Supplementary Figures for "High sensitivity quantitative proteomics using accumulated ion monitoring and automated multidimensional nano-flow chromatography"

CONTENT

SUPPLEMENTARY FIGURES LEGENDS

Supplementary Figure 1. A) Scanning electron micrograph of a typical nano-electrospray emitter tip, with 2 μm tip diameter. B) Photomicrograph (4X) of a typical electrospray tip.

Supplementary Figure 2. Ionization efficiency estimation for four target peptides, obtained from optimized 2-3 μm tip emitters.

Supplementary Figure 3. AIM acquisition enables detection of 2-5 yoctomoles/ms of target peptide serially diluted in neat solvent, and seven order of magnitude of linear dynamic range. The MS intensity within 10 ppm from the theoretical m/z was recorded for peptides SPEVSPPR (A), YTEYNEPHESR (B), and LFEVIETEK (C) with maximum injection time (max IT) 25 (red), 250 (black), and 2500 (grey) ms (n=7, error bars = standard deviation). Noise levels recorded at baseline level (i.e., with no target peptide infused) is displayed for each maximum injection times as continuous line.

Supplementary Figure 4. Reverse phase retention times for 6 targets over 18 consecutive sample injections in A) 1D and B) 2D. Outliers runs were not removed to demonstrate true technical variability.

Supplementary Figure 5. Comparison of sensitivity in AIM and PRM sensitivity following 1D (black) and 2D (red) chromatography. The figures refers to peptides: SEPVSPR (A, B), SEPV(pS)PR (C,D), VLIPGSK (E, F), and YTEYNEPHESR (G, H). Horizontal lines represent average noise levels (n=3) for 1D (black) and 2D (red) in AIM. Error bars represent technical variability as standard deviation (n=3) measured at noise

level, 10 amol and 100 fmol. Overlapping data points are horizontally offset when needed for clarity.

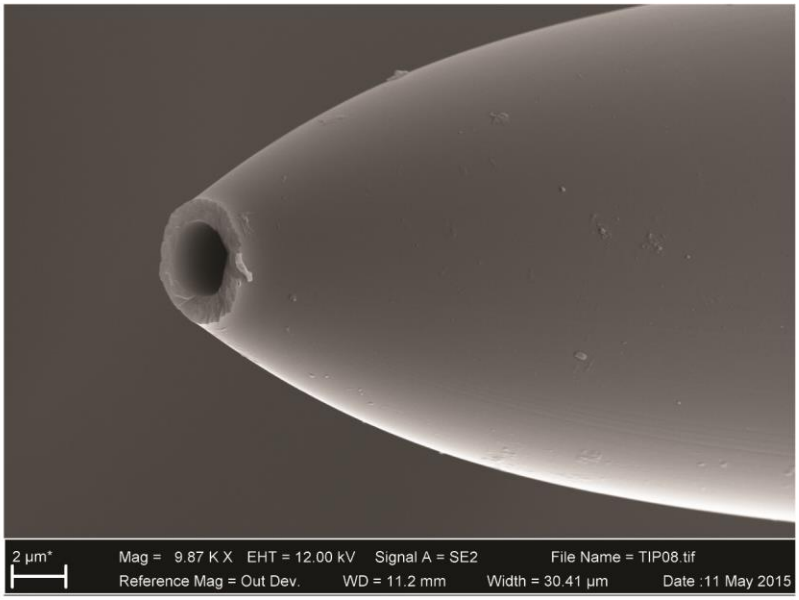
Supplementary Figure 6. Precision of measurement for AIM and PRM under 1D (shades of grey) and 2D (shades of red), expressed as variability (standard error of the mean) of endogenous peptides quantification.

Supplementary Figure 7. High target flow-rate, here demonstrated with peptide N(pS)PGLLVSPGNLNK at 1 pmol, produced deterioration of mass accuracy (black) and reverse phase resolution (evident as peak broadening to several minutes, red).

