant DNA techniques. In the pharmaceutical industry, conjugated biomolecules have been investigated as treatments for a variety of medical conditions. In these cases, the conjugate can provide a number of therapeutic benefits by combining useful properties of two or more precursor molecules into a single entity.

[0005] One particularly successful class of pharmaceutical bioconjugates are antibody conjugates, also called antibody-drug conjugates. These molecules consist of an antibody, typically derived from mammalian cell culture, and a synthetic molecule with biologic or pharmacologic activity. Several antibody-drug conjugates have been approved as cancer therapies, and many more are in clinical and pre-clinical development.

[0006] Pharmaceuticals based on site-specific antibody conjugates, where a synthetic molecule is attached at defined sites in the antibody molecule, can provide therapeutic benefits as well as improved quality control and/or shelf life. Numerous efforts have therefore been undertaken to develop methods for producing site-specific conjugates of antibodies. A common approach to site-specific conjugation is the use of a cysteine mutant antibody (Cys-mAb or Thiomab), in which a new cysteine amino acid is introduced into the antibody primary structure. This engineered cysteine can be used as a site for conjugating a synthetic molecule.

[0007] The conjugation process of a Cys-mAb consists of three key steps: reduction, oxidation, and conjugation. Site-specific conjugation with a Cys-mAb protein requires the engineered cysteine side chain in the reduced thiol form. However, when antibodies are isolated from mammalian cell culture, the

engineered cysteine is generally “capped” as a mixed disulfide with a cytoplasmic thiol such as glutathione. The reduction step removes the cysteamine caps and oxidation reforms disulfide bonds that may have been broken during the reduction. Finally, alkylation is carried out to conjugate the synthetic molecule to the antibody.

[0008] The conjugation process often involves complicated manufacturing processes that require precise control of reaction times and conditions and the hold times of the intermediate pools to achieve the desired reaction products and to mitigate product instability. Because of this, batchwise conjugation poses various drawbacks, including the requirement for additional buffer exchange steps throughout the process, which are challenging to scale-up depending on the time and volume constraints required for a process with unstable intermediates.

[0009] An overview of a typical site-specific conjugation process for an antibody is provided in FIG. 1. In the batchwise manufacturing process, the reduction chemistry step is followed by an Ultrafiltration/Diafiltration (UF/DF) step which buffer exchanges and concentrates the product, resulting in the in-process intermediate “UFDF2.” Buffer exchange is required at this stage because the capped species liberated during reduction needs to be removed from solution before adjusting the pH for the oxidation and conjugation steps, otherwise the capped species will compete with the conjugate for attachment to the monoclonal antibody.

[0010] The UFDF2 pool may exhibit poor product stability if too many interchain disulfide bonds are reduced during the reduction step. Poor product stability is evidenced by a rapid increase in high molecular weight (HMW) species. This instability of the UFDF2 intermediate poses constraints for large-scale manufacturing in terms of minimizing processing time and for process design as the downstream process must be designed in such a way that these product impurities are removed. Additional steps for removing product impurities causes a decrease in product yield.

[0011] An additional challenge of the typical batchwise conjugation process is precise control of the drug antibody ratio (DAR) (alternatively referred to as the peptide antibody ratio (PAR) when the conjugate is a peptide molecule). This represents the molar ratio of the amount of drug (e.g., peptide) conjugated to the monoclonal antibody. Typically, a product is designed to achieve a target DAR (e.g., 2:1 or DAR2), but the process may produce a heterogeneous population of products with different DARs, e.g., 0:1, 1:1, and 3:1, as a function of reaction time and operating pH for the alkylation step of the conjugation process. An increase in products having a higher DAR than the target DAR is typically observed with an increase in reaction time, as free drug continues to conjugate the monoclonal antibody. Elevated levels of non-target DAR species pose a further challenge to downstream processing steps, which must reduce the level of these impurities to an acceptable amount without significantly reducing yield.

[0012] In addition to challenges related to stability of in-process intermediates during a large-scale conjugation process, such reactions may require long processing time which can pose challenges for processing a large mass of product within the allowable schedule of a large-scale facility.

[0013] Accordingly, there remains a need for improved methods of manufacturing antibody conjugates that offer high yield and consistent product quality with shorter processing times.

SUMMARY OF THE INVENTION

[0014] One aspect of the present disclosure is directed to a method for preparing an antigen binding protein conjugate. This method involves the steps of: a) providing an antigen binding protein comprising a mixed disulfide; b) immobilizing the mixed disulfide comprising antigen binding protein on a solid support; c) adding a reducing agent to the immobilized antigen binding protein thereby forming a reduced antigen binding protein; d) adding an oxidizing agent to the reduced antigen binding protein thereby forming an oxidized antibody or oxidized antibody fragment; and e) adding an activated conjugate moiety to the oxidized antigen binding protein thereby forming an antigen binding protein conjugate.

[0015] In one aspect, the method for preparing an antigen binding protein conjugate may further comprise the steps of obtaining an antigen binding protein, and exposing the antigen binding protein to a cysteine blocking agent, where the cysteine blocking agent forms a stable mixed-disulfide with at least one cysteine residue of the antigen binding protein thereby forming the mixed disulfide comprising antigen binding protein.

[0016] In any embodiment of the method for preparing an antigen binding protein conjugate as described herein, the mixed disulfide comprising antigen binding protein is an antigen binding protein comprising one or more capped free cysteines. In any embodiment, the one or more capped free cysteines comprise a cap selected from the group consisting of cysteine, cysteamine, cystamine, and glutathione.

[0017] In any embodiment of the method for preparing an antigen binding protein conjugate as described herein, the solid support is selected from an anion exchange support material, a cation exchange support material, a mixed mode exchange support material, and an affinity ligand support material. In any embodiment, the solid support immobilizes the antigen binding protein at a pH range of 5 to 8. In any embodiment, the solid support immobilizes the antigen binding protein at a salt concentration providing a conductivity of ≤ 15 mS/cm. In any embodiment, the solid support immobilizes the antigen binding protein at a pH range of 5 to 8 and a salt concentration providing a conductivity of ≤ 15 mS/cm.

[0018] In any embodiment of the method for preparing an antigen binding protein conjugate as described herein the solid support comprises an affinity ligand support material. In any embodiment, the affinity ligand support material comprises a ligand that binds an antigen binding protein Fc portion, an antigen binding protein heavy chain portion, or an antigen binding protein light chain portion. In any embodiment, the affinity ligand support material comprises a ligand that binds an antigen binding protein Fc portion.

[0019] In any embodiment of the method for preparing an antigen binding protein conjugate as described herein, the reducing agent is selected from the group consisting of triphenylphosphine-3,3',3''-trisulfonate ("TPPTS"), tris(2-carboxyethyl)phosphine ("TCEP"), and triphenylphosphine-3,3'-disulfonate ("TPPDS"). In any embodiment, the reducing agent is added at a reducing agent to antigen binding protein ratio of 2:1 to 4:1 (mole/mole). In any embodiment, adding the reducing agent to form the reduced antigen binding protein is carried out at a pH of 5.0 to 6.0.

[0020] In any embodiment of the method for preparing an antigen binding protein conjugate as described herein, the oxidizing agent is dehydroascorbic acid ("DHAA"). In any embodiment, the oxidizing agent is added at an oxidizing agent to antigen binding protein ratio of 3:1 to 10:1 (mole/mole).

[0021] In any embodiment of the method for preparing an antigen binding protein conjugate as described herein, the activated conjugate moiety is a peptide comprising a halogen. In any embodiment, the halogen is selected from the group consisting of Br, I, and Cl. In any embodiment, the activated conjugate moiety is added at an activated conjugate moiety to antigen binding protein of 2:1 to 3:1 (mole/mole).

[0022] In any embodiment of the method for preparing an antigen binding protein conjugate as described herein, the reduced antigen binding protein is eluted from the solid support prior to adding said oxidizing agent. In another embodiment, the antigen binding protein is eluted from the solid support prior to adding said activated conjugate moiety.

[0023] Many of the challenges associated with antigen binding protein conjugation discussed above can be addressed by performing either all or a subset of the conjugation reaction steps (*e.g.*, reduction, oxidation, and/or alkylation) using a stationary phase to immobilize the antigen binding protein. Specifically, a solid phase or on-column conjugation process minimizes stability concerns as buffer exchange in a column operation is much faster (occurring in minutes) than buffer exchange during ultra/diafiltration (occurring over hours). This results in producing a higher quality final antigen binding protein conjugate. Additionally, on-column conjugation steps offers the benefit of reducing the overall processing time as compared to a batch-wise process requiring multiple time-intensive UF/DF steps. Finally, the exposure times of the conjugation reagents can be controlled much more precisely in a solid phase approach thereby allowing for more precise control of the DAR ratio which reduces the overall downstream purification burden.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] FIG. 1. is a schematic of a general antibody conjugation process. Conjugation of a synthetic molecule to an antibody involves the steps of reduction, oxidation, and alkylation as described herein.

[0025] FIG. 2 is a graph showing the initial evaluation of on-column reduction of a cysteamine capped anti-GIPR antibody at elevated pH for different reagent conditions and reaction times (30, 60, or 120 minute reduction reaction times). Shown is CEX-HPLC data (an indicator for the extent of reaction)

for three different conditions, (1) the control at 1X reducing agent (RA) at pH 7.2, (2) double the amount of reduction reagent (2X RA), pH 7.2, and (3) 1X RA at lower pH (pH 6.8).

[0026] FIG. 3 shows that evaluation of Protein A chromatography binding capacity of the cysteamine capped anti-GIPR antibody at various pH values. Briefly, the antibody was loaded to 40 g/L resin at pH 7.2 and the pH was equilibrated with wash buffers of varying pH (pH 5.0, 5.5, 6.0, 6.5). The graph shows the washout behavior upon pH adjustment as well as the elution profile upon lowering the pH further, which are qualitatively consistent with the measured step yields. As shown, the yield decreases with respect to pH over the range of 6.0 to 5.0 but remains high within the studied range.

[0027] FIG. 4 shows the evaluation of on-column reduction of the anti-GIPR antibody at different pHs (pH 5.0, 5.5, and 6.0) and reaction times (60 mins and 120 mins). The target ($\leq 10\%$ mono-capped CEX-HPLC) was reached for all conditions demonstrating the requirement for operating the reduction step at low pH..

[0028] FIG. 5 is a graph showing product quality (SE-HPLC high molecular weight) of the anti-GIPR antibody for pH and time evaluations of on-column reduction in FIG. 4. As pH decreases, the product quality increases. Reactions at pH 5.0 are shown by left two columns, reactions at pH 5.5 are shown by middle columns, and reactions at pH 6.0 are shown by right two columns.

[0029] FIG. 6 is a graph showing the evaluation of on-column reduction of the anti-GIPR antibody at different pH, times, and configurations (static hold vs. flowthrough). The target reaction conversion ($\leq 10\%$ mono-capped) was reached for all conditions except for pH 6.0 with 30 minute flowthrough.

[0030] FIG. 7 is a graph of product quality as assessed by the presence of high molecular weight (HMW) species (measured using SE-HPLC) of the anti-GIPR antibody as a function of pH, time, and configuration evaluations of on-column reduction. As pH decreases, the product quality increases.

DETAILED DESCRIPTION OF THE INVENTION

[0031] The present disclosure provides a method of preparing an antigen binding protein conjugate on a solid support. In accordance with this method, the conjugation steps of reduction, oxidation and/or alkylation are carried out on a solid support to improve the product yield, quality, and stability, while decreasing the overall process time.

[0032] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention as claimed.

