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 claim 3, wherein the glycosylated protein comprises at least one attached glycan.~~

4~~5~~. The method of any one of claims 1-34, wherein the protein of interest comprises an antigen binding domain.

5~~6~~. The method of claim 4~~5~~, wherein the protein of interest comprises an antibody, an antibody fragment or an scFv.

6~~7~~. The method of any one of claims 1-5~~6~~, wherein the protein of interest comprises an Fc domain.

7~~8~~. The method of any one of claims 1-6~~7~~, wherein the protein of interest comprises a receptor fusion protein.

8~~9~~. The method of claim 7~~8~~, wherein the receptor fusion protein is a receptor-Fc-fusion protein or a soluble TCR-Fc fusion protein.

~~10. The method of claim 8 or 9, wherein the receptor fusion protein is a trap protein or a mini trap protein.~~

9~~11~~. The method of any one of claims 1-8~~10~~, wherein the protein of interest is a recombinant human protein.

~~12. The method of any one of claims 2-11, wherein the glycosylation site comprises an Asn-X-Ser/Thr consensus sequence.~~

10~~13~~. The method of any one of claims 3-9~~4-12~~, wherein the at least one attached glycan is N-linked.

~~14. The method of claim 13, wherein the at least one attached glycan is N-linked to an asparagine in the glycosylated protein.~~

11~~15~~. The method of any one of claims 1-10~~14~~, wherein the endoglycosidase catalyzes deglycosylation of N-linked glycans.

12~~16~~. The method of claim any one of claims 1-11~~15~~, 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.

~~17. The method of any one of claims 1-15, wherein the endoglycosidase is PNGase F.~~

13~~18~~. The method of claim 12~~17~~, wherein the PNGase F is Rapid PNGase F.

14~~19~~. The method of claim 13~~18~~, wherein the Rapid PNGase F is non-reducing.

15~~20~~. The method of claim 13 or 14 ~~any one of claims 17-19~~, wherein deglycosylating the sample comprises heating the sample to about 50 ºC for 10 minutes.

16~~21~~. The method of any one of claims 1-15~~20~~, 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.

~~22. The method of claim 21, wherein the reaction mixture comprises 0.2 mg labeled protein of interest.~~

~~23. The method of claim 21, wherein the reaction mixture comprises 5 µL Rapid PNGase F.~~

~~24. The method of any one of claims 21-23, wherein the reaction mixture comprises a buffer.~~

17~~25~~. The method of any one of claims- 3-9~~4-11~~, wherein the at least one glycan is an O-linked glycan.

18~~26~~. The method of claim 17~~25~~, wherein the endoglycosidase catalyzes deglycosylation of O-linked glycans.

19~~27~~. The method of claim 17 or 18 ~~25 or 26~~, wherein the endoglycosidase comprises Endo-α-N-acetylgalactosamindase (O-glycosidase).

20~~28~~. The method of any one of claims 1-19~~27~~, wherein labeling the sample with the fluorescent label comprises heating the sample to about 35 ºC for 10-30 minutes.

~~29. The method of any one of claims 1-27, wherein labeling the sample with the fluorescent label comprises heating the sample to about 35 ºC for about 15 minutes.~~

2130. The method of any one of claims 1-2029, wherein the sample is denatured using a reducing solution or a non-reducing solution.

2231. The method of claim 2130, wherein the reducing solution comprises dithiothreitol (DTT), and wherein the non-reducing solution comprises iodoacetamide (IAM).

~~32. The method of any one of claims 1-29, wherein the sample is denatured using a non-reducing solution.~~

~~33. The method of claim 32, wherein the non-reducing solution comprises iodoacetamide (IAM).~~

~~34. The methods of any one of claims 1-33, wherein denaturing the sample comprises heating the sample to between 40 ºC and 99 ºC for between 1 minute and 5 hours.~~

2335. The methods of any one of claims 1-2233, wherein denaturing the sample comprises heating the sample to between 50 ºC and 99 ºC for between 1 to 60 minutes.

2436. The method of any one of claims 1-2335, wherein quenching the un-reacted fluorescent label comprises adding a stop solution.

2537. The method of any one of claims 1-2436, further comprising analyzing a reference standard in parallel to the sample.

2638. The method of any one of claims 1-2537, wherein the electrophoresis is selected from the group consisting of gel electrophoresis, isoelectric focusing, capillary electrophoresis (CE) or microchip capillary electrophoresis (MCE).

~~39. The method of any one of claims 1-37, wherein the electrophoresis is MCE.~~

~~40. The method of claim 39, wherein the MCE is carried out using an MCE instrument.~~

2741. The method of any one of claims 1-2640, 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.

2842. The method of claim 2741, 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.

~~43. The method of claim 41, wherein the endoglycosidase peak is absent in an electropherogram when compared to an electropherogram generated using a sample labeled after deglycosylation.~~

2944. 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).

3045. The method of claim 2944, 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.

3146. The method of claim 3045, 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.

~~47. The method of claim 44, wherein stressing the sample comprises at least one freeze/thaw cycle.~~

~~48. The method of claim 44, wherein stressing the sample comprises exposing the sample to storage conditions.~~

3249. The methods of claim 3048, 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 ~~.~~

~~50. The methods of claim 48, 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.