SUPPLEMENTARY FIGURES

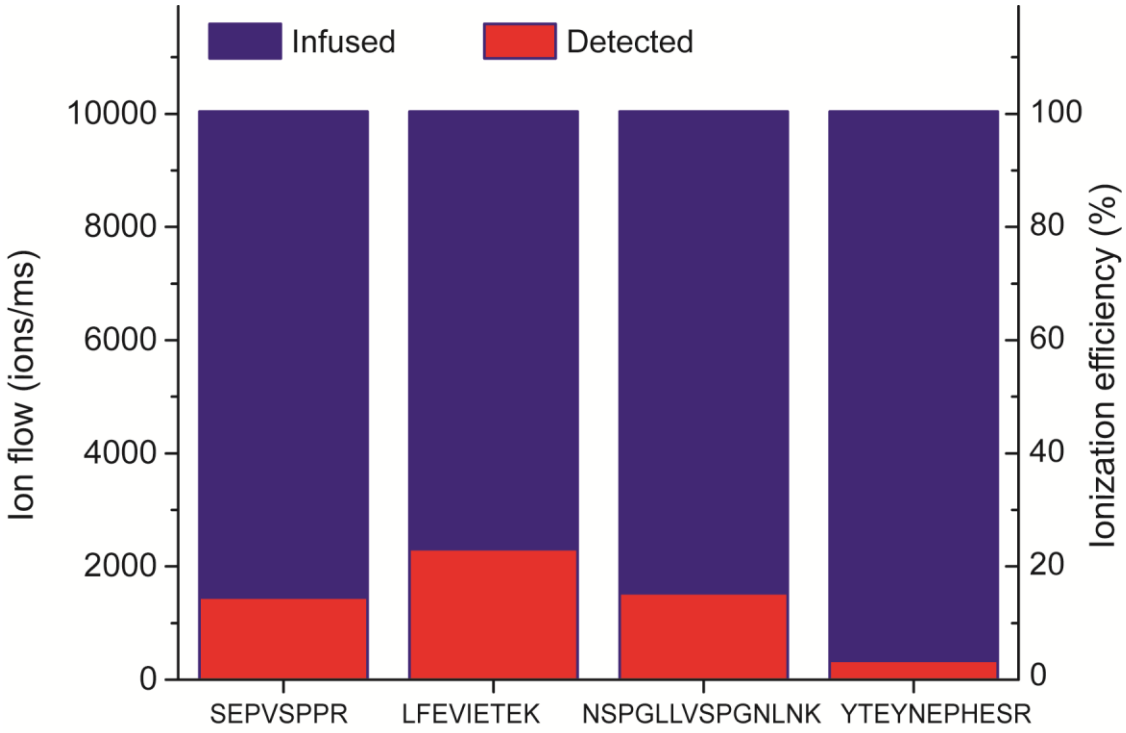
Supplementary Figure 1.

