

What is claimed is:

1. A method of enhancing a mass spectral signal, comprising:
contacting a sample to a separation column under conditions that permit sample components to bind to the substrate;
applying a first mobile phase gradient to the separation column, wherein the first mobile phase gradient comprises trifluoroacetic acid (TFA) and a small molecule additive or formic acid (FA) and a small molecule additive;
applying a second mobile phase gradient to the separation column, wherein the second mobile phase gradient comprises TFA in acetonitrile (ACN) and a small molecule additive or FA in ACN and a small molecule additive; and
performing mass spectrometric analysis on eluted sample components.
2. The method of claim 1, wherein the small molecule additive in the first mobile phase is selected from glycine, alanine, serine, valine, N-acetyl glycine, methionine, β -alanine, aspartic acid, or N-methyl glycine.
3. The method of claim 2, wherein the small molecule additive in the first mobile phase is selected from glycine, alanine, serine, or valine.
4. The method of claim 3, wherein the small molecule additive in the first mobile phase is glycine.
5. The method of any one of claims 1 to 4, wherein the small molecule additive concentration is between about 1 mM and about 2 mM.
6. The method of claim 4, wherein glycine concentration is about 1 mM.
7. The method of claim 4, wherein glycine concentration is about 2 mM.
8. The method of any one of claims 1 to 7, wherein the small molecule additive in the second mobile phase is selected from glycine, alanine, serine, valine, N-acetyl glycine, methionine, β -alanine, aspartic acid, or N-methyl glycine.

9. The method of claim 8, wherein the small molecule additive in the second mobile phase is selected from glycine, alanine, serine, or valine.
10. The method of any one of claims 1 to 9, wherein the small molecule additive in the second mobile phase is glycine.
11. The method of any one of claims 8 to 10, wherein the small molecule additive concentration is between about 1 mM and about 2 mM.
12. The method of claim 10, wherein glycine concentration is about 1 mM.
13. The method of claim 10, wherein glycine concentration is about 2 mM.
14. The method of any one of claims 1-13, wherein TFA concentration in the first mobile phase is about 0.05% to 0.1% TFA in H₂O or the FA concentration in the first mobile phase is about 0.1% FA.
15. The method of any one of claims 1-14, wherein TFA concentration in the second mobile phase comprises about 0.05% TFA in 80% ACN and 20% H₂O or about 0.1% TFA in 80% ACN and 20% H₂O.
16. The method of any one of claims 1-15, wherein the sample comprises peptides or nucleotides.
17. The method of claim 16, wherein the peptides are glycopeptides.
18. The method of claim 17, wherein the glycopeptides are obtained from a monoclonal antibody.
19. The method of claim 18, wherein the monoclonal antibody is of isotype IgG1, IgG2, IgG3, IgG4, or mixed isotype.
20. The method of any one of claims 1-19, further comprising preparing the sample prior to contacting the sample to a separation column under conditions that permit sample components to bind to the substrate.

21. The method of claim 20, wherein preparing the sample comprises:
contacting a sample with a denaturing and reducing solution under conditions that permit sample denaturation and reduction;
contacting denatured and reduced sample with an alkylating solution under conditions that permit sample alkylation;
contacting alkylated sample with a digest solution under conditions that permit sample digestion; and
contacting digested sample with a quenching solution under conditions that stop sample digestion.
22. The method of claim 21, wherein the sample is a monoclonal antibody and the digest solution comprises a protease.
23. The method of claim 22, wherein the protease comprises trypsin.
24. The method of any one of claims 1 to 23, wherein the separation column is a liquid chromatography column.
25. The method of claim 24, wherein the liquid chromatography (LC) separation column comprises a hydrophilic interaction (HILIC) liquid chromatography column.
26. The method of any one of claims 1-25, wherein performing mass spectrometric analysis on eluted sample components comprises applying electrospray ionization to generate charged ions from the eluted sample components and measuring the generated charge ions.
27. The method of any one of claims 1-26, wherein the method enhances the mass spectral signal as indicated by about 5 to 14-fold on average and/or an approximately about 2 to 1000 fold ($z \geq 3$) increase in high charge state species.
28. The method of claim 27, wherein the spectral signal increase by approximately 14-fold and/or approximately 1000-fold increase in high charge state species.

29. The method of any one of claims 17-19, wherein the mass spectral signal obtained on the eluted sample components is enhanced by from 2-fold to 50-fold relative to a mass spectral signal obtained on a control sample in the absence of the small molecule additive.
30. The method of claim 29, wherein the glycopeptide is an O-glycan containing glycopeptide.
31. The method of claim 29, wherein the glycopeptide is an N-glycan containing glycopeptide.
32. The method of claim 30 or 31, wherein O-glycan or N-glycan is linked to a label.
33. The method of claim 32, wherein the label is procainamide.
34. The method of any one of claims 29-33, wherein the small molecule additive is glycine.