[0033] Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art. The terminology

used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard techniques can be used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

[0034] In this application, the use of the singular terms include pluralities and plural terms shall include the singular unless specifically stated otherwise. As used herein, the singular forms “a”, “an”, and “the” include both singular and plural referents unless the context clearly dictates otherwise.

[0035] In this application, the use of “or” means “and/or” unless stated otherwise. Furthermore, the use of the term “including”, as well as other forms, such as “includes” and “included”, is not limiting. Also, terms such as “element” or “component” encompass both elements and components comprising one unit and elements and components that comprise more than one subunit unless specifically stated otherwise. Also, the use of the term “portion” can include part of a moiety or the entire moiety.

[0036] The terms “comprising”, “comprises” and “comprised of” as used herein are synonymous with ‘including’, ‘includes’, ‘containing’, or ‘contains’, are inclusive or open-ended, and do not exclude additional, non-recited members, compounds, products, elements, or method steps. The expression “consisting essentially of” used in the context of a product or method (*e.g.*, “a method consisting essentially of”) means that additional elements, ingredients, or steps may be present but only to the extent that such additional elements, ingredients, or steps do not change/alter the characteristic/activity/functionality of said product, composition or method. The expression “consisting of” used in the context of a product, composition, or method means that the referenced product, composition, or method includes only the elements, steps, or ingredients specifically recited in the particular embodiment or claim.

[0037] In embodiments or claims where the term “comprising” is used as the transition phrase, such embodiments and claims can also be envisioned with replacement of the term “comprising” with the terms “consisting of” or “consisting essentially of”.

[0038] The recitation of numerical ranges by endpoints includes all numbers and fractions subsumed within the respective ranges, as well as the recited endpoints.

[0039] The term “about” as used herein when referring to a measurable value such as a parameter, an amount, a temporal duration, and the like, is meant to encompass variations of +/-10% or less, preferably +/- 1–5% or less from the specified value, insofar such variations are appropriate to perform in the disclosed embodiment. It is to be understood that the value to which the modifier “about” refers is itself also specifically, and preferably, disclosed.

[0040] In one aspect, the present disclosure provides a method for preparing an antigen binding protein conjugate. This method involves the steps of: providing an antigen binding protein comprising a mixed disulfide; immobilizing the mixed disulfide comprising antigen binding protein on a solid support; adding a reducing agent to the immobilized antigen binding protein to form a reduced antigen binding protein; adding an oxidizing agent to the reduced antigen binding protein to form an oxidized antigen

binding protein; and adding an activated conjugate moiety to the oxidized antigen binding protein to form an antigen binding protein conjugate.

[0041] An “antigen binding protein” as used herein means any protein that binds a specified target antigen or protein. The term encompasses (i) full-length and substantially full-length immunoglobulin molecules, *i.e.*, antibodies, (ii) fragments of full-length antibodies (*i.e.*, antibody fragments or epitope binding fragments), and (iii) antibody derivatives.

[0042] An “antibody” as referred to herein encompasses immunoglobulin molecules comprising a tetramer composed of at least two heavy (H) polypeptide chains and at least two light (L) polypeptide chains. Each heavy chain is comprised of a heavy chain variable (VH) region and a heavy chain constant (CH) region. Heavy chains are classified as mu (μ), delta (Δ), gamma (γ), alpha (α), and epsilon (ϵ), and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. The term “heavy chain” in the context of an antibody refers to a polypeptide comprising, from amino terminus to carboxyl terminus, a single immunoglobulin VH, an immunoglobulin heavy chain constant domain 1 (CH1), an immunoglobulin hinge region, an immunoglobulin heavy chain constant domain 2 (CH2), an immunoglobulin heavy chain constant domain 3 (CH3), and optionally an immunoglobulin heavy chain constant domain 4 (CH4). Each light chain of an antibody is comprised of a light chain variable (VL) region and a light chain constant (CL) region. Human light chains are classified as kappa and lambda light chains. The term “light chain” refers to a polypeptide comprising, from amino terminus to carboxyl terminus, a single immunoglobulin VL and a single immunoglobulin CL. The antibody chains are linked together via inter-polypeptide disulfide bonds between the CL domain and the CH1 domain (*i.e.*, between the light and heavy chain) and between the hinge regions of the antibody heavy chains.

[0043] The term “antibody” also includes antibodies comprising two substantially full-length heavy chains and two substantially full-length light chains provided the antibodies retain the same or similar binding and/or function as the antibody comprised of two full length light and heavy chains. For example, antibodies having 1, 2, 3, 4, or 5 amino acid residue substitutions, insertions, or deletions at the N-terminus and/or C-terminus of the heavy and/or light chains are included in the definition provided that the antibodies retain the same or similar binding and/or function as the antibodies comprising two full length heavy chains and two full length light chains.

[0044] Exemplary antibodies useful in the methods of conjugation described herein include, without limitation chimeric, humanized, fully human, and multi-specific antibodies, *e.g.*, bispecific antibodies.

[0045] An “antibody fragment” of an antibody encompasses any polypeptide fragment, region, portion, or domain of a full-length antibody that exhibits some or all of the binding properties of the full-length antibody and can be obtained, for example, by protease cleavage of an intact parental antibody. Exemplary functional immunoglobulin fragments include, but are not limited to, Fab, Fab', F(ab')₂, Fv, Fd, dAb (single-domain), Fc domain fragments.

[0046] An “antibody derivative” is a protein or polypeptide that contains at least one epitope binding domain of an antibody and is typically formed using recombinant techniques or via chemical

modification of a parent antibody or portion thereof. An antibody derivative comprises an amino acid sequence that is substantially similar to at least a portion of the amino acid sequence of one or more parental antibodies. Exemplary antibody derivatives include, without limitation single chain Fv (scFv), including monospecific and bispecific scFvs. Antibody derivatives also encompass antibodies or antibody fragments comprising one or more modified amino acid residues, e.g., one or more amino acid residues chemically modified by alkylation, PEGylation, acylation, ester formation, amide formation, or the like.

[0047] Antigen binding proteins suitable for conjugation using the methods described herein, including antibodies, antibody fragments, antibody derivative, and antibody mimetics may be produced using methods and techniques known in the art including, without limitation, hybridomas, recombinant DNA techniques, or enzymatic or chemical cleavage of intact antibodies.

[0048] In any embodiment of the method of preparing an antigen binding protein conjugate as described herein the antigen binding protein comprises a conjugation site that is amenable to conjugation. A suitable site “amenable to conjugation” can be, for example, a side chain of an amino acid residue at a selected conjugation site that will react with the activated moiety on the conjugate molecule of interest or with the activated moiety on a linker covalently attached to the conjugate molecule of interest, under defined chemical conditions resulting in the formation of a covalent bond between the amino acid side chain and the activated moiety (directly or via the linker).

[0049] In any embodiment, the antigen binding protein of the method described herein is engineered to comprise a site, *i.e.*, an amino acid residue, that is amenable to conjugation. For example, the antigen binding protein is engineered to comprise a free cysteine residue to facilitate site-specific conjugation. Free cysteines are suitable attachment points for conjugation of various activated chemical moieties. A free cysteine is a cysteine residue that is not engaged in an ordinary disulfide bond between two cysteines of one or two polypeptides. Therefore, by introducing a cysteine residue into an antigen binding protein, a free cysteine is usually obtained as no partner for forming a disulfide bond is present in the protein.

[0050] The selection of the placement of the conjugation site in the overall antigen binding protein is an important facet of selecting an internal conjugation site on the antigen binding protein. Any of the exposed amino acid residues on the antigen binding protein can be potentially useful conjugation sites and can be mutated to cysteine or some other reactive amino acid for site-selective coupling, if not already present at the selected conjugation site of the antigen binding protein sequence. The amino acid residue, for example, a cysteinyl residue, at the internal conjugation site that is selected can be one that occupies the same amino acid residue position in a native Fc domain sequence, or the amino acid residue can be engineered into the Fc domain sequence by substitution or insertion.

[0051] In any embodiment, an antigen binding protein engineered to comprise a free cysteine residue is an antigen binding protein wherein at least one amino acid in either the heavy or light chain is deleted, altered, or substituted (preferably with another amino acid) to provide at least one free cysteine. In any embodiment the engineered antigen binding protein comprises at least one amino acid deletion or

substitution of an intrachain or interchain cysteine residue. An interchain cysteine residue refers to a cysteine residue that is involved in a native disulfide bond either between the light and heavy chain of an antigen binding protein or between the two heavy chains of an antigen binding protein. An intrachain cysteine residue is one naturally paired with another cysteine in the same heavy or light chain. In any embodiment, the deleted or substituted interchain cysteine residue is involved in the formation of a disulfide bond between the light and heavy chain. In any embodiment the deleted or substituted cysteine residue is involved in a disulfide bond between the two heavy chains. In one embodiment, due to the complementary structure of an antibody, in which the light chain is paired with the VH and CH1 domains of the heavy chain and wherein the CH2 and CH3 domains of one heavy chain are paired with the CH2 and CH3 domains of the complementary heavy chain, a mutation or deletion of a single cysteine in either the light chain or in the heavy chain would result in two unpaired, free cysteine residues in the engineered antibody.

[0052] In one embodiment, an interchain cysteine residue is deleted to generate a free cysteine residue in the antigen binding protein. In one embodiment, an interchain cysteine is substituted for another amino acid (*e.g.*, a naturally occurring amino acid). For example, the amino acid substitution can result in the replacement of an interchain cysteine with a neutral (*e.g.*, serine, threonine or glycine) or hydrophilic (*e.g.*, methionine, alanine, valine, leucine or isoleucine) residue.

[0053] In one embodiment, an intrachain cysteine residue is deleted to generate a free cysteine residue in the antigen binding protein. In one embodiment, an intrachain cysteine is substituted for another amino acid (*e.g.*, a naturally occurring amino acid). For example, the amino acid substitution can result in the replacement of an intrachain cysteine with a neutral (*e.g.*, serine, threonine or glycine) or hydrophilic (*e.g.*, methionine, alanine, valine, leucine or isoleucine) residue.

[0054] In any embodiment the deleted or substituted cysteine residue is in the light chain (either kappa or lambda) thereby leaving a free cysteine on the heavy chain. In any embodiment the deleted or substituted cysteine residue is in the heavy chain leaving the free cysteine on the light chain constant region.

[0055] In one embodiment of the present disclosure, the antigen binding protein is an antigen binding protein that binds to the glucose-dependent insulinotropic polypeptide receptor (GIPR). In one embodiment, the antigen binding protein is an anti-GIPR antibody. Suitable anti-GIPR antibodies include those known in the art, in particular, those disclosed in U.S. Patent No. 10,905,772 to Cheng et al., which is hereby incorporated by reference in its entirety. In one embodiment, the anti-GIPR antibody comprises the anti-GIPR antibody referred to herein as 2G10_LC1.003 or a functional variant thereof; the anti-GIPR antibody referred to herein as 2G10_LC1.006 or a functional variant thereof; or the anti-GIPR antibody referred to herein as 5G12.006 or a functional variant thereof. The sequences of the anti-GIPR antibody heavy and light chain complementarity determining regions (CDRs), heavy and light chain variable regions, and heavy and light chain are provided in Tables A-D herein. Suitable functional variants of the disclosed anti-GIPR antibodies include those having at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at

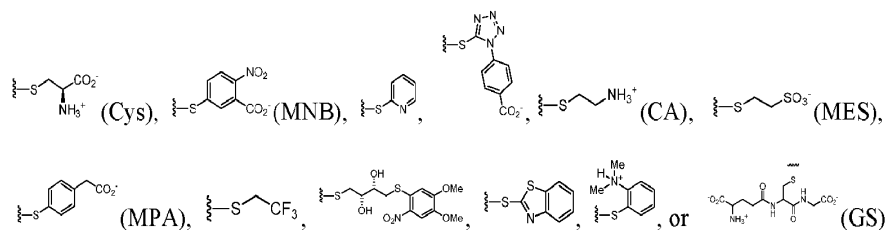
least 99% sequence identity to the heavy and light chain CDRs, variable regions, and full-length chain sequences provided herein.

