CLAIMS

What is claimed is:

1. A method of analyzing a sample comprising a protein of interest, the method comprising:
   a. denaturing the sample;
   b. labeling the sample with a fluorescent label to produce a labeled sample;
   c. quenching un-reacted fluorescent label in the labeled sample;
   d. deglycosylating the labeled sample with an endoglycosidase; and
   e. performing electrophoresis on the labeled sample;
   wherein the sample is denatured, labeled and quenched in steps (a) through (c) prior to deglycosylation in step (d).

2. The method of claim 1, wherein the protein of interest comprises at least one glycosylation site.

3. The method of claim 1 or 2, wherein the protein is of interest is a glycosylated protein comprising at least one attached glycan.

4. The method of any one of claims 1-3, wherein the protein of interest comprises an antigen binding domain.

5. The method of claim 4, wherein the protein of interest comprises an antibody, an antibody fragment or an scFv.

6. The method of any one of claims 1-5, wherein the protein of interest comprises an Fc domain.

7. The method of any one of claims 1-6, wherein the protein of interest comprises a receptor fusion protein.

8. The method of claim 7, wherein the receptor fusion protein is a receptor-Fc-fusion protein or a soluble TCR-Fc fusion protein.

9. The method of any one of claims 1-8, wherein the protein of interest is a recombinant human protein.

10. The method of any one of claims 3-9, wherein the at least one attached glycan is N-linked.

11. The method of any one of claims 1-10, wherein the endoglycosidase catalyzes deglycosylation of N-linked glycans.

12. The method of claim any one of claims 1-11, wherein the endoglycosidase is selected from the group consisting of Peptide-N-Glycosidase F (PNGase F), Endoglycosidase H (Endo

H), Endoglycosidase S (Endo S), Endoglycosidase D, Endoglycosidase F1, Endoglycosidase F2 and Endoglycosidase F4.

13. The method of claim 12, wherein the PNGase F is Rapid PNGase F.

14. The method of claim 13, wherein the Rapid PNGase F is non-reducing.

15. The method of claim 13 or 14, wherein deglycosylating the sample comprises heating the sample to about 50 ºC for 10 minutes.

16. The method of any one of claims 1-15, wherein deglycosylating the sample comprises a reaction mixture comprising between 0.2-1.5 mg labeled protein of interest, and between 1-5 µL Rapid PNGase F in a 10 µL reaction volume, excluding the volume of the Rapid PNGase F.

17. The method of any one of claims 3-9, wherein the at least one glycan is an O-linked glycan.

18. The method of claim 17, wherein the endoglycosidase catalyzes deglycosylation of O-linked glycans.

19. The method of claim 17 or 18, wherein the endoglycosidase comprises Endo-α-N-acetylgalactosamindase (O-glycosidase).

20. The method of any one of claims 1-19, wherein labeling the sample with the fluorescent label comprises heating the sample to about 35 ºC for 10-30 minutes.

21. The method of any one of claims 1-20, wherein the sample is denatured using a reducing solution or a non-reducing solution.

22. The method of claim 21, wherein the reducing solution comprises dithiothreitol (DTT), and wherein the non-reducing solution comprises iodoacetamide (IAM).

23. The methods of any one of claims 1-22, wherein denaturing the sample comprises heating the sample to between 50 ºC and 99 ºC for between 1 to 60 minutes.

24. The method of any one of claims 1-23, wherein quenching the un-reacted fluorescent label comprises adding a stop solution.

25. The method of any one of claims 1-24, further comprising analyzing a reference standard in parallel to the sample.

26. The method of any one of claims 1-25, wherein the electrophoresis is selected from the group consisting of gel electrophoresis, isoelectric focusing, capillary electrophoresis (CE) or microchip capillary electrophoresis (MCE).

27. The method of any one of claims 1-26, wherein method results in reduced free dye interference in the less than 20 kDa range and a reduced or absent endoglycosidase peak in an

electropherogram when compared to an electropherogram generated using a sample labeled after deglycosylation.

28. The method of claim 27, wherein the endoglycosidase peak is reduced by at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80% or at least 90% when compared to an electropherogram generated using a sample labeled after deglycosylation.

29. A method of determining stability of a protein of interest comprising:

a. stressing a sample comprising the protein of interest;

b. denaturing the stressed sample and a non-stressed sample comprising the protein of interest;

c. labeling the stressed sample and the non-stressed sample with a fluorescent label to produce a labeled stressed sample and a labeled non-stressed sample;

d. quenching un-reacted fluorescent label in the labeled stressed sample and the labeled non-stressed sample;

e. deglycosylating the labeled stressed sample and the labeled non-stressed sample with an endoglycosidase;

f. performing microchip capillary electrophoresis (MCE) on the labeled stressed sample and the labeled non-stressed sample to generate electropherograms for the stressed sample and the non-stressed sample; and

g. comparing the electropherograms from the stressed sample and the non-stressed sample, thereby determining the stability of the protein of interest;

wherein the stressed sample and the non-stressed sample are denatured, labeled and quenched in steps (b) through (d) prior to deglycosylation in step (e).

30. The method of claim 29, wherein stressing the sample comprises thermally stressing the sample, at least one freeze/thaw cycle, exposing the sample to storage conditions, mechanically agitating the sample, lyophilizing and rehydrating the sample, or exposing the sample to light, radiation, singlet oxygen species, free radicals, high pH conditions or low pH conditions.

31. The method of claim 30, wherein thermally stressing the sample comprises holding the sample at between about 30 ºC and about 45 ºC for at least 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 5 weeks, at least 6 weeks, at least 7 weeks or at least 8 weeks.

32. The methods of claim 30, wherein the storage conditions comprise a temperature of about -80 ºC to -30 ºC for at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2

months, at least 3 months, at least 6 months, at least 8 months, at least 12 months, at least 18 months, at least 24 months or at least 30 months; or wherein the storage conditions comprise a temperature of about 2 ºC to 8 ºC for at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 6 months, at least 8 months, at least 12 months or at least 18 months.

33. The method of any one of claims 29-32, wherein the protein is of interest is a glycosylated protein comprising at least one attached glycan.

34. The method of any one of claims 29-33, wherein comparing the electropherograms for the stressed and non-stressed samples comprises comparing peak number, height, position, area, or a combination thereof.