A

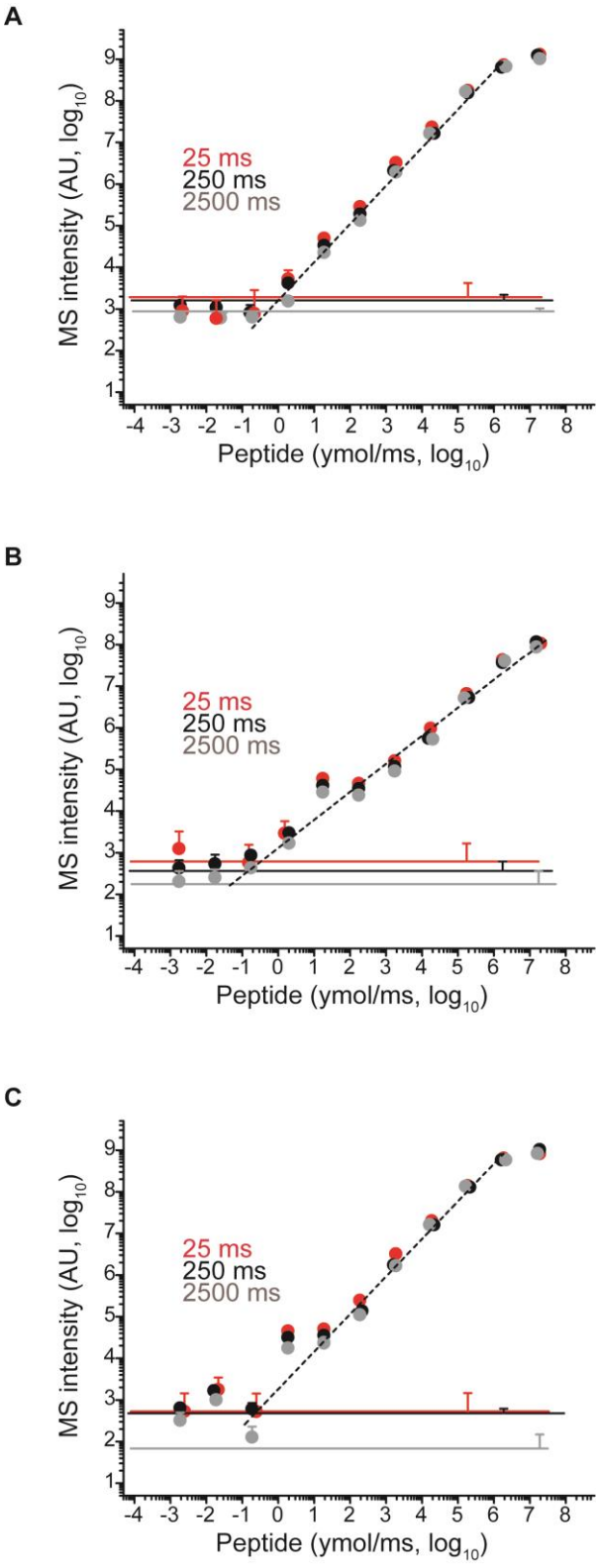


B

Supplementary Figure 2.

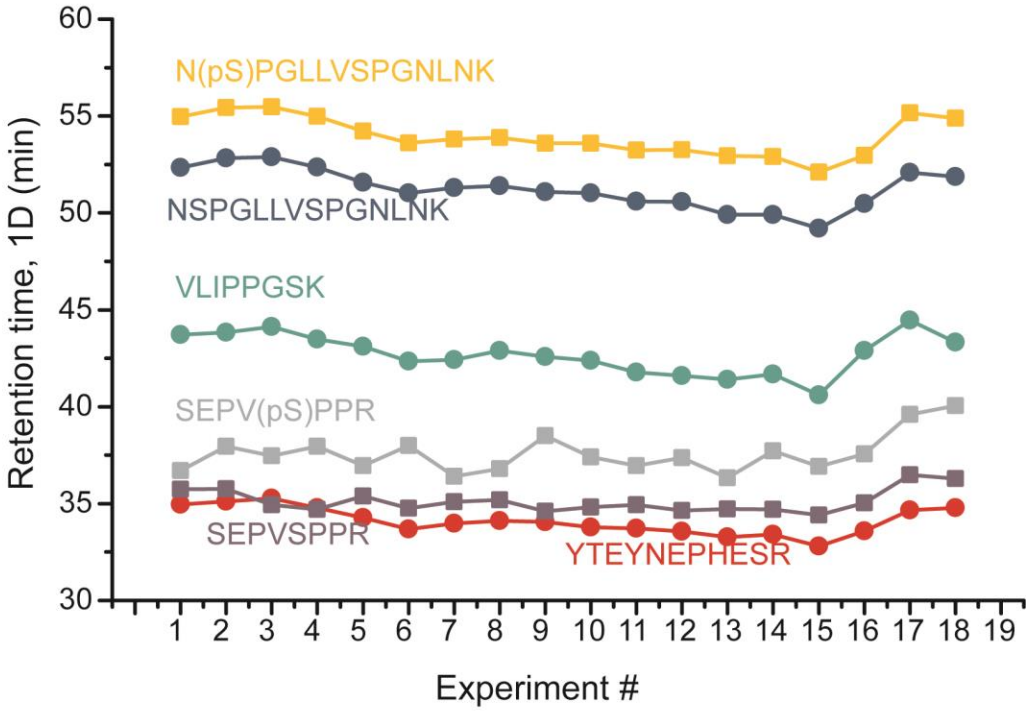


Supplementary Figure 3.

