What is claimed is:

1. A method for quantifying or characterizing conjugation of at least one attachment linked to at least one specific conjugation site of a partially conjugated peptide or protein in a sample, comprising:

cleaving a portion of the at least one attachment to generate a peptide or protein containing a cleaved linker, wherein the attachment comprises the cleaved linker;

adding a modified linker to an unconjugated conjugation site of the partially conjugated peptide or protein;

subjecting the sample to mass analysis to identify the peptide or protein containing the cleaved linker and/or the modified linker.

2. The method of claim 1, wherein the at least one attachment comprises a linker and a payload, wherein the cleaved portion of the attachment comprises the payload and wherein the linker comprises the cleaved linker.

3. The method of claim 1, further comprising quantifying or characterizing the site-specific conjugation of the attachment based on quantifying the cleaved linker and the modified linker.

4. The method of claim 1, wherein the mass analysis is conducted using a mass spectrometer, electrospray ionization mass spectrometer, nano-electrospray ionization mass spectrometer or a triple quadrupole mass spectrometer, wherein the mass spectrometer can be coupled to a liquid chromatography system and wherein the mass spectrometer is capable of performing a LC-MS (liquid chromatography-mass spectrometry), a LC-MRM-MS (liquid chromatography-multiple reaction monitoring-mass spectrometry) or a LC-MS/MS analyses.

5. The method of claim 1, further comprising treating the peptide or protein with an enzyme prior to cleaving the portion of the attachment and/or prior to adding the modified linker to the unconjugated conjugation site.

6. The method of claim 1, further comprising treating the sample with an enzyme prior to subjecting the sample to the mass analysis.

7. The method of claim 1, wherein the portion of the attachment is cleaved using an enzyme, a protease, a chemical, an acid, a base, or a reducing agent.

8. The method of claim 1, wherein the step of adding the modified linker to the unconjugated conjugation site is performed prior to conducting the step of cleaving the portion of the attachment.

9. The method of claim 1, wherein the step of cleaving a portion of the attachment is performed prior to conducting the step of adding a modified linker and the step of subjecting the sample to mass analysis.

10. The method of claim 1, wherein a molecular weight of the modified linker is different from a molecular weight of the cleaved linker.

11. The method of claim 5, wherein the enzyme is trypsin.

12. The method of claim 1, wherein the portion of the attachment is cleaved using papain, cathepsin B, or plasmin.

13. The method of claim 6, wherein the enzyme is Glu-C.

14. The method of claim 1, wherein the specific conjugation site or the unconjugated conjugation site is located within a cysteine residue of the peptide or the protein.

15. The method of claim 1, wherein the attachment is linked to the at least one specific conjugation site through a maleimide attachment group.

~~16. The method of claim 1, wherein the peptide or protein is an antibody, an antibody fragment, a Fab region of an antibody, a Fc region of an antibody, or a fusion protein.~~

~~17. The method of claim 2, wherein the linker is an acid-labile linker, a protease-cleavable linker, a disulfide-containing linker, a pyrophosphate-diester linker, or a hydrazone linker.~~

~~18. The method of claim 2, wherein the linker comprises a peptide.~~

19. The method of claim 2, wherein the linker comprises valine-alanine, phenylalanine-lysine, valine-citrulline, or derivatives thereof.

20. The method of claim 2, wherein the linker comprises polyethylene glycol.

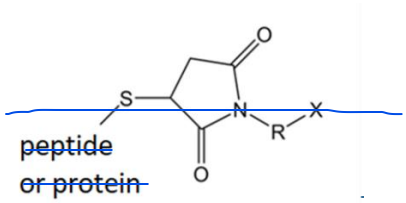
21. The method of claim 2, wherein the linker comprises para-aminobenzyloxycarbonyl (PABC) or para-aminobenzylalcohol (PABA).

22. The method of claim 1, wherein the modified linker comprises polyethylene glycol.

23. The method of claim 1, wherein the modified linker is added to the unconjugated conjugation site through a maleimide attachment group.

24. The method of claim 2, wherein the payload is a drug, a compound, a toxin, a cytotoxic agent, an anti-mitotic agent, a microtubule inhibitor, a DNA-damaging agent, a topoisomerase inhibitor, a RNA polymerase inhibitor, an amanitins analog, a tubu-lysin analog, a chemotherapeutic drug, a microtubule polymerization inhibitor, or a microtubule polymerization promoter.

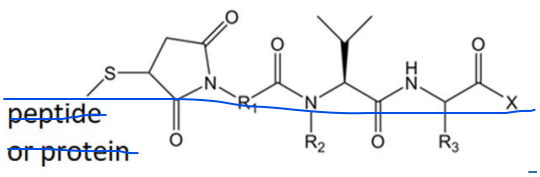
25. The method of claim 1, wherein the partially conjugated peptide or protein is selected from the group consisting of a conjugated peptide or protein of formula I,



wherein R is a linker, wherein X is a payload.

