presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophotometer (Aviv Instruments) or a 8000-series SLM-Aminco spectrophotometer (ThermoSpectronic) with a stirred cuvette.

"Biologically active" and "biological activity" and "biological characteristics" with respect to a polypeptide of this invention, such as an antibody, fragment, or derivative thereof, means having the ability to bind to a biological molecule, except where specified otherwise.

"Peptibody" or "peptibodies" refers to a fusion of randomly generated peptides with an Fc domain. See U.S. Pat. No. 6,660,843, issued Dec. 9, 2003 to Feige et al. ~~(incorporated by reference in its entirety)~~. They include one or more peptides linked to the N-terminus, C-terminus, amino acid sidechains, or to more than one of these sites. Peptibody technology enables design of therapeutic agents that incorporate peptides that target one or more ligands or receptors, tumor-homing peptides, membrane-transporting peptides, and the like. Peptibody technology has proven useful in design of a number of such molecules, including linear and disulfide-constrained peptides, "tandem peptide multimers" (i.e., more than one peptide on a single chain of an Fc domain). See, for example, U.S. Pat. No. 6,660,843; U.S. Pat. App. No. 2003/0195156, published Oct. 16, 2003 (corresponding to WO 02/092620, published Nov. 21, 2002); U.S. Pat. App. No. 2003/0176352, published Sep. 18, 2003 (corresponding to WO 03/031589, published Apr. 17, 2003); U.S. Ser. No. 09/422,838, filed Oct. 22, 1999 (corresponding to WO 00/24770, published May 4, 2000); U.S. Pat. App. No. 2003/0229023, published Dec. 11, 2003; WO 03/057134, published Jul. 17, 2003; U.S. Pat. App. No. 2003/0236193, published Dec. 25, 2003 (corresponding to PCT/US04/010989, filed Apr. 8, 2004); U.S. Ser. No. 10/666,480, filed Sep. 18, 2003 (corresponding to WO 04/026329, published Apr. 1, 2004)~~, each of which is hereby incorporated by reference in its entirety~~.

"Affibodies" or "Affibody" refers to the use of a protein liked by peptide bond to an Fc region, wherein the protein is used as a scaffold to provide a binding surface for a target molecule. The protein is often a naturally occurring protein such as staphylococcal protein A or IgG-binding B domain, or the Z protein derived therefrom (see Nilsson et al (1987), Prot Eng 1, 107-133, and U.S. Pat. No. 5,143,844) or a fragment or derivative thereof. For example, affibodies can be created from Z proteins variants having altered binding affinity to target molecule(s), wherein a segment of the Z protein has been mutated by random mutagenesis to create a library of variants capable of binding a target molecule. Examples of affibodies include U.S. Pat. No. 6,534,628, Nord K et al, Prot Eng 8:601-608 (1995) and

WO94/11026.

Conjugates of an antibody and one or more small molecule toxins, such as a calicheamicin, maytansinoids, dolastatins, aurostatins, a trichothecene, and CC1065, and the derivatives of these toxins that have toxin activity, are also contemplated herein.

i. Maytansine and maytansinoids

In some embodiments, the immunoconjugate comprises an antibody (full length or fragments) of the invention conjugated to one or more maytansinoid molecules.

Maytansinoids are mitototic inhibitors which act by inhibiting tubulin polymerization. Maytansine was first isolated from the east African shrub *Maytenus serrata* (U.S. Patent No. 3,896,111). Subsequently, it was discovered that certain microbes also produce maytansinoids, such as maytansinol and C-3 maytansinol esters (U.S. Patent No. 4,151,042). Synthetic maytansinol and derivatives and analogues thereof are disclosed, for example, in U.S. Patent Nos. 4,137,230; 4,248,870; 4,256,746; 4,260,608; 4,265,814; 4,294,757; 4,307,016; 4,308,268; 4,308,269; 4,309,428; 4,313,946; 4,315,929; 4,317,821; 4,322,348; 4,331,598; 4,361,650; 4,364,866; 4,424,219; 4,450,254; 4,362,663; and 4,371,533.

