

We claim:

1. A method for identifying noncovalent interaction sites or dimerization interfaces in a protein drug product, comprising:

employing limited enzymatic digestion of a protein drug product under native conditions to form a protein drug dimer sample mixture comprising monomers and noncovalently linked dimers;

introducing detectable modifications into the dimers and monomers of the protein drug dimer sample mixture to produce modified dimers and modified monomers, wherein the detectable modifications are introduced by fast photochemical oxidation of proteins;

separating the modified dimers and modified monomers using native size exclusion chromatography into modified dimer and modified monomer fractions;

digesting the modified dimer and modified monomer fractions to form peptide samples;

separating the peptide samples using liquid chromatography/mass spectrometry (LC-MS) to obtain mass spectrometry data of the peptide samples;

determining modification sites in each peptide in the peptide samples using the mass spectrometry data of the peptides compared to known mass data of the protein drug product by calculating the mass of each peptide and modified peptide of such protein drug product, wherein regions showing decreased labeling in the dimer fractions than the monomer fractions indicates sites involved or in close proximity to the dimerization interfaces.

2. The method of claim 1, wherein native limited digestion conditions retain disulfide bonds between polypeptides and noncovalent interactions between dimers.
3. The method of any one of claims 1-2, wherein the protein drug product comprises an antibody or an antigen binding fragment thereof, a recombinant protein, a fusion protein, or a combination thereof.
4. The method of any one of claims 1-3, wherein the protein drug product is an antibody, and the dimers in the mixture consist essentially of two interacting F(ab') or F(ab) fragments.
5. The method of any one of claims 1-4, wherein the protein drug product is an antibody, and the monomers in the mixture consist essentially of F(ab) fragments and Fc fragments.
6. The method of any one of claims 1-5, wherein the modification is an amino acid side chain modification.
7. The method of any one of claims 3-6, wherein the antibody is a monoclonal antibody, a bispecific antibody, or an antigen binding fragment thereof.
8. The method of any one of claims 1-7, wherein the native size exclusion chromatography is performed using a mobile phase comprising ammonium acetate and ammonium bicarbonate.
9. The method of any one of claims 1-8, wherein the digested protein sample is separated with reverse phase liquid chromatography.
10. The method of any one of claims 1-9, wherein the modification is selected from the group consisting of oxidation, dioxidation, trioxidation, kynurenine, dithiomethyl, decarboxylation, hydroxykynurenine, and combinations thereof.

11. The method of any one of claims 1 or 2 wherein the enzyme is selected from the group consisting of papain, ficain, endoprotease Lys-C and IdeS or a modified form thereof.
12. The method of claim 1, wherein the modified dimer and modified monomer fractions are digested by an enzyme that breaks covalent chemical bonds linking two consecutive amino acid residues to form peptides.
13. The method of any one of claims 1-12, wherein the protein drug product sample is from a fed-batch culture, a purification process step, or a formulated drug substance.