[0056] In one embodiment, the anti-GIPR antibody comprises a cysteine or non-canonical amino acid substitutions at one or more conjugation site(s). Suitable conjugation sites include, without limitation, the amino acid residue corresponding to residue D70 of the antibody light chain comprising sequence of SEQ ID NO: 34; the amino acid residue corresponding to residue E276 of the antibody heavy chain comprising the sequence of SEQ ID NO: 32; and the amino acid residue corresponding to residue T363 of the antibody heavy chain comprising the sequence of SEQ ID NO: 32. For sake of clarity, the residue D70 of SEQ ID NO: 34 is the same substitution site as AHo position D88 and Kabat position D70 of SEQ ID NO: 34; the residue E276 of SEQ ID NO: 32 is the same substitution site as AHo position E384 and Kabat position E285 of SEQ ID NO: 32; and the residue T363 of SEQ ID NO: 32 is the same substitution site as AHo position T487 and Kabat position T382 of SEQ ID NO: 32.

[0057] In order to be an effective target of a conjugation reaction, the free cysteine must be in the reduced form. A protein with a free cysteine may be difficult to produce, and is thus frequently obtained as a mixed disulfide including a small organic moiety or "Cap". In the present application the term "mixed disulfide" refers to a disulfide bond linking two different entities, *e.g.*, polypeptide and non-polypeptide entities. Thus, in any embodiment, the antigen binding protein of the method described herein comprises a capped free cysteine in the form of a mixed disulfide that is represented as an antigen binding protein-S-S-Cap molecule. The antigen binding protein may additionally include "ordinary" disulfides bonds (*e.g.*, disulfide bonds linking two cysteine amino acid residues within one polypeptide or two different polypeptides) in addition to the mixed disulfide bonds.

[0058] As described above, the Cap is usually derived from a small organic moiety, including at least one sulfur atom that is part of the disulfide bond of the mixed disulfide. Such organic moieties can exist as monomers in the reduced form or as dimers in the oxidized form. In the mixed disulfide, -S-Cap is thus the oxidized form of the monomer or half a dimer. In one embodiment the -S-Cap is derived from cysteine/cystine, cysteamine/cystamine (which is a decarboxylated cystine), or glutathione (G-SH)/glutathione disulfide (GS-SG). Thus, in any embodiment, the mixed disulfide can be, for example, antigen binding protein-S-S-cys, antigen binding protein-S-S-cyst, or antigen binding protein-S-S-G, where cys refers to half of a cystine, cyst refers to half of cystamine, and G refers to half of glutathione disulfide. In other words, in one embodiment the Cap of the free cysteine is a Cap derived from cysteine, cysteamine or glutathione.

[0059] Alternatively suitable Caps can be derived from the following small organic moieties:



[0060] The conjugation process involves three key steps, i.e., reduction, oxidation, and conjugation via alkylation, that can individually or in combination be carried out on a solid phase support. In other words, the antigen binding protein comprising the mixed disulfide can be immobilized on a solid support and the reduction, oxidation, and/or alkylation (conjugation) steps are carried out on the immobilized antigen binding protein. In one embodiment, the antigen binding protein comprising a mixed disulfide is immobilized on a solid support prior to the reducing step and remains immobilized for the reduction, oxidation, and alkylation (conjugation) steps. In another embodiment, the antigen binding protein comprising a mixed disulfide is immobilized on a solid support prior to the reducing step and remains immobilized for the reduction and oxidation steps, but is eluted prior to the alkylation step and the alkylation step is carried out in solution. In another embodiment, the antigen binding protein comprising a mixed disulfide is immobilized on a solid support prior to the reducing step and remains immobilized for only the reduction step. In accordance with this embodiment, the reduced antigen binding protein is eluted from the solid support prior to the oxidation and alkylation steps and these steps are carried out in solution.

[0061] In accordance with the method of preparing an antigen binding protein conjugate as described herein, the solid support material utilized in this method encompasses any solid support material capable of immobilizing the antigen binding protein and maintaining the antigen binding protein in an immobilized state for the reduction, oxidation, and/or alkylation steps of the process. Generally, the solid support may be any suitable insoluble, functionalized material to which an antigen binding protein can be reversibly attached, either directly or indirectly, allowing it to be separated from unwanted materials, for example, excess reagents, contaminants, and solvents. Examples of suitable solid supports include, for example and without limitation, functionalized polymeric materials, *e.g.*, agarose, or its bead form Sepharose®, dextran, polystyrene and polypropylene, or mixtures thereof; compact discs comprising microfluidic channel structures; protein array chips; pipet tips; membranes, *e.g.*, nitrocellulose or PVDF membranes; and microparticles, *e.g.*, paramagnetic or non-paramagnetic beads. The solid support may take any form, *e.g.*, beads, particles, membrane, sheet, column, plate, tube, bottle, flask, and the like, comprising a material suitable for antigen binding protein capture and immobilization. In any embodiment, the solid support material is a material that immobilizes the antigen binding protein at a pH range of pH 5 to pH 8. This pH range is particularly suitable for methods where the reduction, oxidation, and alkylation steps are carried out on the solid support. In another embodiment, the solid support material is a material that immobilizes the antigen binding protein at a pH range of pH 5 to pH 7. This pH range is particularly suitable for methods where the reduction and oxidation steps are carried out on the solid support and alkylation is carried out in solution phase. In yet another embodiment, the solid support material is a material that immobilized the antigen binding protein at a pH range of pH 5 to pH 6. This pH range is particularly suitable for methods where the reduction step is carried out on the solid support and oxidation and alkylation steps are carried out in solution phase.

[0062] In any embodiment, the solid support material is a material that immobilizes the antigen binding protein at a salt concentration providing a conductivity of $\leq 15\text{mS/cm}$.

[0063] In any embodiment, the solid support material is a material that immobilizes the antigen binding protein at a pH range of pH 5 to pH 8 and a salt concentration providing a conductivity of $\leq 15\text{mS/cm}$.

[0064] In any embodiment, the solid support material is a material that immobilizes the antigen binding protein at a pH range of pH 5 to pH 7 and a salt concentration providing a conductivity of $\leq 15\text{mS/cm}$.

[0065] In any embodiment, the solid support material is a material that immobilizes the antigen binding protein at a pH range of pH 5 to pH 6 and a salt concentration providing a conductivity of $\leq 15\text{mS/cm}$.

[0066] Suitable solid support materials capable of immobilizing the antigen binding protein at the above noted pH and salt content include, without limitation, cation exchange resins, anion exchange resins, mixed mode exchange resins, and affinity ligand resins. Thus, in any embodiment, the solid support material is a cation exchange support material capable of immobilizing the antigen binding protein at a pH of 5 to 8, at a pH of 5 to 7, or at a pH of 5 to 6. In another embodiment, the solid support material is an anion exchange support material capable of immobilizing the antigen binding protein at a pH of 5 to 8, at a pH of 5 to 7, or at a pH of 5 to 6. In another embodiment, the solid support material is mixed mode exchange support material capable of immobilizing the antigen binding protein at a pH of 5 to 8, at a pH of 5 to 7, or at a pH of 5 to 6. In another embodiment, the solid support material is an affinity ligand support material capable of immobilizing the antigen binding protein at a pH of 5 to 8, at a pH of 5 to 7, or at a pH of 5 to 6.

[0067] In any embodiment, the antigen binding protein is loaded or immobilized on the solid support material at a concentration of about 20 g/L of resin to about 60 g/L of resin. In any embodiment, the antigen binding protein is loaded onto the solid support material at a concentration of about 20 g/L resin, about 25 g/L resin, about 30 g/L resin, about 35 g/L resin, about 40 g/L resin, about 45 g/L resin, about 50 g/L resin, about 55 g/L resin, or about 60 g/L resin.

[0068] In one embodiment the solid support material comprises an affinity medium comprising a ligand that binds an antigen binding protein Fc portion, an antigen binding protein heavy chain portion, or an antigen binding protein light chain portion. Suitable affinity ligands include, without limitation, Protein A and Protein G, which are bacterial proteins that bind to the Fc region of immunoglobulins; artificial protein A (ApA); Protein A/G, which is a fusion protein of Proteins A and G that also binds the Fc region of immunoglobulins; Protein L, another bacterial protein that binds kappa variable light chains of immunoglobulins; and artificial protein L (PpL). Other suitable affinity ligands for immobilization of antibodies and antibody fragments include the lectin, jacalin; M protein family members; protein Sir22; protein Arp4; and the hexamer peptide ligand HWRGWV. Additional affinity ligands that can be employed in the solid support material used in the methods described herein include immobilized metal ions. Chelating agents, such as iminodiacetic acid (IDA) or nitrilotriacetic acid (NTA), generate metal

ions (e.g., Ni^{2+} , Zn^{2+} , Co^{2+} , Cu^{2+} , and Fe^{2+}) on the solid phase matrix to facilitate the affinity reaction between the metal ion and side chains or termini of amino acids, such as histidine, serine, aspartate, cysteine, glutamate, methionine, tyrosine, and tryptophan of the antibody or antibody fragment. Other affinity ligands suitable for capturing and immobilizing the antigen binding protein as described herein are described in Cassedy and O'Kennedy, *AFFINITY CHROMATOGRAPHY METHODS AND PROTOCOLS in Methods in Molecular Biology* (Springer Science, 2022), which is hereby incorporated by reference in its entirety).

[0069] As noted above, the conjugation process starts with a reduction step to remove the cap moiety from the cysteine to obtain an antigen binding protein with a free reduced cysteine (-SH) that is reactive in a conjugation reaction. In order to obtain an antigen binding protein with a reactive sulfur atom, a reducing agent is added to the immobilized antigen binding protein comprising the mixed disulfide. The desired reaction time to produce the reduced antigen binding protein can be achieved via a static hold, *i.e.*, introducing the reducing agent to the solid support material comprising the immobilized antigen binding protein followed by a pause with no flow or wash for a specified period of time. Alternatively, the desired reaction time can be achieved via continuous flow, where the reducing agent is continuously flowed over the solid support material comprising the antigen binding protein.

[0070] Suitable reducing agents that can be utilized in the methods described herein include negatively charged reducing agents that can effectively reduce the positively charged cysteine cap. In any embodiment, the reducing agent is a redox buffer selected from the group of glutathione, gamma-glutamylcysteine, cysteinylglycine, cysteine, N-actylcystein, cysteamine and lipamide. In any embodiment, the reducing agent is a small molecule reducing agent, e.g., such as dithiothreitol (DTT). In any embodiment, the reducing agent is a phosphine, e.g., an aromatic phosphine such as a triarylphosphine, a substituted triarylphosphine such as tris(2-carboxyethyl)phosphine ("TCEP"), trisodium triphenylphosphine-3,3',3''-trisulfonate (TPPTS), or disodium triphenylphosphine-3,3'-disulfonate (TPPDS). In a preferred embodiment, the reducing agent is TPPTS.