Maytansinoid drug moieties are attractive drug moieties in antibody drug conjugates because they are: (i) relatively accessible to prepare by fermentation or chemical modification, derivatization of fermentation products, (ii) amenable to derivatization with functional groups suitable for conjugation through the non-disulfide linkers to antibodies, (iii) stable in plasma, and (iv) effective against a variety of tumor cell lines.

Immunoconjugates containing maytansinoids, methods of making same, and their therapeutic use are disclosed, for example, in U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1~~, the disclosures of which are hereby expressly incorporated by reference~~. Liu et al., Proc. Natl. Acad. Sci. USA 93:8618-8623 (1996) described immunoconjugates comprising a maytansinoid designated DM1 linked to the monoclonal antibody C242 directed against human colorectal cancer. The conjugate was found to be highly cytotoxic towards cultured colon cancer cells, and showed antitumor activity in an *in vivo* tumor growth assay. Chari et al., Cancer Research 52:127-131 (1992) describe immunoconjugates in which a maytansinoid was conjugated via a disulfide linker to the murine antibody A7 binding to an antigen on human colon cancer cell lines, or to another murine monoclonal antibody TA.1 that binds the HER-2/neu oncogene. The cytotoxicity of the TA.1-maytansinoid conjugate was tested *in vitro* on the human breast cancer cell line SK-BR-3, which expresses 3 x 10^5 HER-2 surface antigens per cell. The drug conjugate achieved

a degree of cytotoxicity similar to the free maytansinoid drug, which could be increased by increasing the number of maytansinoid molecules per antibody molecule. The A7-maytansinoid conjugate showed low systemic cytotoxicity in mice.

Antibody-maytansinoid conjugates are prepared by chemically linking an antibody to a maytansinoid molecule without significantly diminishing the biological activity of either the antibody or the maytansinoid molecule. See, e.g., U.S. Patent No. 5,208,020 ~~(the disclosure of which is hereby expressly incorporated by reference)~~. An average of 3-4 maytansinoid molecules conjugated per antibody molecule has shown efficacy in enhancing cytotoxicity of target cells without negatively affecting the function or solubility of the antibody, although even one molecule of toxin/antibody would be expected to enhance cytotoxicity over the use of naked antibody. Maytansinoids are well known in the art and can be synthesized by known techniques or isolated from natural sources. Suitable maytansinoids are disclosed, for example, in U.S. Patent No. 5,208,020 and in the other patents and nonpatent publications referred to hereinabove. Preferred maytansinoids are maytansinol and maytansinol analogues modified in the aromatic ring or at other positions of the maytansinol molecule, such as various maytansinol esters.

There are many linking groups known in the art for making antibody-maytansinoid conjugates, including, for example, those disclosed in U.S. Patent No. 5,208,020 or EP Patent 0 425 235 B1, Chari et al., Cancer Research 52:127-131 (1992), and U.S. Patent Application Publication No. 2005/0169933~~, the disclosures of which are hereby expressly incorporated by reference~~. Antibody-maytansinoid conjugates comprising the linker component SMCC may be prepared as disclosed in U.S. Patent Application Publication No. 2005/0169933. The linking groups include disulfide groups, thioether groups, acid labile groups, photolabile groups, peptidase labile groups, or esterase labile groups, as disclosed in the above-identified patents, disulfide and thioether groups being preferred. Additional linking groups are described and exemplified herein.

Conjugates of the antibody and maytansinoid may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-

diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). Particularly preferred coupling agents include N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP) (Carlsson et al., Biochem. J. 173:723-737 (1978)) and N-succinimidyl-4-(2-pyridylthio)pentanoate (SPP) to provide for a disulfide linkage.

The linker may be attached to the maytansinoid molecule at various positions, depending on the type of the link. For example, an ester linkage may be formed by reaction with a hydroxyl group using conventional coupling techniques. The reaction may occur at the C-3 position having a hydroxyl group, the C-14 position modified with hydroxymethyl, the C-15 position modified with a hydroxyl group, and the C-20 position having a hydroxyl group. In a preferred embodiment, the linkage is formed at the C-3 position of maytansinol or a maytansinol analogue.

ii. Auristatins and dolastatins

In some embodiments, the immunoconjugate comprises an antibody of the invention conjugated to dolastatins or dolostatin peptidic analogs and derivatives, the auristatins (U.S. Patent Nos. 5,635,483 and 5,780,588). Dolastatins and auristatins have been shown to interfere with microtubule dynamics, GTP hydrolysis, and nuclear and cellular division (Woyke et al., Antimicrob. Agents and Chemother. 45(12):3580-3584 (2001)) and have anticancer (U.S. Patent No. 5,663,149) and antifungal activity (Pettit et al., Antimicrob. Agents Chemother. 42:2961-2965 (1998)). The dolastatin or auristatin drug moiety may be attached to the antibody through the N- (amino) terminus or the C- (carboxyl) terminus of the peptidic drug moiety (WO 02/088172).