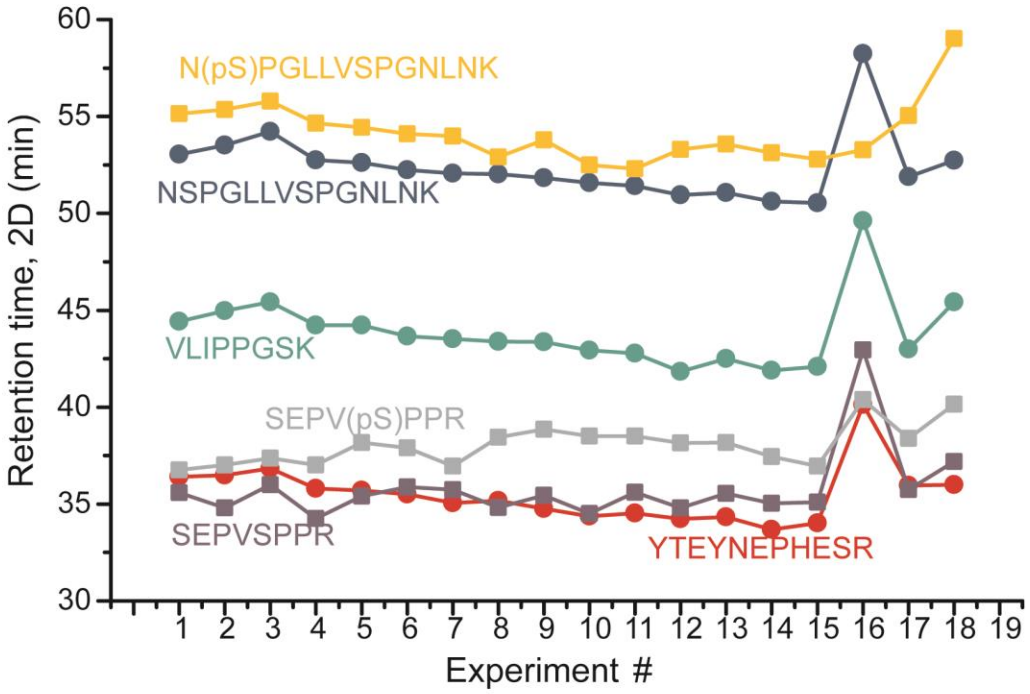


Supplementary Figure 4.

