Claims

1. A method of conjugating a therapeutic agent to a thiol moiety in an isolated protein that contains at least one disulfide bond and at least one trisulfide bond, comprising the steps of:
   (a) reducing at least one sulfide bond in the isolated protein under nondenaturing conditions to form a composition comprising a reactive sulfide and a reduced protein comprising at least one thiol group;
   (b) decreasing the reactive sulfide content of the composition by contacting the composition with a nitrogen containing gas and,
   (c) conjugating the therapeutic agent to the at least one thiol group of the reduced protein to form a protein-agent conjugate.
2. The method of claim 1, wherein the reducing step comprises contacting the isolated protein with a chemical reducing agent in a concentration of 0.1 to 8mM.
3. The method of claim 2, wherein the reducing agent is at least one of dithiothreitol (DTT), beta-mercaptoethanol (βME), tris(2-carboxyethyl)phosphine (TCEP), cysteine, L-cysteine, reduced glutathione (GSH) and L-GSH.
4. The method of any preceding claim, wherein, prior to the reducing step, the pH of the isolated protein is adjusted to a pH between pH 5.0 and pH 8.0.
5. The method of any preceding claim, wherein the decreasing step comprises:
   (a) allowing the reduced protein in the composition to associate with a solid support; and,
   (b) replacing at least 90% of the composition with a replacement solution lacking the reactive sulfide.
6. The method of any preceding claim, wherein the decreasing step comprises contacting the composition with nitrogen gas.
7. The method of any preceding claim, wherein the isolated protein is an antibody or antibody fragment.
8. The method of claim 7, wherein the reducing step results in reducing only interchain disulfide bonds and not intrachain disulfide bonds.

9. The method of any preceding claim, wherein the therapeutic agent is at least one therapeutic agent selected from a chemotherapeutic agent, a nucleic acid, a cytokine, an immunosuppressant, a radioisotope, an antibiotic, and a therapeutic antibody.

10. The method of claim 9, wherein the chemotherapeutic agent is at least one anti-tubulin agent selected from an auristatin, a vinca alkaloid, a podophyllotoxin, a taxane, a baccatin derivative, a cryptophysin, a maytansinoid, a combretastatin, and a dolastatin.

11. The method of claim 9, wherein the chemotherapeutic agent is at least one of an auristatin, a DNA minor groove binding agent, a DNA minor groove alkylating agent, an enediyne, a lexitropsin, a duocarmycin, a taxane, a puromycin, a dolastatin, a maytansinoid, and a vinca alkaloid.

12. The method of claims 10 or 11, wherein the auristatin is MMAE or MMAF.

13. The method of any preceding claim, wherein the therapeutic agent comprises a linker moiety adapted to link the at least one thiol group of the reduced protein to the therapeutic agent.

14. The method of claim 13, wherein the linker moiety is a dipeptide linker, such as valine-citrulline (val-cit) or a phenylalanine-lysine (phe-lys) linker.

15. The method of claim 14, wherein the dipeptide linker comprises a maleimide functional group that reacts with free thiols to form a covalent bond.

16. The method of any preceding claim, wherein the protein-agent conjugate is one of aCD22-val-cit-MMAE, aCD22-val-cit-MMAF, aLy6E-val-cit-MMAE, aLy6E-val-cit-MMAF, aCD79b-val-cit-MMAE, aCD79b-val-cit-MMAF, aNaPi2b-val-cit-MMAE, aNaPi2b-val-cit-MMAF, aMUC16-val-cit-MMAE, a MUC16-val-cit-MMAF, aSTEAP1-val-cit-MMAE, aSTEAP1-val-cit-MMAF, aETBR-val-cit-MMAF and aETBR-val-cit-MMAE.

17. A method for converting trisulfide bonds to disulfide bonds in an isolated antibody comprising the steps of:

    (a) reducing at least one isolated antibody containing at least one trisulfide bond under nondenaturing conditions in a solution with TCEP at a pH between pH 5.0 and pH 8 to form a reduced antibody;

    (b) contacting the solution with nitrogen gas; and,

(c) conjugating an auristatin, or a derivative thereof, to the reduced antibody to form an antibody-drug conjugate (ADC).