

What is Claimed is:

1. A method of detecting an analyte in a sample, comprising:
 - (a) performing high pH reversed phase liquid chromatography (RPLC) survey scan with detection by mass spectrometry (MS) on the analyte at previously determined concentration in the presence of an internal standard, to determine the peak apex (T_{apex}) of the analyte;
 - (b) obtaining an experimental sample comprising the analyte;
 - (c) performing high pH RPLC on the sample of (b);
 - (d) collecting at least a portion of the high pH RPLC eluate of (c) corresponding to T_{apex} for the analyte +/- average peak width of the analyte or of the internal standard in the survey scan of (a), wherein the at least a portion is optionally collected in a trap column;
 - (e) performing low pH RPLC on the collected portion of the high pH RPLC eluate of (d); and
 - (f) detecting the analyte by MS.
2. The method of claim 1, wherein the analyte is a peptide.
3. A method of detecting a peptide analyte in a sample, comprising:
 - (a) obtaining an experimental sample and performing an affinity-based capture to isolate a protein in the sample;
 - (b) digesting the captured protein with a protease to obtain a peptide analyte for detection;
 - (c) performing high pH reversed phase liquid chromatography (RPLC) survey scan with detection by mass spectrometry (MS) on the peptide analyte at previously determined concentration in the presence of an internal standard peptide, to determine the peak apex (T_{apex}) of the analyte;
 - (d) performing high pH RPLC on the digested sample of (b) comprising the peptide analyte;
 - (e) collecting at least a portion of the high pH RPLC eluate of (d) corresponding to T_{apex} for the analyte +/- average peak width of the analyte, the internal standard, or a peptide in the survey scan of (c), wherein the at least a portion is optionally collected in a trap column;
 - (f) performing low pH RPLC on the collected portion of the high pH RPLC eluate of (e); and

- (g) detecting the analyte by MS.
4. The method of any one of claims 1-3, wherein the high pH RPLC and the low pH RPLC are each performed at microflow rates.
 5. The method of claim 4, wherein the high pH RPLC and/or the low pH RPLC is performed at a flow rate of 4-10 $\mu\text{L}/\text{min}$, 5-10 $\mu\text{L}/\text{min}$, 6-9 $\mu\text{L}/\text{min}$, 7-10 $\mu\text{L}/\text{min}$, 6-8 $\mu\text{L}/\text{min}$, 5 $\mu\text{L}/\text{min}$, 6 $\mu\text{L}/\text{min}$, 7 $\mu\text{L}/\text{min}$, 8 $\mu\text{L}/\text{min}$, 9 $\mu\text{L}/\text{min}$, or 10 $\mu\text{L}/\text{min}$.
 6. The method of any one of claims 1-5, wherein the high pH RPLC and/or the low pH RPLC are performed on a C18 column.
 7. The method of claim 6, wherein both the high pH RPLC and the low pH RPLC are performed on a C18 column.
 8. The method of any one of claims 1-7, wherein the high pH RPLC and survey scan are performed at a pH between 8 and 10, between 8 and 9, between 9 and 10, or at pH 8.0, pH 8.5, pH 9.0, pH 9.5, or pH 10, and optionally with a mobile phase A comprising ammonium formate and a mobile phase B comprising ammonium formate and acetonitrile (e.g., ammonium formate in 90% acetonitrile).
 9. The method of any one of claims 1-8, wherein the high pH RPLC survey scan and the high pH RPLC performed on the sample or digested sample are each performed at substantially the same flow rate, pH, temperature, pressure, and/or column type (e.g. C8, C18, etc.).
 10. The method of any one of claims 1-9, wherein the low pH RPLC is performed at a pH between 2 and 4.5, between 2 and 4, between 2 and 3, between 2 and 2.5, between 2.5 and 3, between 3 and 4, or at pH 2.0, pH 2.5, pH 3.0, pH 3.5, pH 4.0, or pH 4.5, and optionally with a mobile phase A comprising formic acid and a mobile phase B comprising formic acid and acetonitrile (e.g., formic acid in acetonitrile).
 11. The method of any one of claim 1-10, wherein the sample is a biological sample.
 12. The method of claim 11, wherein the sample is a biological fluid sample, such as tears, saliva, lymph fluid, urine, serum, cerebral spinal fluid, pleural effusion, ascites, or plasma.
 13. The method of claim 12, wherein the sample is a serum sample, optionally wherein the sample is treated to enrich or extract components comprising the analyte prior to use in the method.