[0071] To achieve an effective reduction of the mixed disulfide comprising antigen binding protein, an excess of the reducing agent in molar concentrations is usually applied. The amount of reducing agent may be expressed in equivalents of the amount of the mixed disulfide comprising antigen binding protein such that where the amount of reducing agent is 1 equivalent of the amount of disulfide comprising antigen binding protein, the molar concentration of the reducing agent and the mixed disulfide antigen binding protein are equal. In one embodiment, the amount of the reducing agent added is from about 2 molar equivalents to about 4 molar equivalents of the molar amount of the mixed disulfide containing antigen binding protein. In other words, the reducing agent is added to the solid support comprising the mixed disulfide comprising antigen binding protein at a reducing agent to mixed disulfide comprising antigen binding protein ratio of 2:1, 3:1, or 4:1 (mole/mole). In any embodiment, the reducing agent is added to the solid support at reducing agent to antigen binding protein ratio of >4:1 (mole/mole).

[0072] In any embodiment, the pH during the reduction step is maintained at a pH of about 5.0 to about 8.0. In any embodiment, the pH during the reduction step is maintained at a pH of about 5.0 to about 7.0. In a preferred embodiment, the pH during the reduction step is maintained at a pH of about 5.0 to about 6.0. More preferably, the pH during the reduction step is maintained at a pH of about 5.0 to about 5.5.

[0073] In any embodiment, the salt concentration during the reduction step is maintained to provide a conductivity of ≤ 15 mS/cm. A suitable salt concentration provides a conductivity of 15 mS/cm, 10 mS/cm, 9 mS/cm, 8 mS/cm, 7 mS/cm, 6 mS/cm, 5 mS/cm, 4 mS/cm, 3 mS/cm, 2 mS/cm, or 1 mS/cm. In any embodiment, a suitable salt concentration provides a conductivity of about 3 mS/cm to about 7 mS/cm, or of about 4 mS/cm to about 6 mS/cm, or of about 5 mS/cm.

[0074] In order to reduce the amount of over-reduction of the antigen binding protein, it is advantageous to reduce the amount required, which as described herein is possible if the process steps are optimized. An effective reduction reaction using lower amounts of the reducing agent requires that remaining reaction conditions are carefully selected as is provided by the present disclosure.

[0075] The reduction of the mixed disulfide comprising antigen binding protein may, depending on the conditions, take minutes, *e.g.*, 5 minutes, 10 minutes, 20 minutes, 30 minutes, 60 minutes, 120 minutes, 180 minutes, or hours, *e.g.*, 1 hour, 1.5 hours, 2 hours, 2.5 hours, 3 hours, 3.5 hours, 4 hours, 4.5 hours, 5 hours, 5.5 hours, 6 hours, 6.5 hours, 7 hours, 7.5 hours, or > 7.5 hours. In exemplary embodiment, the reduction of the mixed disulfide comprising antigen binding protein is carried out for a duration of 30 minutes to 120 minutes. The skilled person will know that different conditions will result in different efficacy and thus the time and conditions needed to obtain complete or almost complete reduction of the mixed-disulfide are described in more detailed in the Examples below.

[0076] A sufficient reduction process should result in at least a 70% reduction of all capped cysteines in the antigen binding protein. In any embodiment, the reduction step results in at least 75% reduction of all capped cysteines, at least 80% reduction of all capped cysteines, at least 85% reduction of all capped cysteines, at least 90% reduction of all capped cysteines, at least 95% reduction of all capped cysteines, or $>95\%$ reduction of all capped cysteines. Thus, the reduction is considered satisfactory when the amount of non-reduced mixed disulfide comprising antigen binding protein is at most 30%, at most 25%, at most 20%, at most 15%, at most 10%, or at most 5% of the amount of non-reduced mixed disulfide.

[0077] In one embodiment, the antigen binding protein comprises two capped cysteines, *i.e.*, two sites of conjugation. In this embodiment, the reduction process results in $\leq 20\%$ mono-capped species, $\leq 19\%$ mono-capped species, $\leq 18\%$ mono-capped species, $\leq 17\%$ mono-capped species, $\leq 16\%$ mono-capped species, $\leq 15\%$ mono-capped species, $\leq 14\%$ mono-capped species, $\leq 13\%$ mono-capped species, $\leq 12\%$ mono-capped species, $\leq 11\%$ mono-capped species, $\leq 10\%$ mono-capped species. In a preferred embodiment, the reduction process results in $\leq 10\%$ mono-capped species.

[0078] The reduction process may be carried out at a temperature between 1°C and 50°C, such as at room temperature, *i.e.*, at about 18°C to 25°C. In an alternative embodiment, the reduction process may be performed at a colder temperature, such as below 10°C, such as around 4°C to 6°C.

[0079] Once the mixed disulfide comprising antigen binding protein has been reduced the solid support comprising the reduced antigen binding protein can optionally be subject to one or more washes, *e.g.*, using a sodium acetate buffer solution, to remove the reducing agent and/or the released Cap molecules.

[0080] Before proceeding with the conjugation step the reduced antigen binding protein is subject to an oxidation step. Because the reduction step can reduce inter-chain disulfides of paired cysteines in an antigen binding protein (in addition to removing the caps of the engineered cysteine residues), these reduced inter-chain disulfide bonds must be reformed. This is achieved via the oxidation step. Accordingly, an oxidizing agent capable of restoring inter-chain disulfide linkages is introduced or added to the reduced or “over-reduced” antigen binding protein.

[0081] Accordingly, in any embodiment, an excess of the oxidizing agent in molar concentrations is added to the solid phase comprising the immobilized reduced antigen binding protein or solution comprising the reduced antigen binding protein (if eluted from the solid phase) to ensure an effective oxidation of the over-reduced antigen binding protein is achieved. In embodiments where oxidation of the reduced antigen binding protein is carried out on the solid support, the solid support may be subject to one or more pre-oxidation washes, *e.g.*, using an alkalinizing buffer solution, to adjust the conditions, *e.g.*, increase pH, of the solid support.

[0082] The amount of oxidizing agent added for the oxidation step may be expressed as the ratio of oxidizing agent (moles) to reduced antigen binding protein (moles). In any embodiment, the oxidizing agent is added to the reduced antigen binding protein, whether immobilized on the solid support or in solution, at an oxidizing agent to reduced antigen binding protein ratio of 3:1 to 10:1. In particular, a suitable oxidizing agent to antigen binding protein ratio is 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, or 10:1 (mole/mole).

[0083] Suitable oxidizing agents include those known in the art. An exemplary oxidizing agent for use in the methods described herein is dehydroascorbic acid.

[0084] The oxidation of the over-reduced antigen binding protein may, depending on the conditions, take minutes or hours. The skilled person will know that different conditions will result in different efficacy and thus the time and conditions needed to obtain complete or almost complete oxidation of the over-reduced antigen binding protein are described in more detailed in the Examples below.

[0085] In any embodiment, the pH during the oxidation step is maintained at a pH of about 5.0 to about 8.0. In any embodiment, the pH during the oxidation step is maintained at a pH of about 5.0 to about 7.0. In a preferred embodiment, the pH during the oxidation step is maintained at a pH of about 5.0 to about 6.0. More preferably, the pH during the oxidation step is maintained at a pH of about 5.0 to about 5.5.

[0086] The oxidation may be carried out for a duration of at least 30 minutes, at least 1 hour, or at least 2 hours. In one embodiment the oxidation is carried out for a duration of about 2 to about 10 hours, or a duration of about 3 to about 6 hours, a duration of about 3 to about 4 hours, or a duration of about 1 to about 2 hours after addition of the oxidation agent. In certain embodiments, the oxidation may be carried out for >10 hours. The desired reaction time to produce the oxidized antigen binding protein on the solid support can be achieved via a static hold, *i.e.*, introducing the oxidizing agent to the solid support material comprising the immobilized antigen binding protein followed by a pause with no flow or wash for a specified period of time. Alternatively, the desired reaction time can be achieved via continuous flow, where the oxidizing agent is continuously flowed over the solid support material comprising the antigen binding protein.

[0087] The oxidation process may be carried out at a temperature between 1°C and 50°C, such as at room temperature, *i.e.*, at about 18°C to about 25°C. In an alternative embodiment, the reduction process may be performed at a colder temperature, such as below 10°C, *e.g.*, around 4°C to 6°C.

[0088] In order to have a sufficiently effective process, the oxidation should result in at least a 70% oxidation of the total amount of over-reduced antigen binding protein. In any embodiment, the oxidation step results in at least 75% oxidation of the total amount of over-reduced antigen binding protein, at least 80% oxidation of the total amount of over-reduced antigen binding protein, at least 85% oxidation of the total amount of over-reduced antigen binding protein, at least 90% oxidation of the total amount of over-reduced antigen binding protein, at least 95% oxidation of the total amount of over-reduced antigen binding protein, or >95% oxidation of the total amount of over-reduced antigen binding protein. Thus, oxidation is considered satisfactory when the amount of over-reduced antigen binding protein is at most 30%, at most 25%, at most 20%, at most 15%, at most 10%, or at most 5% of the total antigen binding protein following oxidation.

[0089] Once the antigen binding protein has been oxidized, the solid support comprising the oxidized antigen binding protein can optionally be subject to one or more washes, *e.g.*, using an alkalizing buffer solution, to remove the oxidizing reagent.

[0090] In the conjugation reaction, an activated conjugate moiety is covalently bonded to the sulfur atom of the free cysteine of the reduced and oxidized antigen binding protein (protein-SH). The conjugate moiety may be any moiety suitable for conjugation to the antigen binding protein. Suitable moieties include, without limitation, peptides, polypeptides, proteins, prodrugs which are metabolized to an active agent *in vivo*, polymers, nucleic acid molecules, small molecules, binding agents, mimetic agents, synthetic drugs, inorganic molecules, organic molecules and radioisotopes. In one embodiment, the conjugate moiety is a protein modifying moiety. The protein modifying moiety may be a chemical moiety capable of altering one or more features of the antigen binding protein. In one embodiment the conjugate moiety is a protein modifying group, such as a chemical moiety capable of stabilizing the antigen binding protein, increasing the circulatory half-life of the antigen binding protein, or increasing potency of the antigen binding protein. In an alternative embodiment, the conjugate moiety comprises a therapeutic moiety, *e.g.*, a chemical or biological moiety that exerts a therapeutic effect. For example, a

therapeutic moiety may be a small molecule, peptide, polypeptide, or oligonucleotide that functions as a target receptor agonist, a target receptor antagonist, a recombinant protein, a protein mimetic, or a chemical or peptide that otherwise modulates a signaling pathway. In yet another embodiment, the conjugate moiety comprises a cytotoxic or cytostatic agent. Suitable cytotoxic agents include, without limitation, any substance that inhibits or prevents the function of cells and/or causes destruction of cells. Exemplary cytotoxic agents include radioactive isotopes (e.g., ^{211}At , ^{131}I , ^{125}I , ^{90}Y , ^{186}Rd , ^{188}Re , ^{153}Sm , ^{212}Bi , ^{32}P , ^{60}Co , and radioactive isotopes of Lu), chemotherapeutic agents, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including synthetic analogs and derivatives thereof

[0091] In order for the conjugation to occur effectively, the conjugate moiety is utilized in an activated form, *i.e.*, comprising an active moiety amenable to conjugation to antigen binding protein comprising the free cysteine. Alternatively, the conjugate moiety is attached to a linker moiety, e.g., a peptide linker, that comprises an active moiety suitable for conjugation. Suitable linker moieties are disclosed in more detail herein. Such activate moieties may include a sulfhydryl-reactive chemical group, such as a maleimide, a haloacetyl, an aziridine, an acryloyl, an arylating agent, a vinylsulfone, a pyridyl disulfide, an iodoacetamide, a TNB-thiol, or other thiol-reactive conjugation partner which are known in the art. Alternatively, the activated conjugate moiety may comprise a halogenated conjugate moiety, such as a halogenated peptide ligand. Such halogenated conjugate moiety, *e.g.*, halogenated peptide ligand, may comprise a halogen moiety selected from bromine (Br), iodine (I), fluorine (F), or chlorine (Cl). According, adding an activated conjugate moiety to the oxidized antigen binding protein immobilized to the solid support or eluted from the solid support results in the conjugation of the conjugate moiety via alkylation to the oxidized antigen binding protein to form an antigen binding protein conjugate as disclosed herein.