Exemplary auristatin embodiments include the N-terminus linked monomethylauristatin drug moieties DE and DF, disclosed in "Monomethylvaline Compounds Capable of Conjugation to Ligands," U.S. Application Publication No. 2005/0238649~~, the disclosure of which is expressly incorporated by reference in its entirety~~.

Typically, peptide-based drug moieties can be prepared by forming a peptide bond between two or more amino acids and/or peptide fragments. Such peptide bonds can be prepared, for example, according to the liquid phase synthesis method (see E. Schröder and K. Lübke, "The Peptides," volume 1, pp. 76-136, 1965, Academic Press) that is well known in the field of peptide chemistry. The auristatin/dolastatin drug moieties may be prepared according to the methods of: U.S. Patent Nos. 5,635,483 and 5,780,588; Pettit et al., J. Nat. Prod. 44:482-485 (1981); Pettit et al., Anti-Cancer Drug Design 13:47-66 (1998); Poncet, Curr. Pharm. Des. 5:139-162 (1999); and Pettit, Fortschr. Chem. Org. Naturst. 70:1-79

(1997). See also Doronina, Nat. Biotechnol. 21(7):778-784 (2003); and "Monomethylvaline Compounds Capable of Conjugation to Ligands," U.S. Application Publication No. 2005/0238649~~, hereby incorporated by reference in its entirety~~ (disclosing, e.g., linkers and methods of preparing monomethylvaline compounds such as MMAE and MMAF conjugated to linkers).

iii. Calicheamicin

In other embodiments, the immunoconjugate comprises an antibody of the invention conjugated to one or more calicheamicin molecules. The calicheamicin family of antibiotics are capable of producing double-stranded DNA breaks at sub-picomolar concentrations. For the preparation of conjugates of the calicheamicin family, see U.S. Patent Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296 (all to American Cyanamid Company). Structural analogues of calicheamicin which may be used include, but are not limited to, γ_1^I, α_2^I, α_3^I, N-acetyl-γ_1^I, PSAG and θ^I_1 (Hinman et al., Cancer Research 53:3336-3342 (1993), Lode et al., Cancer Research 58:2925-2928 (1998) and the aforementioned U.S. patents to American Cyanamid). Another anti-tumor drug that the antibody can be conjugated is QFA, which is an antifolate. Both calicheamicin and QFA have intracellular sites of action and do not readily cross the plasma membrane. Therefore, cellular uptake of these agents through antibody mediated internalization greatly enhances their cytotoxic effects.

iv. Other cytotoxic agents

Other antitumor agents that can be conjugated to the antibodies of the invention or made according to the methods described herein include BCNU, streptozoicin, vincristine and 5-fluorouracil, the family of agents known collectively LL-E33288 complex described in U.S. Patent Nos. 5,053,394 and 5,770,710, as well as esperamicins (U.S. Patent No. 5,877,296).

Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes (see, for example, WO 93/21232, published October 28, 1993).

The present invention further contemplates an immunoconjugate formed between an antibody and a compound with nucleolytic activity (e.g., a ribonuclease or a DNA

The compounds of the invention expressly contemplate, but are not limited to, ADC prepared with cross-linker reagents: BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A). See pages 467-498, 2003-2004 Applications Handbook and Catalog.

v. Preparation of conjugated antibodies

In the conjugated antibodies of the invention, an antibody is conjugated to one or more moieties (for example, drug moieties), e.g. about 1 to about 20 moieties per antibody, optionally through a linker. The conjugated antibodies may be prepared by several routes, employing organic chemistry reactions, conditions, and reagents known to those skilled in the art, including: (1) reaction of a nucleophilic group of an antibody with a bivalent linker reagent via a covalent bond, followed by reaction with a moiety of interest; and (2) reaction of a nucleophilic group of a moiety with a bivalent linker reagent via a covalent bond, followed by reaction with the nucleophilic group of an antibody. Additional methods for preparing conjugated antibodies are described herein.