14. The method of claim 11, wherein the sample is a tissue sample, optionally wherein the sample is treated to enrich or extract components comprising the analyte prior to use in the method.
15. The method of any one of claims 1-14, wherein the portion of the high pH RPLC eluate on which the low pH RPLC is performed corresponds to a portion comprising T_{apex} for the analyte ± 0.25 minutes at a flow rate of 8 $\mu\text{l}/\text{min}$ in a C18 column.
16. The method of any one of claims 1-14, wherein the portion of the high pH RPLC eluate on which the low pH RPLC is performed corresponds to a portion comprising T_{apex} for the analyte ± 0.3 minutes at a flow rate of 8 $\mu\text{l}/\text{min}$ in a C18 column.
17. The method of claim 15 or 16, wherein the portion of the high pH RPLC eluate on which the low pH RPLC is performed corresponds to a portion consisting of T_{apex} for the analyte $\pm$ between 0.25 and 0.30 minutes at a flow rate of 8 $\mu\text{l}/\text{min}$ on a C18 column.
18. The method of any one of claims 3-17, wherein the protein is captured by attachment to particles coated with a binding partner for the protein, such as a ligand or antigen.
19. The method of claim 18, wherein the particles are coated with protein A, protein G, or a protein A and G chimera.
20. The method of any one of claims 3-19, wherein the protein is an antibody.
21. The method of any one of claims 2-20, wherein the analyte is a portion of an antibody (e.g., an antigen binding fragment such as a Fab, Fv, scFv, or F(ab')_2 fragment).
22. The method of any one of claims 2-19, wherein the analyte is an antigen or a drug binding target.
23. The method of any one of claims 3-22, wherein the protease comprises one or more of: trypsin, endoproteinase LysC, endoproteinase ArgC, staph aureus V8, endoproteinase GluC, chymotrypsin, or papain.
24. The method of any one of claims 1-23, wherein the method detects more than one analyte in the sample, optionally wherein levels of two or more analytes are determined, and optionally wherein the more than one analyte comprises a modified and unmodified form of a molecule.

25. The method of claim 24, wherein the method detects a modified and unmodified form of a molecule, wherein the modified and unmodified forms comprise a modified and unmodified protein, such as an alkylated and nonalkylated protein or a glycosylated and unglycosylated protein, or a molecule and a metabolite of the molecule.
26. The method of any one of claims 1-25, wherein the high pH RPLC and low pH RPLC and MS detection are automated.
27. The method of any one of claims 1-26, wherein detecting the analyte by MS following the low pH RPLC comprises quantitatively determining the amount or concentration of the analyte in the sample.
28. The method of any one of claims 1-27, wherein the MS is tandem MS ("MS/MS") (such as multiple reaction monitoring (MRM), single ion monitoring (SIM), triple stage quadrupole (TSQ), quadrupole/time of flight (QTOF), quadrupole linear ion trap (QTRAP), hybrid ion trap/FTMS, time of flight/time of flight (TOF/TOF), or tandem in time MS/MS).
29. The method of any one of claims 1-28, wherein the at least a portion of high pH RPLC eluate is diluted in a low pH mobile phase prior to performing low pH RPLC, optionally further wherein the dilution is automatically controlled.
30. The method of any one of claims 1-29, wherein the method allows for at least 30-fold or for 30 to 100-fold greater sensitivity in analyte detection than a single dimension HPLC-tandem MS (1D-LC-MS/MS) process at microflow rates, and/or wherein the method allows for at least 100-fold greater sensitivity in analyte detection than a single dimension HPLC-tandem MS (1D-LC-MS/MS) process at analytical flow rates.
31. The method of any one of claims 1-30, wherein the method allows for detection of an analyte in a tissue sample at a concentration of less than 10 fmol/ μ g total protein, less than 1 fmol/ μ g total protein, 1-10 fmol/ μ g total protein, less than 0.1 fmol/ μ g total protein, 0.1-1 fmol/ μ g total protein, or between 0.01 and 0.1 fmol/ μ g total protein; and/or wherein the method allows for detection of an analyte in a fluid sample at a concentration of less than 10 ng/mL, less than 1 ng/mL, less than 100 pg/mL, or less than 10 pg/mL, for example, such as 1-10 ng/mL, 100pg/mL to 1 ng/mL, or 10-100 pg/mL.
32. A system for performing a two-dimensional liquid chromatography method for detecting an analyte in a sample, wherein the system comprises:

(a) an injection valve for injecting a sample containing the analyte into the system, wherein the injection valve is connected to a high performance liquid chromatography (HPLC) column and a first dimension pump to control flow of sample and mobile phase through the column;

(b) a trap column for collecting at least a pre-determined portion of a first dimension HPLC column eluate for analysis in a second dimension HPLC process;

(c) a trap valve for controlling flow path of mobile phases from a first dimension HPLC process to a second dimension HPLC process;

(d) a second dimension pump to control flow of mobile phases through a second dimension HPLC column; and

(e) a mass spectrometer for analysis of analyte following a second dimension HPLC process.