[0092] In one embodiment the molar concentration of the activated conjugate moiety is at least twice the molar concentration of the antigen binding protein to be conjugated. The amount of activated conjugate moiety to be added for the conjugation step may be expressed as the ratio of activated conjugate moiety (moles) to oxidized antigen binding protein (moles). In any embodiment, the activated conjugate moiety is added to the oxidized antigen binding protein, whether immobilized on the solid support or in solution, at an activated conjugate moiety to antigen binding protein ratio of 2 to 5: 1. In particular, a suitable activated conjugate moiety to antigen binding protein ratio is 2:1, 3:1, 4:1, or 5:1 (mole/mole). In a preferred embodiment, the activated conjugate moiety to antigen binding protein ratio is 2:1 to 3:1.

[0093] Conjugation of the activated conjugate moiety to the oxidized antigen binding protein may be carried out for a duration of at least 5 hours, at least 10 hours, at least 15 hours, at least 20 hours, or at least 25 hours. In one embodiment the conjugation step is carried out for a duration of about 15 to about 30 hours, or a duration of about 18 to about 27 hours, a duration of about 20 to about 25 hours after addition of the activated conjugate moiety to the oxidized antigen binding protein. The desired reaction time to produce the conjugated antigen binding protein on the solid support can be achieved via a static

hold, *i.e.*, introducing the activated conjugate moiety to the solid support material comprising the immobilized antigen binding protein followed by a pause with no flow or wash for a specified period of time. Alternatively, the desired reaction time can be achieved via continuous flow, where the activated conjugate moiety is continuously flowed over the solid support material comprising the antigen binding protein. In embodiments where the oxidized antigen binding protein is eluted from the solid support prior to conjugation, the activated conjugate moiety can be added as concentrate to the solution comprising the oxidized antigen binding protein. In any embodiment the activated conjugate moiety can be dissolved in a suitable solution prior to adding to the solution comprising the oxidized antigen binding protein.

[0094] The conjugation reaction is considered satisfactorily completed when the amount of conjugated antigen binding protein comprises at least 80%, at least 85%, at least 90%, at least 95%, or >95% of the total antigen binding protein available for conjugation. The amount of conjugated antigen binding protein can be measure using techniques known in the art and described herein including, without limitation, reduced fabricator SDS, hydrophobic interaction chromatography (HIC), and mass spectrometry.

[0095] Methods of eluting the antigen binding protein conjugate from the solid support are known in the art and the skilled practitioner will be able to select an appropriate buffer for elution. For example, in embodiments, where the solid support comprises protein A or protein G resin, the antigen binding protein conjugates can be eluted with standard low pH buffers for elution from protein A or protein G columns. Similarly, in embodiments where the reduced antigen binding protein is eluted prior to the oxidation step or the oxidized antigen binding protein is eluted prior to the conjugation step, such elution is carried out using a buffer having the appropriate pH, salt content, or other biochemical property suitable for elution. Suitable elution buffers can be selected by one of skill in the art based on the type of solid support utilized for the antigen binding protein immobilization.

[0096] A “linker moiety” as used herein refers to a biologically acceptable peptidyl or non-peptidyl organic group that covalently joins or conjugates the antigen binding protein to the activated conjugate moiety. The presence of any linker moiety is optional. The linker may be covalently bound to an amino acid residue of the antigen binding protein or to an amino acid residue of the activated conjugate moiety. When present, its chemical structure is not critical, since it serves primarily as a spacer to position, join, connect, or optimize presentation or position of one functional moiety (*i.e.*, antigen binding protein) in relation to one or more other functional moieties (*i.e.*, activated conjugate moiety) of the antigen binding protein conjugate as described herein. The presence of a linker moiety can be useful in optimizing pharmacological activity of the antigen binding protein conjugate. The linker moiety, if present, can be independently the same or different from any other linker, or linkers, that may be present in the antigen binding protein conjugate. In some embodiments the linker can be a multivalent linker that facilitates multivalent display of the activated conjugate moiety.

[0097] The linker moiety, if present, can be “peptidyl” in nature (*i.e.*, made up of amino acids linked together by peptide bonds) and made up in length, preferably, of from 1 up to about 40 amino acid

residues, more preferably, of from 1 up to about 20 amino acid residues, and most preferably of from 1 to about 10 or fewer amino acid residues. Preferably, but not necessarily, the amino acid residues in the linker are from among the twenty canonical amino acids, more preferably, cysteine, glycine, alanine, proline, asparagine, glutamine, and/or serine. Even more preferably, a peptidyl linker is made up of a majority of amino acids that are sterically unhindered, such as glycine, serine, and alanine linked by a peptide bond. It is also desirable that, if present, a peptidyl linker is selected that avoids rapid proteolytic turnover in circulation *in vivo*. Some of these amino acids may be glycosylated, as is well understood by those in the art.

[0098] In any embodiment, the amino acid residues of the peptidyl linker moiety are selected from glycine, alanine, proline, asparagine, glutamine, and lysine. Preferably, a linker is made up of a majority of amino acids that are sterically unhindered, such as glycine, serine, and alanine. Thus, preferred linkers include polyglycines, polyserines, and polyalanines, or combinations of any of these.

[0099] Non-peptidyl linker moieties are also useful for conjugating the activated conjugate moiety to the antigen binding protein as described herein. For example, alkyl linkers such as -NH-(CH₂)_s-C(O)-, wherein s = 2-20 can be used. These alkyl linkers may further be substituted by any non-sterically hindering group such as lower alkyl (e.g., C₁-C₆), lower acyl, halogen (e.g., Cl, Br), CN, NH₂, phenyl, *etc.* Exemplary non-peptidyl linkers also include PEG linkers.

[0100] The linker moieties can optionally be chemically differentiated on either end to accommodate orthogonal coupling chemistries (e.g., thioether formation by alkylation with maleimide or haloacetamide, oxime formation, reductive amination, Click chemistry, etc.).

[0101] The above is merely illustrative and not an exhaustive treatment of the kinds of linkers that can optionally be employed in accordance with the present invention.

EXAMPLES

Example 1: Evaluation of Binding Capacity and Reduction Reaction Using Affinity Ligand Column

[0102] The use of resins with affinity ligands for performing on-column reduction reaction was assessed. Specifically, an alkaline-stable Protein A chromatography resin (manufactured by Purolite®) was tested. A 0.66 cm x 25 cm column was packed, and a cysteamine capped anti-GIPR antibody (antibody 2G10_LC1.006 engineered to have an E275C mutation in heavy chain sequence of SEQ ID NO: 40) was loaded at pH 7.2. The capped antibody was loaded on the Protein A column to a target of 40 g/L resin. The sequence of steps for on-column reduction are provided in Table 1 below. The reduction agent (RA) was TPPTS dissolved in Tris (1X), pH 7.2 and added to the reaction in a 2:1 (RA:antibody) ratio.

Table 1. Sequence of steps for on-column reduction of cysteamine capped anti-GIPR antibody on Protein A column

Phase	Buffer / Solution	Length in Column Volumes (CVs)
Equilibration (EQ)	100 mM Tris, pH 7.2	3
Loading	Conditioned CEX pool	As needed (40 g/L resin)
Reduction	TPPTS dissolved in 100mM Tris (1X RA) pH 7.2	30 mins, 60 mins, 120 mins (300 cm/hr w/ recirculation)
Post Reduction Wash	100 mM Tris, pH 7.2	3
Elution	50 mM Acetate, pH 3.7	4
Strip	100 mM Phosphoric Acid	3
Flush	100 mM Tris pH 7.2	2
Sanitization	0.3 M Sodium Hydroxide	3

[0103] The Protein A resin was capable of binding the product at the target loading over the entire sequence and eluting the product at high yield (>95%, not shown). However, the reduction reaction was much slower at the higher pH values as shown in FIG. 2 with the fraction of mono-capped species being above the target limit (<10%) for all conditions over the time points tested.

[0104] This slowing of the reaction rate is likely caused by an increase in the reverse reaction rate with increasing pH. The caps that are reduced from the monoclonal antibody in this step are attached at an earlier stage in the process at pH 7.6. Therefore, methods to increase the reaction rate were investigated.

[0105] As shown in FIG. 2, the possibility of lowering the reaction pH to 6.8 and doubling the amount of reducing agent (2X RA) was considered. While both changes increased the overall reaction rate as measured by a greater fraction of fully reduced species across all timepoints, neither of the changes were enough to result in a reaction rate that was as fast as the batchwise process, in which the target limit of <10% mono-capped species is generally reached within 120 minutes. Optimization efforts focused on further increasing this reaction rate thus reducing the required reaction time.

[0106] Leveraging Static Binding Capacity Increased Binding Capacity of Affinity

Column: While increasing the concentration of the reduction reagent increased the reaction rate to a greater extent than decreasing the pH in the range studied, dramatically increasing the reduction reagent consumption is less desirable due to both cost of goods and the potential for off-target reduction of disulfides. Because of this, the binding capacity of the affinity ligand as a function of pH was studied to determine if a lower operating pH was feasible. Protein A ligands typically have high binding at elevated / neutral pH with a low pH elution being used to recover the protein. Because of this, a trend of decreasing binding capacity with decreasing pH was expected as shown in Table 2 for a different antibody (Antibody B) tested for dynamic binding capacity to a different Protein A column (Cytiva medium capacity resin) having similar properties and alkaline stability.

Table 2. Dynamic binding capacity of Antibody B as a function of pH on Protein A column (Cytiva®)

Load pH	Binding Capacity at 5% breakthrough (mg Protein / mL Resin)	Capacity loss (%) (relative to load pH 7.5)
5.0	28.6	35.3
5.5	35.2	20.4
6.0	38.1	13.8
6.6	41.3	6.7
7.0	42.4	4.1
7.5	44.2	N/A

[0107] As shown in Table 2, loss (>30%) of *dynamic* binding capacity was expected at pH 5.0, so the next set of experiments attempted to increase total binding by leveraging the *static* binding capacity at pH 5.0 by loading protein at high binding conditions (pH 7.2), and adjusting the pH to the desired reaction pH as shown in Table 3.

Table 3. Sequence of steps for on-column reduction of cysteamine capped anti-GIPR antibody on Protein A column (Purolite®)

Phase	Buffer / Solution	Length in Column Volumes (CVs)
Equilibration (EQ)	100 mM Tris, pH 7.2	3
Load	Conditioned CEX pool	40 g/L resin
Pre-Reduction Wash	100 mM Acetate / MES (Various pHs)	3
Reduction	TPPTS dissolved Acetate / MES (1X RA) (Various pHs)	30 mins, 60 mins, 120 mins (300 cm/hr w/ recirculation)
Post Reduction Wash	100 mM Acetate / MES (Various pHs)	3
Elution	50 mM Acetate, pH 3.7	4
Strip	100 mM Phosphoric Acid	3
Flush	100 mM Tris pH 7.2	2
Sanitization	0.3 M Sodium Hydroxide	3

[0108] FIG. 3 shows the results of this evaluation with the UV absorbance as measured at 280 nanometers post loading being shown. As the target reduction pH drops from 6.5 to 5.0, the yield drops to below 95%, and the area of the pH adjustment peak increases. However, the overall yield is much higher than would be expected based on the dynamic binding capacity changes shown in Table 2 thus showing the utility of this post-loading pH adjustment approach to increase overall binding and improve technical feasibility.