~~51. The method of claim 44, wherein stressing the sample comprises mechanically agitating the sample.~~

~~52. The method of claim 44, wherein stressing the sample comprises lyophilizing and rehydrating the sample.~~

~~53. The method of claim 44, wherein stressing the sample comprises exposing the sample to light, radiation, singlet oxygen species, free radicals, high pH conditions or low pH conditions.~~

~~54. The method of any one of claims 44-53, wherein the protein of interest comprises at least one glycosylation site.~~

33~~55~~. The method of any one of claims 29-32~~44-53~~, wherein the protein is of interest is a glycosylated protein comprising at least one attached glycan.

~~56. The method of claim 55, wherein the glycosylated protein comprises at least one attached glycan.~~

~~57. The method of any one of claims 44-56, wherein the protein of interest comprises an antigen binding domain.~~

~~58. The method of claim 57, wherein the protein of interest comprises an antibody, an antibody fragment or an scFv.~~

~~59. The method of any one of claims 44-58, wherein the protein of interest comprises an Fc domain.~~

~~60. The method of any one of claims 44-59, wherein the protein of interest comprises a receptor fusion protein.~~

~~61. The method of claim 60, wherein the receptor fusion protein is a receptor-Fc-fusion protein or a soluble TCR-Fc fusion protein.~~

~~62. The method of claim 60 or 61, wherein the receptor fusion protein is a trap protein or a mini trap protein.~~

~~63. The method of any one of claims 44-62, wherein the protein of interest is a recombinant human protein.~~

64. The method of any one of claims 54-63, wherein the glycosylation site comprises an Asn-X-Ser/Thr consensus sequence.

65. The method of any one of claims 56-64, wherein the at least one attached glycan is N-linked.

66. The method of claim 65, wherein the at least one attached glycan is N-linked to an asparagine in the glycosylated protein.

67. The method of any one of claims 44-66, wherein the endoglycosidase catalyzes deglycosylation of N-linked glycans.

68. The method of claim any one of claims 44-67, 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.

69. The method of any one of claims 44-67, wherein the endoglycosidase is PNGase F.

70. The method of claim 69, wherein the PNGase F is Rapid PNGase F.

71. The method of claim 70, wherein the Rapid PNGase F is non-reducing.

72. The method of any one of claims 44-71, wherein deglycosylating the stressed and non-stressed samples comprises heating the samples to about 50 ºC for 10 minutes.

73. The method of any one of claims 44-72, wherein deglycosylating the stressed and non-stressed samples comprises a reaction mixture for each sample 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.

74. The method of claim 73, wherein the reaction mixture for each of the stressed and non-stressed sample comprises 5 µL Rapid PNGase F.

75. The method of claim 73 or 74, wherein each of the stressed and non-stressed sample comprise 0.2 mg labeled protein of interest.

76. The method of any one of claims 73-75, wherein the reaction mixture for each of the stressed and non-stressed sample comprises a buffer.

77. The method of any one of claims 44-63, wherein the at least one glycan is an O-linked glycan.

78. The method of claim 77, wherein the endoglycosidase catalyzes deglycosylation of O-linked glycans.

~~79. The method of claim 77 or 78, wherein the endoglycosidase comprises Endo-α N-acetylgalactosamindase (O-glycosidase).~~

~~80. The method of any one of claims 44-79, wherein labeling the stressed and non-stressed samples with the fluorescent label comprises heating each sample to about 35 ºC for 10-30 minutes.~~

~~81. The method of any one of claims 44-79, wherein labeling the stressed and non-stressed samples with the fluorescent label comprises heating each sample to about 35 ºC for about 15 minutes.~~

~~82. The method of any one of claims 44-81, wherein the stressed and non-stressed samples are denatured using a reducing solution.~~

~~83. The method of claim 82, wherein the reducing solution comprises dithiothreitol (DTT).~~

~~84. The method of any one of claims 44-81, wherein the stressed and non-stressed samples are denatured using a non-reducing solution.~~

~~85. The method of claim 84, wherein the non-reducing solution comprises iodoacetamide (IAM).~~

~~86. The method of any one of claims 44-85, wherein denaturing the stressed and non-stressed samples comprises heating the samples to between 40 ºC and 99 ºC for between 1 minute and 5 hours.~~

~~87. The method of any one of claims 44-85, wherein denaturing the stressed and non-stressed samples comprises heating the samples to between 50 ºC and 99 ºC for between 1 to 60 minutes.~~

~~88. The method of any one of claims 44-87, wherein quenching the un-reacted fluorescent label comprises adding a stop solution.~~

~~89. The method of any one of claims 44-88, further comprising analyzing a reference standard in parallel to the stressed and non-stressed samples.~~

34~~90~~. The method of any one of claims 29-33~~44-89~~, wherein comparing the electropherograms for the stressed and non-stressed samples comprises comparing peak number, height, position, area, or a combination thereof.