The linker reagent may be composed of one or more linker components. Exemplary linker components include 6-maleimidocaproyl ("MC"), maleimidopropanoyl ("MP"), valine-citrulline ("val-cit"), alanine-phenylalanine ("ala-phe"), p-aminobenzyloxycarbonyl ("PAB"), N-Succinimidyl 4-(2-pyridylthio) pentanoate ("SPP"), N-Succinimidyl 4-(N-maleimidomethyl) cyclohexane-1 carboxylate ("SMCC'), and N-Succinimidyl (4-iodo-acetyl) aminobenzoate ("SIAB"). Additional linker components are known in the art and some are described herein. See also "Monomethylvaline Compounds Capable of Conjugation to Ligands," U.S. Application Publication No. 2005/0238649~~, the contents of which are hereby incorporated by reference in its entirety~~.

In some embodiments, the linker may comprise amino acid residues. Exemplary amino acid linker components include a dipeptide, a tripeptide, a tetrapeptide or a pentapeptide. Exemplary dipeptides include: valine-citrulline (vc or val-cit), alanine-phenylalanine (af or ala-phe). Exemplary tripeptides include: glycine-valine-citrulline (gly-val-cit) and glycine-glycine-glycine (gly-gly-gly). Amino acid residues which comprise an amino acid linker component include those occurring naturally, as well as minor amino acids and non-naturally occurring amino acid analogs, such as citrulline. Amino acid linker

Claims

What is claimed is:

1. An antibody comprising:

(a) a first polypeptide comprising a VH domain linked to a constant domain and a first coiled coil domain (CC), wherein the first CC comprises a heptad repeat of Formula I:

$(X_1\ X_2\ X_3\ X_4\ X_5\ X_6\ X_7)_n$ (Formula I) (SEQ ID NO:29)

X_1 is a hydrophobic amino acid residue or Asparagine,

X_2, X_3, and X_6 are each any amino acid residue,

X_4 is a hydrophobic amino acid residue, and

X_5 and X_7 are each a charged amino acid residue; and

(b) a second polypeptide comprising a VH domain linked to a constant domain and a second coiled coil domain (CC), wherein the second CC comprises a heptad repeat of Formula Π:

$(X'_1\ X'_2\ X'_3\ X'_4\ X'_5\ X'_6\ X'_7)_n$ (Formula II) (SEQ ID NO:30)

X'_1 is a hydrophobic amino acid residue or Asparagine,

X'_2, X'_3, and X'_6 are each any amino acid residue,

X'_4 is a hydrophobic amino acid residue, and

X'_5 and X'_7 are each a charged amino acid residue;

wherein n in Formula I and Π is greater than or equal to 2; and

wherein, in each heptad repeat, the first CC comprises an X_5 residue that is opposite in charge to the X'_7 residue in the second CC and the first CC comprises an X_7 residue that is opposite in charge to the X'_5 residue in the second CC,

wherein the CC is linked to the constant domain by a linker and wherein the linker cleavable.

2. The antibody of claim 1, wherein the first and second polypeptides each comprise a VH and a CH1 domain.

3. The antibody of claim 2, wherein the first and second polypeptides each further comprise a hinge domain.

4. The antibody of any one of claims 1-3, wherein said first and second polypeptides each further comprise a CH2 and a CH3 domain.

5. The antibody of claim 1 , wherein the first and second polypeptides each comprise VH, CH1, hinge, CH2, and CH3 domains positioned relative to each other in an N-terminal to C-

terminal direction: VH-CHl-hinge-CH2-CH3.

6. The antibody of any one of claims 1-5, wherein said antibody further comprises a third and a fourth polypeptide, wherein said third polypeptide comprises a first VL domain and said fourth polypeptide comprises a second VL domain.

7. The antibody of claim 6, wherein said VH domain of the first polypeptide is linked to the VL domain of the third polypeptide by a tether and the VH domain of the second polypeptide is linked to the VL domain of the fourth polypeptide by a tether.