[0109] **Evaluation of Reduction Rate using Affinity Ligand Column at lower pH.** Based on these results at 40 g/L resin load factor, a load factor of 35 g/L resin was used for the proof of concept runs at pH 5.0 and 5.5 and 40 g/L resin for pH 6.0. These different pH conditions were then screened at various times and molar equivalents of reducing agent (i.e., a RA:antibody ratio of about 2:1 to 4:1). As the pH was increased, longer reaction times or greater equivalents were preferentially screened based on pH vs reaction rate concerns.

[0110] The resulting CEX-HPLC results (measuring extent of reaction) and SE-HPLC (measuring product quality via levels of high molecular weight (HMW) species) are shown in FIG. 4 and FIG. 5, respectively. The target limit for % mono-capped was met for all conditions, and the product quality improved (via lower levels of HMW %) at lower pH and reduction agent reaction equivalents. These conditions were run with the reduction phase in flowthrough mode (i.e., washing the column with the reagent solution for the target incubation time, as opposed to a short wash, in order to subject the entire column to the reagent solution followed by an on-column hold).

[0111] The final proof of concept for on-column reduction examined on-column holds as well as shortening the total reaction time as shown in FIG. 6 and FIG. 7. The pH 5.0 condition was studied more thoroughly as it tended to have better product quality (lower HMW%) compared to the pH 6.0 condition. For the on-column hold, the dilution of TPPTS was adjusted to ensure the amount of TPPTS available for reaction was at least 4 molar equivalents. The on-column hold had an overall faster reaction rate compared to the flowthrough configuration, which is attributed to the greater concentration of the reactants. Similarly, the on-column hold had improved overall product quality as measured by lower high molecular weight species. This observation may be related to differences in the generation of over-reduced species between the two configurations (not characterized).

Example 2: Evaluation of Antibody Conjugation using Affinity Ligand Column

[0112] Following successful demonstration of the on-column reduction, the feasibility of performing on-column reduction, oxidation, and conjugation was evaluated on two different affinity ligand columns, the alkaline-stable Protein A column from Purolite® (Resin 1) and a higher capacity resin from Cytiva® (Resin 2). In this example, the peptide conjugate was a bromoacetyl-GLP-1 peptide ({H2}H[Aib]EGTFTSDYSSYLEEQAAKEFIAWLKGGG; SEQ ID NO: 43 (Aib = α amino-isobutyric acid)) comprising a C-terminal linker (GGGGSGGGSGGGGS; SEQ ID NO: 44). For these runs, the sequence of steps for the on-column conjugation are shown in Table . Based on the better product quality

for an on-column hold as well as the lower complexity of setup for on-column hold versus flowthrough, all steps (reduction, oxidation, and conjugation) were executed in an on-column hold manner.

Table 4. Sequence of steps for on-column reduction, oxidation, and conjugation of the cysteamine capped anti-GIPR antibody using Protein A chromatography.

Step	Buffer / Solution	Column Volumes or Load Factor	Linear velocity (cm/hr)	Hold time (If Any)
Equilibration	100 mM Tris pH 7.2	5	250	
Load	CEX Pool Titrated to pH 7.2 w/ 2 M Tris Base	35 g/L	125	
Pre-Reduction Wash	100 mM Sodium Acetate pH 5.0	3	125	
Reduction	100 mM Sodium Acetate pH 5.0 + TPPTS (1X)	2	250	30 mins
Post-Reduction Wash	100 mM Sodium Acetate pH 5.0	3	250	
Pre-Oxidation Wash	100 mM Tris pH 7.2	5	250	
Oxidation	100 mM Tris pH 7.2 w/ DHAA	2	250	2 hours
Pre-Conjugation Wash	100 mM Tris pH 7.6	3	250	
Conjugation	100 mM Tris pH 7.6 + Peptide	2	250	20 hours
Post-Conjugation Wash	100 mM Tris pH 7.6	3	250	
Elution	50 mM Sodium Acetate pH 3.7	4	125	
Strip	0.1 M Phosphoric Acid	3	250	
Flush	Water	3	250	
Cleaning	0.3 M NaOH	3	250	

[0113] The TPPTS solution was diluted in sodium acetate pH 5.0, keeping the molar amount of reactant during the static hold the same as the molar amount supplied for the batchwise reaction.

[0114] The governing equations for the DHAA (oxidizing agent) and peptide solutions are shown in equations 3 and 4.

$$DHAA \text{ dilution } \left(\frac{L}{L}\right) = \left(\frac{35 \frac{g}{L}}{145 \frac{g}{mmol}}\right) \left(\frac{5.0 \text{ (equivalents of DHAA)}}{4 \text{ mM (concentration of DHAA solution)}}\right) \quad (3)$$

$$Peptide \text{ dilution } \left(\frac{L}{L}\right) = \left(\frac{35 \frac{g}{L}}{145 \frac{g}{mmol}}\right) \left(\frac{2.5 \text{ (equivalents)}}{1.5 \text{ mM (conc. of peptide solution)}}\right) \quad (4)$$

[0115] The DHAA was diluted with 100 mM Tris pH 7.2, and the peptide solution was titrated to pH 7.6 with 2 M Tris Base and then diluted with 100 mM Tris pH 7.6. The titration was required for the peptide solution as it is dissolved in acetic acid compared to the other solutions that were formulated in water.

[0116] A lower binding capacity (30 g/L resin) was targeted and successfully utilized as shown in Table . Product quality was measured by SEC-HPLC (HMW%) and extent of conjugation was measured by a fabricator non-reduced SDS assay (% heavy chain (HC) conjugated). In general, the conjugation was successful with >90% HC conjugated, and the yields demonstrate very high recovery.

[0117] The step yield is a good surrogate for successful conjugation – it is routinely above 100% which is correlated to the increased mass from addition of the peptide, which also has UV absorbance, as well as the fact that the extinction coefficient used for calculations is reported on a mass basis rather than a molar basis (*i.e.*, the yield should be >100% on a mass basis since the total mass of species with UV absorbance is increased).

Table 5. Product quality and conjugation efficiency for on-column reduction, oxidation, and conjugation using Protein A affinity column

Protein A Resin	Loading [g/L-r]	%HMW	% HC Conjugated	%Yield
Protein A Resin 1	30	5.4	94.1	102.6
Protein A Resin 2	50	10.3	94.2	104.3

*HMW for conjugated pool in the corresponding large-scale production run was 5.6% and 2.7% in load

[0118] Also shown in Table are data for on-column conjugation with the Cytiva® high capacity affinity resin (Resin 2), which is another affinity resin with similar alkaline stability but greater binding capacity compared Resin 1 (manufactured by Purolite®). Resin 2 was evaluated to further improve facility fit by increasing the total amount of material that could be processed in a single run. As noted, the load factor was increased from 30 g/L resin to 50 g/L resin based on this increased capacity. Yields and conjugation efficiency remained high, but product quality decreased slightly as demonstrated by elevated levels of HMW%. The data in Table 5 confirms successful proof of concept and high efficiency (>90%) conjugation with moderate increases in high molecular weight dependent on resin loading.

[0119] While the fabricator non-reduced capillary electrophoresis (CE)-SDS method provides an indicator of the extent of conjugation, an additional critical performance indicator for conjugation is the peptide antibody ratio (PAR). The target PAR ratio is 2 peptides for each monoclonal antibody (PAR-2).

[0120] Table shows the distribution of PAR species obtained for different configurations of on-column conjugation as well as batch-wise conjugation and the reference standard. As shown, a wider variety of PAR species is obtained with both the batchwise, and on-column conjugation trials as compared to the reference standard. The reference standard consists of Drug Substance produced with a conjugated pool that was purified over a hydrophobic interaction chromatography (HIC) column, a mode of chromatography that is adept at resolving PAR variants, so the overall distribution is much lower compared to on-column and batchwise.

Table 6. Peptide antibody ratio (PAR) of GIPR antibody conjugate for on-column conjugation with Protein A affinity column

Resin	Loading	Par-0	Par-1	Par-2	Par -3	Par-4	Par-5	Par-6	Par-7	Par-8
Reference Standard		4.5	2.1	93.4	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Batchwise	N/A	0.86	13.52	74.01	4.66	4.08	N.D.	N.D.	N.D.	N.D.
Protein A Resin 1	30	0.2	9.3	68.5	15.5	3.5	0.4	2.4	N.D	0.3
Protein A Resin 2	50	N.D.	6.5	64.7	19.8	5.4	0.8	2.5	N.D.	0.4

N.D. = Not Detected

While the distribution of PAR species for on-column conjugation is wider than what is seen in the reference standard and in the batchwise reaction, these data demonstrate the potential for on-column chemistry steps after further optimization.

[0121] While this initial proof of concept studied all three steps (reduction, oxidation, conjugation) on the column, the strategy could be applied for a subset of these reactions. Specifically, the peptide is a relatively expensive feedstock, and efficient delivery in an on-column application is difficult. The peptide is fed at a molar excess (2-3 molar equivalents versus 2.0 target PAR ratio), and loss in peptide due to line priming of a chromatography skid and excess wash volumes to ensure effective exposure could result in significant costs. Therefore, alternative iterations of this process could involve performing the conjugation steps in a flowthrough recirculation mode to minimize loss in peptide due to line priming, or it could consider batchwise conjugation following on-column reduction and oxidation.

[0122] As discussed above, on-column conjugation of cys-mAbs has significant advantages to the batchwise approach. A comparison of facility and product quality parameters for these processes is provided Table 7 below. In particular, on-column conjugation can replace two ultra-filtration / diafiltration steps with one chromatography step saving a substantial amount of time (process time and hold time) and reagents (hold tank volume, total buffer), while producing similar yields and level of conjugation. The on-column conjugation process further minimizes stability concerns as buffer exchange in a column operation is much faster (~minutes) than buffer exchange during ultra/diafiltration (~hours). Finally, since the exposure times of the conjugation reagents can be controlled more precisely in the on-column process, the approach affords better control of the PAR ratio, which reduces the downstream purification burden.

Table 7. Comparison of facility considerations and product quality parameters of the on-column conjugation process as disclosed herein to the prior art batchwise process.

		Facility Considerations				Product Quality		
	Yield	Reagent Use	Process Time (hrs)	Intermediate Hold Time (h)	Intermediate Hold Tank Volume (L)	SEC-HMW (%)	% Conjugated	Higher order PAR
Batchwise	>95%	1X	50	10	4X	1X	>90%	1X
On-Column	>95%	1X	30	0.2	1X	2X	>90%	2X

[0123] Exemplary nucleic acid and amino acid GIPR antibody sequences are provided in Tables A-D.