26. The method of claim 25, wherein the linker comprises polyethylene glycol and wherein the payload is a drug, a compound, a toxin, a cytotoxic agent, an anti-mitotic agent, a microtubule inhibitor, a DNA-damaging agent, a topoisomerase inhibitor, a RNA polymerase inhibitor, an amanitins analog, a tubu-lysin analog, a chemotherapeutic drug, a microtubule polymerization inhibitor, or a microtubule polymerization promoter.

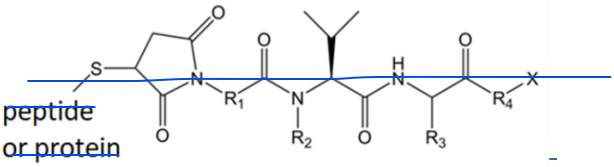
27. The method of claim 1, wherein the partially conjugated peptide or protein is selected from the group consisting of a conjugated peptide or protein of formula II,



wherein R_1 is a spacer, wherein R_2 is H or CH_3, wherein R_3 is CH_3, or $(CH_2)_3NHC(O)NH_2$, wherein X is a payload.

28. The method of claim 27, wherein the spacer comprises polyethylene glycol and wherein the payload is a drug, a compound, a toxin, a cytotoxic agent, an anti-mitotic agent, a microtubule inhibitor, a DNA-damaging agent, a topoisomerase inhibitor, a RNA polymerase inhibitor, an amanitins analog, a tubu-lysin analog, a chemotherapeutic drug, a microtubule polymerization inhibitor, or a microtubule polymerization promoter.

29. The method of claim 1, wherein the partially conjugated peptide or protein is selected from the group consisting of a conjugated peptide or protein of formula III,



wherein R_1 is a first spacer, wherein R_2 is H or CH_3, wherein R_3 is CH_3, or $(CH_2)_3NHC(O)NH_2$, wherein R_4 is a second spacer, wherein X is a payload.

30. The method of claim 29, wherein the first spacer comprises polyethylene glycol and wherein the second space comprises para-aminobenzyloxycarbonyl (PABC) or para-aminobenzylalcohol (PABA); wherein the payload is a drug, a compound, a toxin, a cytotoxic agent, an anti-mitotic agent, a microtubule inhibitor, a DNA-damaging agent, a topoisomerase inhibitor, a RNA polymerase inhibitor, an amanitins analog, a tubu-lysin analog, a

chemotherapeutic drug, a microtubule polymerization inhibitor, or a microtubule polymerization promoter.

31. A method for quantifying or characterizing conjugation of at least one attachment linked to at least one specific conjugation site of a partially conjugated protein in a sample, comprising:

cleaving a portion of the at least one attachment using a first enzyme to generate a protein containing a cleaved linker, wherein the attachment comprises the cleaved linker;

subjecting the sample to a second enzyme to obtain a peptide mixture; and

subjecting the peptide mixture to mass analysis to quantify or characterize the at least one specific conjugation site based on quantifying or characterizing the peptide containing the cleaved linker and/or the peptide which does not contain the cleaved linker.

32. The method of claim 31, wherein the at least one specific conjugation site is located within a lysine residue of the protein.

33. The method of claim 31, wherein the at least one attachment comprises a linker and a payload, wherein the cleaved portion of the at least one attachment comprises the payload and wherein the linker comprises the cleaved linker.

34. The method of claim 31, wherein the mass analysis is conducted using a mass spectrometer, electrospray ionization mass spectrometer, nano-electrospray ionization mass spectrometer or a triple quadrupole mass spectrometer, wherein the mass spectrometer can be coupled to a liquid chromatography system and wherein the mass spectrometer is capable of performing a LC-MS (liquid chromatography-mass spectrometry), a LC-MRM-MS (liquid chromatography-multiple reaction monitoring-mass spectrometry) or a LC-MS/MS analyses.

35. The method of claim 31, further comprising treating the peptide mixture with a third enzyme prior to subjecting the peptide mixture to the mass analysis.

36. The method of claim 31, wherein the first enzyme is papain, cathepsin B, or plasmin; and/or wherein the second enzyme is Glu-C or trypsin.

~~37. The method of claim 35, wherein the third enzyme is Asp-N or Glu-C.~~

~~38. The method of claim 31, wherein the protein is an antibody, an antibody fragment, a Fab region of an antibody, a Fc region of an antibody, or a fusion protein.~~

~~39. The method of claim 33, wherein the linker comprises valine-alanine, phenylalanine-lysine, valine-citrulline, or derivatives thereof.~~

~~40. The method of claim 33, wherein the payload is a drug, a compound, a toxin, a cytotoxic agent, an anti-mitotic agent, a microtubule inhibitor, a DNA-damaging agent, a topoisomerase inhibitor, a RNA polymerase inhibitor, an amanitins analog, a tubu-lysin analog, a chemotherapeutic drug, a microtubule polymerization inhibitor, or a microtubule polymerization promoter.~~