8. The antibody of claim 6, wherein the third polypeptide further comprises a first CL domain wherein said first VL and CL domains are positioned relative to each other within the third polypeptide in an N-terminal to C-terminal direction: VL-CL, and the fourth polypeptide further comprises a second CL domain, and wherein said second VL and CL domains are positioned relative to each other within the fourth polypeptide in an N-terminal to C-terminal direction: VL-CL.

9. The antibody of any one of claims 6-8, wherein the sequences of said first VL domain and said second VL domain are the same.

10. The antibody of any one of claims 1-9, wherein the N-terminus of the VH of at least one of said first or said second polypeptides is connected to the C-terminus of a CL with a tether.

11. An antibody comprising:

(a) a first polypeptide comprising a VH domain linked to a constant domain and a first coiled coil domain (CC), wherein the first CC comprises a heptad repeat of Formula I:

$(X_1\ X_2\ X_3\ X_4\ X_5\ X_6\ X_7)_n$ (Formula I) (SEQ ID NO:29)

X_1 is a hydrophobic amino acid residue or Asparagine,

X_2, X_3, and X_6 are each any amino acid residue,

X_4 is a hydrophobic amino acid residue, and

X_5 and X_7 are each a charged amino acid residue; and

(b) a second polypeptide comprising a constant domain comprising a CH2 and CH3 domain and a second coiled coil

domain (CC), wherein the second CC comprises a heptad repeat of Formula II:

$(X'_1\ X'_2\ X'_3\ X'_4\ X'_5\ X'_6\ X'_7)_n$ (Formula Π) (SEQ ID NO:30)

X'_1 is a hydrophobic amino acid residue or Asparagine,

X'_2, X'_3, and X'_6 are each any amino acid residue,

X'_4 is a hydrophobic amino acid residue, and

X'_5 and X'_7 are each a charged amino acid residue;

wherein n in Formula I and Π is greater than or equal to 2; and

wherein, in each heptad repeat, the first CC comprises an X_5 residue that is opposite in charge to the X'_7 residue in the second CC and the first CC comprises an X_7 residue that is opposite in charge to the X'_5 residue in the second CC,

wherein the CC is linked to the constant domain by a linker and wherein the linker cleavable.

12. The antibody of claim 11, wherein the first polypeptide comprises a VH and CH1 domain.

13. The antibody of claim 12, wherein the first polypeptide further comprises a hinge domain.

14. The antibody of claim 12 or 13, wherein the first polypeptide further comprises a CH2 and a CH3 domain.

15. The antibody of claim 11, wherein the first polypeptide comprises VH, CH1, hinge, CH2, and CH3 domains positioned relative to each other in an N-terminal to C-terminal direction: VH-CH1-hinge-CH2-CH3.

16. The antibody of any one of claims 11-15, wherein the antibody further comprises a third polypeptide, wherein the third polypeptide comprises a VL domain.

17. The antibody of claim 16, wherein said third polypeptide further comprises a CL domain, and the VL and CL domains are positioned relative to each other in an N-terminal to C-terminal direction: VL-CL.

18. The antibody of any one of claims 11-17, wherein the N-terminus of the VH of said first polypeptide is connected to the C-terminus of a CL with a tether.

24. The antibody of claim 23, wherein n is greater than or equal to 4.

~~25. The antibody of any one of claims 1-24, wherein at least one of said first or said second CC is linked C-terminal to a constant domain of the antibody.~~

~~26~~25. The antibody of any one of claim ~~25~~1-24, wherein said constant domain is a CH3 domain and the first CC is linked C-terminal to a CH3 domain of the first polypeptide and the second CC is linked C-terminal to a CH3 domain of the second polypeptide.

~~27. The antibody of claim 25 or 26, wherein linkage is by a cleavable linker sequence.~~

~~28~~26. The antibody of any one of claims 1-~~27~~25, wherein a Lys-C endopeptidase cleavage site is located N-terminal to at least one of said first or said second CC.