Table A. Amino Acid Sequences of Heavy Chain Complementarity Determining Regions (HCDR) of GIPR Antibody

iPS No.	Ab	HCDR1		HCDR2		HCDR2	
		SEQ	ID	SEQ	ID	SEQ	ID
336067	5G12.006	SYGIH	1	VIWYDGSNKFHADSVKG	2	GKVAGMPEAFEI	3
361172	2G10_LC1.003	NYGMH	4	AIWFDASDKYYADAVKG	5	DQAIFGVVPDY	6
361192	2G10_LC1.003	NYGMH	7	AIWFDASDKYYADAVKG	8	DQAIFGVVPDY	9

Table B. Amino Acid Sequences of Light Chain Complementarity Determining Regions (LCDR) of GIPR Antibody

iPS No.	Ab	LCDR1		LCDR2		LCDR2	
		SEQ	ID	SEQ	ID	SEQ	ID
336067	5G12.006	RASQTISRFLN	10	VASSLQS	11	QQSYSTLIS	12
361172	2G10_LC1.003	RASQSVSSNLA	13	GAATRAT	14	QQYNNWPLT	15
361192	2G10_LC1.006	RASQSVSSNLA	16	GAATRAT	17	QQYNNWPLT	18

Table C. Amino Acid (aa) and Nucleic Acid (na) Sequences of Heavy Chain Variable Regions (VH) and Light Chain Variable Regions (VL) of Exemplary GIPR Antibodies

iPS No.	Ab	Region	Type	Sequence	SEQ ID NO
336067	5G12.006	VH	na	CAGGTGCAGTTGGTGGAGTCTGGGGAGGCGTGGTCCAGCCTG GGAGGTCCCTGAGACTCTCCTGTACAGCGTCTGGATTACCTTCA GTAGCTATGGCATACTGGGTCCGCCAGGCTCCAGGCAAGGGG CTGGAGTGGGTGGCAGTTATATGGTATGATGGAAGTAATAAGTTC CATGCAGACTCCGTGAAGGGCCGATTACCATCTCCAGAGACAAT TCCAAGAACACGCTGTATCTGCAATGAACAGCCTGAGAGCCGAG GACTCGGCTATGTACTTCTGTGCGAGAGGAAAAGTGGCTGGTATG CCTGAAGCTTTTGAATCTGGGGCCAAGGGACAAAGGTCACCGTC TCTTCA	19

IPS No.	Ab	Regi on	Type	Sequence	SEQ ID NO
			aa	QVQLVESGGGVVQPGRSRLRLSCTASGFTFSSYGIHWVRQAPGKGLE WVAVIWDGNSNKFHADSVMKGRFTISRDNKNTLYLQMNSLRAEDSA MYFCARGKVAGMPEAFEIWGQGTVSVSS	20
			VL na	GACATCCAGCTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTA GGAGACAGAGTCACTATCACTTGCCGGGCAAGTCAGACCATTAGC AGGTTTTTAAATTGGTATCAGCAGAAACCTGGGAAAGCCCTGAG CTCCTGATCTATGTTGCATCCAGTTTGCAAAGTGGGGTCCCATCA AGATTCAGTGGCAGTGGTCTGGGACAGATTTCCTCTCACCATC AGCAGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAACAGA GTTACAGTACCCTGATCAGTTTGGCCAGGGACCAAGCTGGAGA TCACACGA	21
			aa	DIQLTQSPSSLSASVGRVTITCRASQTISRFLNWWYQKPGKAPELLI YVASSLQSGVPSRFSGSGSGDFTLTISLQPEDFATYYCQSQSYSLI SFGQGTKEITR	22
361172	2G10_L C1.003	VH	na	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTG GGAGGTCCCTGAGACTCTCCTGTGCAGCATCTGGATTCACTTCA GTAATATGGCATGCACTGGGTCCGCCAGGCTCCAGGCGAGGGG CTGGAGTGGGTGGCAGCTATATGTTTTGATGCAAGTGATAAATAC TATGCAGACGCCGTGAAGGGCCGATTACCATCTCCAGAGACAAC TCCAAGAACACGCTGTATCTGCAATGAACAGCCTGAGAGCCGAG GACACGGCTGTGTATTACTGTGCGAGAGATCAGGCGATTTTGGGA GTGGTCCCGACTACTGGGGCCAGGGAACCTGGTCACCGTCTC CTCA	23
			aa	QVQLVESGGGVVQPGRSRLRLSCAASGFTFSNYGMHWVRQAPGEGL EWVAAIWFDASDKYYADAVKGRFTISRDNKNTLYLQMNSLRAEDTA VYYCARDQAIFGVVPDYWGQGTLLTVSS	24
		VL	na	GAAATAGTGATGACGCACTCTCCAGCCACCCTGTCTGTCTCCA GGGGAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAG CAGCAACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCA GGCTCCTCATCTATGGTGCAGCCACCAGGGCCACTGGTATCCCA GCCAGGTTCACTGGCAGTGGTCTGGGACAGAGTTCCTCTCAC CATCAGCAGCCTGCAGTCTGAAGATTTGCAGTTTATTACTGTGAG CAGTATAATAACTGGCCTCTCACTTTCGCGGAGGGACCAAGGTG GAGATCAAACGA	25
			aa	EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQKPGQAPRL LIYGAATRTATGIPARFSGSGSGTEFTLTISLQSEDFAVYYCQQYNNW PLTFGGGTKEIKR	26
361192	2G10_L C1.006	VH	na	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTG GTCCAGCCTGGGAGGTCCCTGAGACTCTCCTGT GCAGCATCTGGATTCACTTCAGTAACTATGGC ATGCACTGGGTCCGCCAGGCTCCAGGCGAGGGG CTGGAGTGGGTGGCAGCTATATGTTTTGATGCA AGTGATAAATACTATGCAGACGCCGTGAAGGGC CGATTCACCATCTCCAGAGACAACCTCAAGAAC ACGCTGTATCTGCAATGAACAGCCTGAGAGCC GAGGACACGCTGTGTATTACTGTGCGAGAGAT CAGGCGATTTTGGAGTGGTCCCGACTACTGG GGCCAGGGAACCTGGTCACCGTCTCCTCA	27
			aa	QVQLVESGGGWQPGRSRLRLSCAASGFTFSNYGM HWVRQAPGEGLEWVAAIWFDASDKYYADAVKG RFTISRDNKNTLYLQMNSLRAEDTAVYYCARD AIFGVVPDYWGQGTLLTVSS	28
		VL	na	GAAATAGTGATGACGCACTCTCCAGCCACCCTG TCTGTGTCTCCAGGGGAAGAGCCACCCTCTCC TGCAGGGCCAGTCAGAGTGTTAGCAGCAACTTA GCCTGGTACCAGCAGAAACCTGGCCAGGCTCCC AGGCTCCTCATCTATGGTGCAGCCACCAGGGCC ACTGGTATCCAGCCAGGTCAGTGGCAGTGGG TCTGGGACAGAGTTCCTCTCACCATCAGCAGC	29

iPS No.	Ab	Region	Type	Sequence	SEQ ID NO
				CTGCAGTCTGAAGATTTGCAGTTTATTACTGTC AGCAGTATAATAACTGGCCTCTCACTTTTCGGCG GAGGGACCAAGGTGGAGATCAAACGA	
			aa	EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAW YQQKPGQAPRLLIYGAATRATGIPARVSGSGSGTE FTLTSSLOSEDFAVYYCQQYNNWPLTFGGGTKVE IKR	30

Table D. Amino Acid (aa) and Nucleic Acid (na) Sequences of the Heavy Chains (HC) and Light Chains (LC) of Exemplary GIPR Antibodies

iPS No.	Ab		Type	Sequence	SEQ ID NO
336067	5G12.006	HC	na	CAGGTGCAGTTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCT GGGAGGTCCCTGAGACTCTCCTGTACAGCGTCTGGATTACCT TCAGTAGCTATGGCATACACTGGGTCCGCCAGGCTCCAGGCCAA GGGGCTGGAGTGGGTGGCAGTTATATGGTATGATGGAAGTAAT AAGTTCCATGCAGACTCCGTGAAGGGCCGATTACCATCTCCA GAGACAATTCCAAGAACACGCTGTATCTGCAATGAACAGCCTG AGAGCCGAGGACTCGGCTATGTACTTCTGTGCGAGAGGAAAAG TGGCTGGTATGCCTGAAGCTTTTGAATCTGGGGCCAAGGGAC AAAGGTCACCGTCTCTTCAGCCTCCACCAAGGGCCCATCGGT TCCCCCTGGCACCTCCTCCAAGAGCACCTCTGGGGGCACAG CGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCGAACCGG TGACGGTGTCTGGAACCTCAGGCGCCCTGACCAGCGCGTGC ACACCTTCCCGGCTGTCTACAGTCTCAGGACTCTACTCCCTC AGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAG ACCTACATCTGCAACGTGAATCACAAGCCAGCAACACCAAGG TGGACAAGAAAGTTGAGCCCAATCTTGACAAAACACACACA TGCCCAACCGTGCCAGCACCTGAACTCCTGGGGGACCGTCA GTCTTCTCTTCCCCCAAAACCAAGGACACCTCATGATCTC CCGGACCCCTGAGGTCACATGCGTGGTGGTGGAGCTGAGCCA CGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTG GAGGTGCATAATGCCAAGACAAAGCCGTGTGAGGAGCAGTACG GCAGCAGTACCGTTGTGTCAGCGTCTCACCCTCCTGCACCA GGACTGGCTGAATGGCAAGGAGTACAAGTGAAGGTCTCCAAC AAAGCCCTCCAGCCCCATCGAGAAAACCATCTCCAAAGCCA AAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCAT CCCGGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCC TGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGA GAGCAATGGGCAGCCGAGAACAACTACAAGACCACGCCTCC CGTCTGGACTCCGACGGCTCCTTCTCTCTATAGCAAGCTC ACCGTGGACAAGACAGGTGGCAGCAGGGGAACGTCTTCTCAT GCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAA GAGCCTCTCCCTGTCTCCGGGTAAG	31
			aa	QVQLVESGGGVQPRSLRLSCTASGFTFSSYGIHWVRQAPGKG LEWVAVIWDGSKNFHADS VKGRFTISRDNKNTLYLQMNSLRAE DSAMYFCARGKVAGMPEAFEIWQGQTKVTSSASTKGPSVFPLA PSSKSTSGGTAAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS CDKTHTCPPELPGPSVFLFPPKPKDTLMISRTPEVTCVVD VSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLD SDGSFFLYSKLTVDKSRWQQGNVSCFVSVMHEALHNHYTQKSLSL SPGK	32
		LC	na	GACATCCAGCTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGT AGGAGACAGAGTCACTATCACTTGCCTGGCAAGTCAGACCATT	33

