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, an antigen binding fragment, an antigen, or a drug binding target.

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 claim 1, wherein the high pH RPLC and the low pH RPLC are each performed at microflow rates.

5. The method of claim 1, wherein the high pH RPLC is performed on a C18 column, the low pH RPLC is performed on a C18 column, or both the high pH RPLC and the low pH RPLC are performed on a C18 column.

6. The method of claim 1, 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.

7. The method of claim 1, 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.

8. The method of claim 1, 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.

9. The method of claim 1, wherein the sample is a biological sample.

10. The method of claim 9, wherein the sample is a biological fluid sample, optionally comprising tears, saliva, lymph fluid, urine, serum, cerebral spinal fluid, pleural effusion, ascites, or plasma, or 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.

11. The method of claim 1, 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 µl/min in a C18 column, T_{apex} for the analyte +/- 0.3 minutes at a flow rate of 8 µl/min in a C18 column, or T_{apex} for the

analyte +/- between 0.25 and 0.30 minutes at a flow rate of 8 µl/min on a C18 column.

12. The method of claim 3, wherein the protein is captured by attachment to particles coated with a binding partner for the protein, optionally wherein the particles are coated with protein A, protein G, or a protein A and protein G chimera.

13. The method of claim 3, wherein the protease comprises one or more of: trypsin, endoproteinase LysC, endoproteinase ArgC, staph aureus V8, endoproteinase GluC, chymotrypsin, or papain.

14. The method of claim 1, 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.

15. The method of claim 14, wherein the method detects a modified and unmodified form of a molecule, wherein the modified and unmodified forms comprise a modified and unmodified protein.

16. The method of claim 1, wherein detecting the analyte by MS following the low pH RPLC comprises quantitatively determining the amount or concentration of the analyte in the sample.

17. The method of claim 1, wherein the MS is tandem MS (MS/MS), 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.

18. The method of claim 1, wherein the method allows for at least 30-fold or for 30 to 100-fold greater sensitivity in analyte detection than a single dimension high performance liquid chromatography-tandem (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.

19. The method of claim 1, wherein the method allows for detection of an analyte in a tissue sample at a concentration of less than 10 fmol/µg total protein, less than 1 fmol/µg total protein, 1-10 fmol/µg total protein, less than 0.1 fmol/µg total protein,

0.1-1 fmol/µg total protein, or between 0.01 and 0.1 fmol/µg 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, less than 10 pg/mL, or at a concentration of 1-10 ng/mL, 100pg/mL to 1 ng/mL, or 10-100 pg/mL.

20. 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,

wherein the injection valve and the trap valve positions are 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.

21. The system of claim 20, wherein the system is capable of performing 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.