~~29. An antibody comprising a first polypeptide comprising a VL, CL, tether, VH, CHI, CH2, and CH3 domain positioned relative to each other in an N-terminal to C-terminal direction: VL-CL-tether-VH-CHl-CH2-CH3 (Formula III).~~

~~30. The antibody of claim 29, wherein said antibody further comprises a second polypeptide of Formula III.~~

~~31~~27. The antibody of any one of claims 1-9 ~~or 30~~, wherein the antibody is multispecific.

~~32~~28. The antibody of claim ~~31~~27, wherein the antibody is capable of binding at least 2 antigens.

~~33~~29. The antibody of claim ~~31~~27, wherein the antibody a capable of binding at least 2 epitopes on the same antigen.

~~34~~30. The antibody of any one of claims 1-9 ~~or 30~~, wherein said antibody is bispecific.

~~35~~31. The antibody of any one of claims 7, 10, or 18~~, 29, or 30~~, wherein said tether comprises Glycine (G) and Serine (S) residues.

~~36~~32. The antibody of any one of claims 7, 10, 18, ~~29, 30,~~ or ~~35~~31, wherein said tether is between 15 and 50 amino acids in length.

~~37~~33. The antibody of claim ~~36~~32, wherein said tether is between 20 and 26 amino acids in length.

~~38~~34. The antibody of any one of claims 7, 10, 18, ~~29, 30,~~ or ~~35-37~~31-33, wherein said tether comprises GGS repeats.

~~39~~35. The antibody of any one of claims 7, 10, 18, ~~29, 30,~~ or ~~35-38~~31-34, wherein said tether is cleavable.

~~40~~36. The antibody of any one of claims ~~28-39~~26-35, wherein said antibody comprises a mutation that removes a Lys-C endopeptidase cleavage site.

~~41~~37. The antibody of claim ~~40~~36, wherein said mutation that removes a Lys-C endopeptidase cleavage site is in a hinge domain.

~~42~~38. The antibody of claim ~~41~~37, wherein said antibody has a K222A substitution (EU numbering system).

~~43~~39. The antibody of any one of claims 1-25~~27~~ or ~~39~~35, wherein said tether or said linker is cleavable by one or more of the following endopeptidases: Furin, Thrombin, Genenase, Lys-C, Arg-C, Asp-N, Glu-C, Factor Xa, Tobacco Etch Virus Protease (TEV), Enterokinase, Human Rhinovirus C3 protease (HRV C3), and Kininogenase.

~~44~~40. The antibody of any one of claims 1-25~~27~~ or ~~39~~35, wherein said tether or said linker comprises an Asparagine-Glycine peptide bond.

~~45~~41. The antibody of claim ~~44~~40, wherein said Asparagine-Glycine peptide bond is cleavable by hydroxylamine.

~~46~~42. The antibody of any one of claims 1-~~45~~41, wherein said antibody comprises a constant region conjugated to a cytotoxic agent.

~~47~~43. The antibody of any one of claims 1-~~45~~41, wherein said antibody is expressed by a mammalian cell.

~~48~~44. The antibody of claim ~~47~~43, wherein said mammalian cell is a CHO cell.

~~49~~45. The antibody of any one of claims 1-~~45~~41, wherein said antibody is expressed by a prokaryotic cell.

~~50~~46. The antibody of claim ~~49~~45, wherein said prokaryotic cell is an E. coli cell.

~~51~~47. A method of producing an antibody, said method comprising the step of culturing a cell comprising a vector encoding the antibody of any one of claims 1-~~45~~41 in a culture medium.

~~52~~48. The method of claim ~~51~~47, wherein said method further comprises recovering said antibody from said cell or said culture medium.

~~53~~49. The method of claim ~~52~~48, further comprising the steps of

(a) capturing said antibody on a column comprising Protein A,

(b) eluting said antibody from said column, and

(c) diluting said eluted antibody into a solution containing a chaotropic agent or mild detergent.

~~54. A method of maintaining a coiled-coil-containing antibody in solution, said method comprising maintaining said antibody in the presence of a chaotropic agent or mild detergent.~~

~~55~~50. The method of claim ~~53 or 54~~49, wherein said chaotropic agent or mild detergent is Arginine, Guanidine-HCl, urea, lithium perchlorate, Histidine, Sodium Dodecyl Sulfate (SDS), Tween, Triton, or NP-40.