IPS No.	Ab		Type	Sequence	SEQ ID NO
				AGCAGGTTTTTAAATTGGTATCAGCAGAAACCTGGGAAAGCCCC TGAGCTCCTGATCTATGTTGCATCCAGTTTGCAAAGTGGGGTCC CATCAAGATTCACTGGCAGTGGTTCTGGGACAGATTTCACTCTC ACCATCAGCAGTCTGCAACCTGAAGATTTTGAACCTTACTACTG TCAACAGAGTTACAGTACCCTGATCAGTTTGGCCAGGGGACC AAGCTGGAGATCACACGAACGGTGGCTGCACCATCTGTCTTCA TCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACGTCCTCT GTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGT ACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAG GAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGC CTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACGAGAAAC ACAAAGTCTACGCTGCGAAGTACCCATCAGGGCCTGAGCTC GCCCCTCACAAAGAGCTTCAACAGGGGAGAGTGT	
			aa	DIQLTQSPSSLASVGDRTITCRASQTISRFLNWWYQKPGKAPEL LIYVASSLQSGVPSRFSGSGSGTDFLTIISSLPEDFATYYCQSY STLISFGQGTKLEITRTVAAPSVFIFPPSDEQLKSGTASVVLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	34
361172	2G10_L C1.003	HC	na	CAGGTCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCT GGGAGGTCCCTGAGACTCTCCTGTGCAGCATCTGGATTACCT TCAGTAACTATGGCATGCACTGGGTCCGCCAGGCTCCAGGGCA GGGGCTGGAGTGGGTGGCAGCTATATGGTTTGATGCAAGTGAT AAATACTATGACAGACGCCGTGAAGGGCCGATTACCATCTCCA GAGACAACCTCCAAGAACACGCTGTATCTGCAATGAACAGCCT GAGAGCCGAGGACACGGCTGTGTATTACTGTCCGAGAGATCAG GCGATTTTGGAGTGGTCCCCGACTACTGGGGCCAGGGAACCC TGGTCACCGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTT CCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGC GGCCCTGGGCTGCCGTGGTCAAGGACTACTTCCCCGAACCGGT GACGGTGTCTGGAACTCAGGCGCCCTGACCAGCGCGTGCA CACCTTCCCGGTGTCTACAGTCTCAGGACTTACTCCCTCA GCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGA CCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGT GGACAAGAAAGTTGAGCCCAAATCTTGACAAAACCTACACAT GCCACCGTGCCAGCACCTGAACCTCTGGGGGACCGTCAG TCTTCTCTTCCCCCAAAACCAAGGACACCCCTCATGATCTCC CGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCAC GAAGACCGTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGG AGGTGCATAATGCCAAGACAAGCCGTGTGAGGAGCAGTACGG CAGCACGTACCGTTGTGTGACGCTCCTACCGTCTGCACCAG GACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACA AAGCCCTCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATC CCGGGAGGAGATGACCAAGAACCAGGTACGCTGACCTGCCT GGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAG AGCAATGGGACGCGGAGAACTACAAGACCACGCTCCCTCC GTGCTGACTCCGACGGCTCCTTCTTCTCTATAGCAAGCTCA CCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCAT GCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAA GAGCCTCTCCCTGTCTCCGGGTA	35
			aa	QVQLVESGGGVVQPGRSLRLSCAASGFTFSNYGMHWVRQAPGE GLEWVAIIWFDASDKYYADAVKGRFTISRDNKNTLYLQMNSLRA EDTAVYYCARDQAIFGVVPDYWGQGLVTVSSASTKGPSVFPLAP SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSVTVPSSSLGTQTYICNVNHKPSNTKVDKKEPKSCD KHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVS HEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSD	36

IPS No.	Ab		Type	Sequence	SEQ ID NO
				GSFFLYSKLTVDKSRWQQGNVFSQVMHEALHNHYTQKSLSLSP GK	
		LC	na	GAAATAGTGATGACGCAGTCTCCAGCCACCCTGTCTGTGTCTC CAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTG TTAGCAGCAACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGC TCCCAGGCTCCTCATCTATGGTGCAGCCACCAGGGCCACTGGT ATCCCAGCCAGGTTCACTGGCAGTGGGTCTGGGACAGAGTTCA CTCTCACCATCAGCAGCCTGCAGTCTGAAGATTTTGCAGTTAT TACTGTCAGCAGTATAATAACTGGCCTCTCACTTTTCGGCGGAGG GACCAAGGTGGAGATCAAACGAACGGTGGCTGCACCATCTGTC TTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGC CTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCA AAGTACAGTGGAAAGGTGGATAACGCCCTCCAATCGGGTAACTC CCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTA CAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAG AAACACAAAGTCTACGCTGCGAAGTCACCATCAGGGCCTGA GCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT	37
			aa	EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPGQAP RLLIYGAAATRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQY NNWPLTFGGGTQVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLS KADYEHKHKVYACEVTHQGLSSPVTKSFINRGE	38
361192	2G10_L C1.006	HC	na	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCT GGGAGGTCCCTGAGACTCTCCTGTGCAGCATCTGGATTCACCT TCAGTAACTATGGCATGCACTGGGTCCGCCAGGCTCCAGGCGA GGGGCTGGAGTGGGTGGCAGTATATGGTTTATGCAAGTAT AAATACTATGCAGACGCCGTGAAGGGCCGATTACCATCTCCA GAGACAACTCCAAGAACACGCTGTATCTGCAAATGAACAGCCT GAGAGCC GAGGACACGCTGTGTATTACTGTGCGAGAGAT CAGGCGATTTTGGAGTGGTCCCCGACTACTGGGGCCAGGGAA CCCTGGTCACCGTCTCCTCAGCCAATGAGCAGTTGAAATCTGG AACTGCCCTCTGTTGCCCTCCTCCAAGAGCACCTCTGGGGGCAC AGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCGAACC GGTGACGGTGTGCTGGAAGTCAAGGCGCCTGACCAGCGCGCT GCACACCTTCCCGGCTGTCTACAGTCTCAGGACTCTACTCC CTCAGCAGC GTGGTGACCGTGCCCTCCAGCAGCTTGGGCACC CAGACCTACATCTGCAACGTGAATCACAAGCCAGCAACACCA AGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTAC ACATGCCACCGTGCCAGCACCTGAACCTCTGGGGGGACCG TCAGTCTTCTCTTCCCCCAAAACCAAGGACACCCCTCATGAT TCCCCGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAG CCACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGACGGC GTGGAGGTGCATAATGCCAAGACAAAGCCGTGTGAGGAGCAGT ACGGCAGCACGTACCGTTGTGTGACGCTCTCACCCTCCTGCA CCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCC AACAAAGC CCTCCCAGCCCCATCGAGAAAACCATCTCAA AGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCC CCCATCCCGGGAGGAGATGACCAAGAACAGGTACAGCCTGAC CTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAG TGGGAGAGCAATGGGAGCCGGAGAACAACTACAAGACCACG CCTCCCGTCTGGACTCCGACGCTCCTTCTCCTCTATAGCA AGCTCACCCTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCT TCTCATGC TCCGTGATGATGAGGCTCTGCACAACCACTAC ACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA	39
			aa	QVQLVESGGGVVQPRSLRLSCAASGFTFSNYGMHWVRQAPGE GLEWVAIIWFDASDKYYADAVKGRFTISRDNKNTLYLQMNSLR AEDAVYYCARDQAIFGVVDPYWGQGLVTVSSASTKGPSVFPLAP SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKVEPKSCD	40

IPS No.	Ab		Type	Sequence	SEQ ID NO
				KTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVS HEDPCVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQ DWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSD GSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSP GK	
		LC	na	GAAATAGTGATGACGCAGTCTCCAGCCACCCTG TCTGTGTCTCCAGGGGAAAGAGCCACCCTCTCC TGCAGGGCCAGTCAGAGTGTTAGCAGCAACTTA GCCTGGTACCAGCAGAAACCTGGCCAGGCTCCC AGGCTCCTCATCTATGGTGCAGCCACCAGGGCC ACTGGTATCCCAGCCAGGTCAGTGGCAGTGGG TCTGGACAGAGTTCACTCTCACCATCAGCAGC CTGCAGTCTGAAGATTTTGCAGTTTATTACTGTC AGCAGTATAATAACTGGCCTCTCACTTTTCGGCG GAGGGACCAAGGTGGAGATCAAACGAACGGTG GCTGCACCATCTGTCTTCATCTTCCCGCCATCTG TCCACCAAGGGCCCATCGGTCTTCCCCTGGCA TGTGCCTGCTGAATAACTTCTATCCCAGAGAGG CCAAAGTACAGTGGAAGGTGGATAACGCCCTCC AATCGGGTAACTCCCAGGAGAGTGTCACAGAGC AGGACAGCAAGGACAGCACCTACAGCCTCAGC AGCACCTGACGCTGAGCAAAGCAGACTACGAG AAACACAAAGTCTACGCCTGCGAAGTCACCCAT CAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTC AACAGGGGAGAGTGT	41
			aa	EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPGQAP RLLIYGAATRATGIPARVSGSGSTEFTLTISLQSEDFAVYYCQQ YNNWPLTFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYLSSTLT LSKADYEKHKVYAC EVTHQGLSSPVTKSFNRGEC	42

CLAIMS

1. A method for preparing an antigen binding protein conjugate, the method comprising:
 - a) providing an antigen binding protein comprising a mixed disulfide;
 - b) immobilizing the mixed disulfide comprising antigen binding protein on a solid support;
 - c) adding a reducing agent to the immobilized antigen binding protein thereby forming a reduced antigen binding protein;
 - d) adding an oxidizing agent to the reduced antigen binding protein thereby forming an oxidized antigen binding protein; and
 - e) adding an activated conjugate moiety to the oxidized antigen binding protein thereby forming an antigen binding protein conjugate.
2. The method according to claim 1, wherein the mixed disulfide comprising antigen binding protein is an antigen binding protein comprising one or more capped free cysteines.
3. The method according to claim 2, wherein the one or more capped free cysteines comprise a cap selected from the group consisting of cysteine, cysteamine, cystamine, and glutathione.
4. The method according to claim 1, wherein the solid support is selected from an anion exchange support material, a cation exchange support material, a mixed mode exchange support material, and an affinity ligand support material.
5. The method according to claim 4, wherein the solid support immobilizes the antigen binding protein at a pH range of pH 5 to pH 8.
6. The method according to claim 4, wherein the solid support immobilizes the antigen binding protein at a salt concentration providing a conductivity of $\leq 15\text{mS/cm}$.
7. The method according to claim 4, wherein the solid support immobilizes the antigen binding protein at a pH range of pH 5 to pH 8 and a salt concentration providing a conductivity of $\leq 15\text{mS/cm}$.
8. The method according to claim 4, wherein the solid support comprises an affinity ligand column.
9. The method according to claim 8, wherein the affinity ligand column comprises a ligand that binds an antigen binding protein Fc portion, an antigen binding protein heavy chain portion, or an antigen binding protein light chain portion.
10. The method according to claim 9, wherein the affinity ligand column comprises a ligand that binds an antigen binding protein Fc portion.

11. The method according to any one of claims 1-10, wherein the reducing agent is selected from the group consisting of triphenylphosphine-3,3',3''-trisulfonate ("TPPTS"), tris(2-carboxyethyl)phosphine ("TCEP"), and triphenylphosphine-3,3'-disulfonate ("TPPDS").
12. The method according to claim 11, wherein the reducing agent is added at a reducing agent to antigen binding protein ratio of 2 to 4:1 (mole/mole).
13. The method according to claim 11, wherein adding the reducing agent to form the reduced antigen binding protein is carried out at a pH of 5.0 to 6.0.
14. The method according to any one of claims 1-13, wherein the oxidizing agent is dehydroascorbic acid ("DHAA").
15. The method according to claim 14, wherein the oxidizing agent is added at an oxidizing agent to antigen binding protein ratio of 3 to 10:1 (mole/mole).
16. The method according to any one of claims 1-15, wherein the activated conjugate moiety is a peptide comprising a halogen.
17. The method according to claim 16, wherein the halogen is selected from the group consisting of Br, I, and Cl.
18. The method according to claim 16, wherein the activated conjugate moiety is added at an activated conjugate moiety to antigen binding protein of 2 to 3:1 (mole/mole).
19. The method according to claim 1, wherein the reduced antigen binding protein is eluted from the solid support prior to adding said oxidizing agent.
20. The method according to claim 1, wherein the oxidized antigen binding protein is eluted from the solid support prior to adding said activated conjugate moiety.
21. The method according to claim 1 further comprising:
obtaining an antigen binding protein; and
exposing the antigen binding protein to a cysteine blocking agent, wherein the cysteine blocking agent forms a stable mixed disulfide with at least one cysteine residue of the antigen binding protein thereby forming the antigen binding protein comprising the mixed disulfide.
22. The method according to claim 1, wherein the antigen binding protein is an antibody.
23. The method according to claim 1, wherein the antigen binding protein is an antibody fragment.

24. The method according to claim 1, wherein the antigen binding protein is an antibody derivative.