33. The system of claim 32, wherein the system is capable of performing the method of any one of claims 1-31.

34. The system of claim 32 or 33, wherein the mass spectrometer is a tandem mass spectrometer.

35. The system of any one of claims 32-34, wherein the injection valve and the trap valve positions can be automatically controlled during a two-dimensional liquid chromatography process, and wherein the automatic control allows for only a pre-determined portion of eluate from the first dimension to be separated in the second dimension.

36. The system of any one of claims 32-35, wherein the pre-determined portion of eluate from the first dimension corresponds to a portion comprising T_{apex} for the analyte ± 0.25 minutes at a flow rate of 8 $\mu\text{l}/\text{min}$ in a C18 column.

37. The system of any one of claims 32-36, wherein the pre-determined portion of eluate from the first dimension corresponds to a portion comprising T_{apex} for the analyte ± 0.3 minutes at a flow rate of 8 $\mu\text{l}/\text{min}$ in a C18 column.

38. The system of claim 36 or 37, wherein the pre-determined portion of eluate from the first dimension corresponds to a portion consisting of T_{apex} of an analyte $\pm$ between 0.25 and 0.30 minutes at a flow rate of 8 $\mu\text{l}/\text{min}$ on a C18 column.

39. The system of any one of claims 32-38, wherein the system is further capable of performing a survey scan to determine the T_{apex} of an analyte in the first dimension HPLC column.

40. The system of any one of claims 32-39, wherein the mass spectrometer performs multiple reaction monitoring (MRM), single ion monitoring (SIM), triple stage quadrupole (TSQ), quadrupole/time of flight (QTOF), quadrupole linear ion trap (QTRAP), hybrid ion trap/FTMS, time of flight/time of flight (TOF/TOF), or tandem in time MS/MS.
41. The system of any one of claims 32-40, wherein the analyte is a peptide.
42. The system of any one of claims 32-41, wherein the system is capable of diluting a pre-determined portion of eluate from the first dimension process in a mobile phase buffer for the second dimension process, optionally wherein the dilution is automatically controlled.
43. The method of any one of claims 2-31, wherein the analyte is an anti-CD22 antibody or peptide fragment thereof, such as one or both of IYPGDGDTNYS GK and LSCAASGYEFSR.
44. The method of any one of claims 2-31, wherein the analyte is PD-L1 or a peptide fragment thereof, such as an IgV domain fragment, such as LQDAGVYR, optionally wherein the sample is obtained from a subject previously treated with an immune checkpoint molecule, such as atezolizumab, and further optionally wherein the sample is a serum sample.
45. A method of detecting a peptide analyte for human PD-L1 in a biological sample from a human subject, comprising:
- (a) obtaining a biological sample (e.g., a serum sample) from the subject and performing an affinity-based capture to isolate PD-L1 in the sample, optionally wherein the affinity-based capture comprises capturing PD-L1 complexed with an anti-PD-L1 antibody (e.g. atezolizumab);
 - (b) digesting the captured protein with a protease to obtain a PD-L1 peptide analyte for detection (e.g. a PD-L1 IgV domain fragment, such as LQDAGVYR);
 - (c) performing high pH reversed phase liquid chromatography (RPLC) survey scan with detection by mass spectrometry (MS) on the PD-L1 peptide analyte at previously determined concentration in the presence of an internal standard peptide, to determine the peak apex (T_{apex}) of the analyte;
 - (d) performing high pH RPLC on the digested sample of (b) comprising the peptide analyte;
 - (e) collecting at least a portion of the high pH RPLC eluate of (d) corresponding to T_{apex} for the analyte $\pm$ average peak width of the analyte, the

internal standard, or a peptide in the survey scan of (c), wherein the at least a portion is optionally collected in a trap column;

(f) performing low pH RPLC on the collected portion of the high pH RPLC eluate of (e); and

(g) detecting the PD-L1 peptide analyte by MS (e.g., by tandem MS such as multiple reaction monitoring (MRM), single ion monitoring (SIM), triple stage quadrupole (TSQ), quadrupole/time of flight (QTOF), quadrupole linear ion trap (QTRAP), hybrid ion trap/FTMS, time of flight/time of flight (TOF/TOF), or tandem in time MS/MS).

46. The method of any one of claims 1-31 or 43-45, wherein the method is performed in a system of any one of claims 32-42.