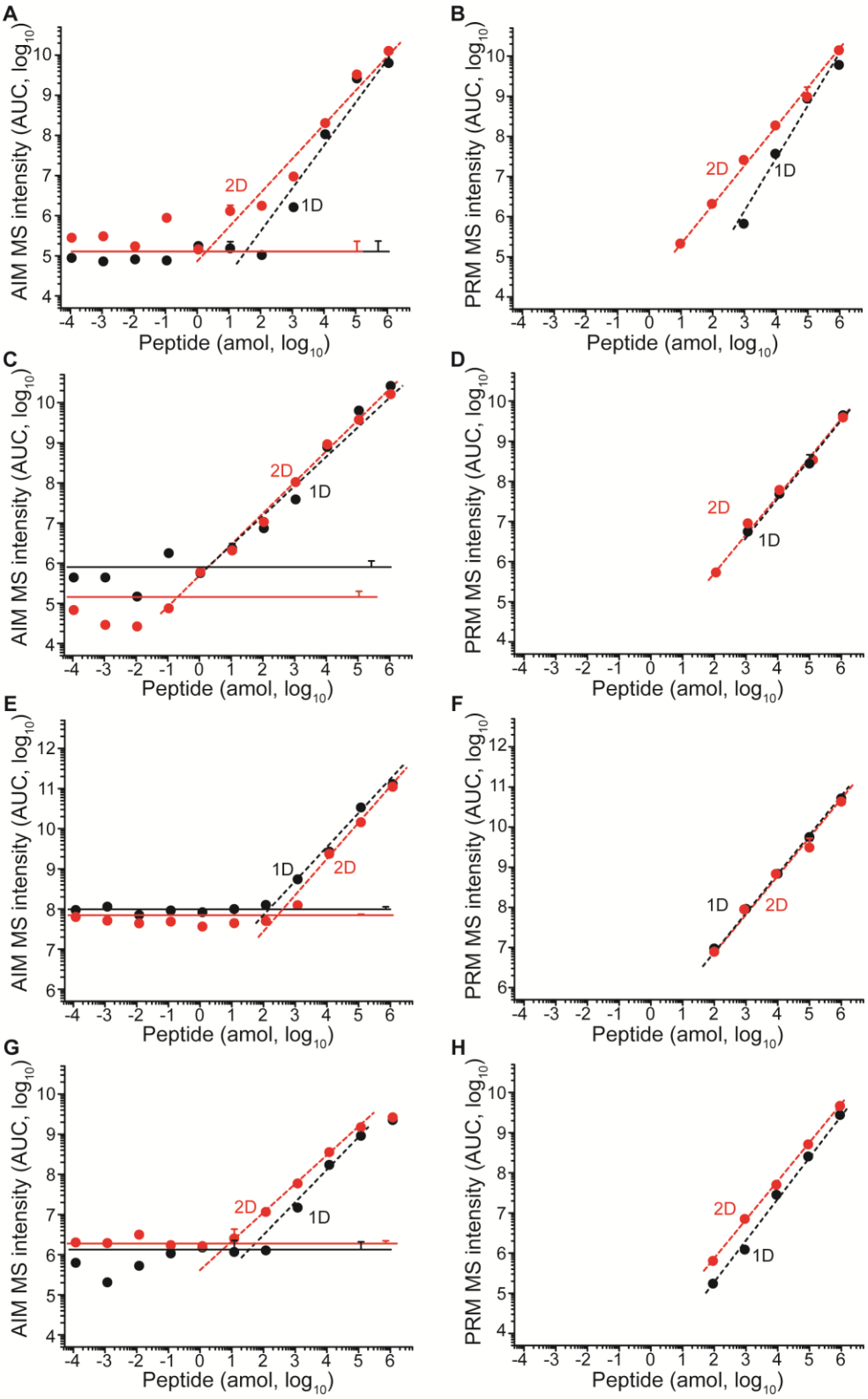
A



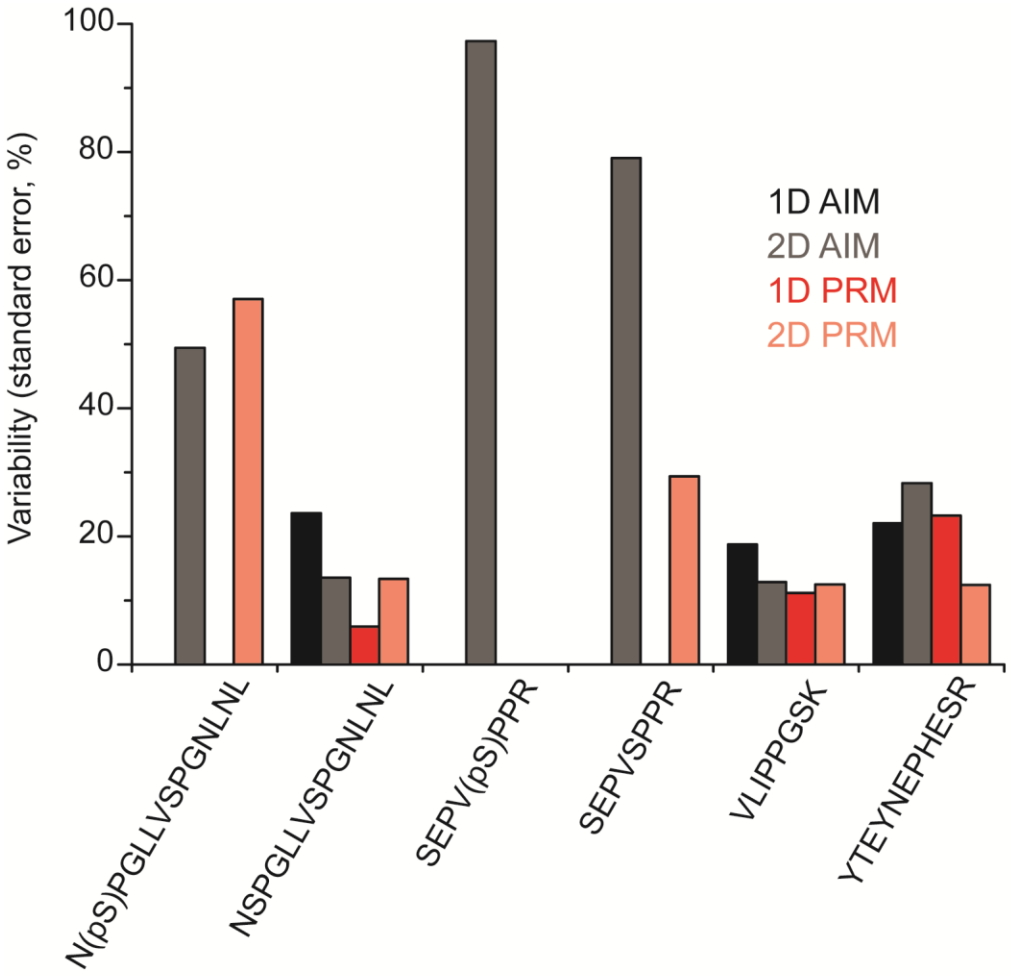
B



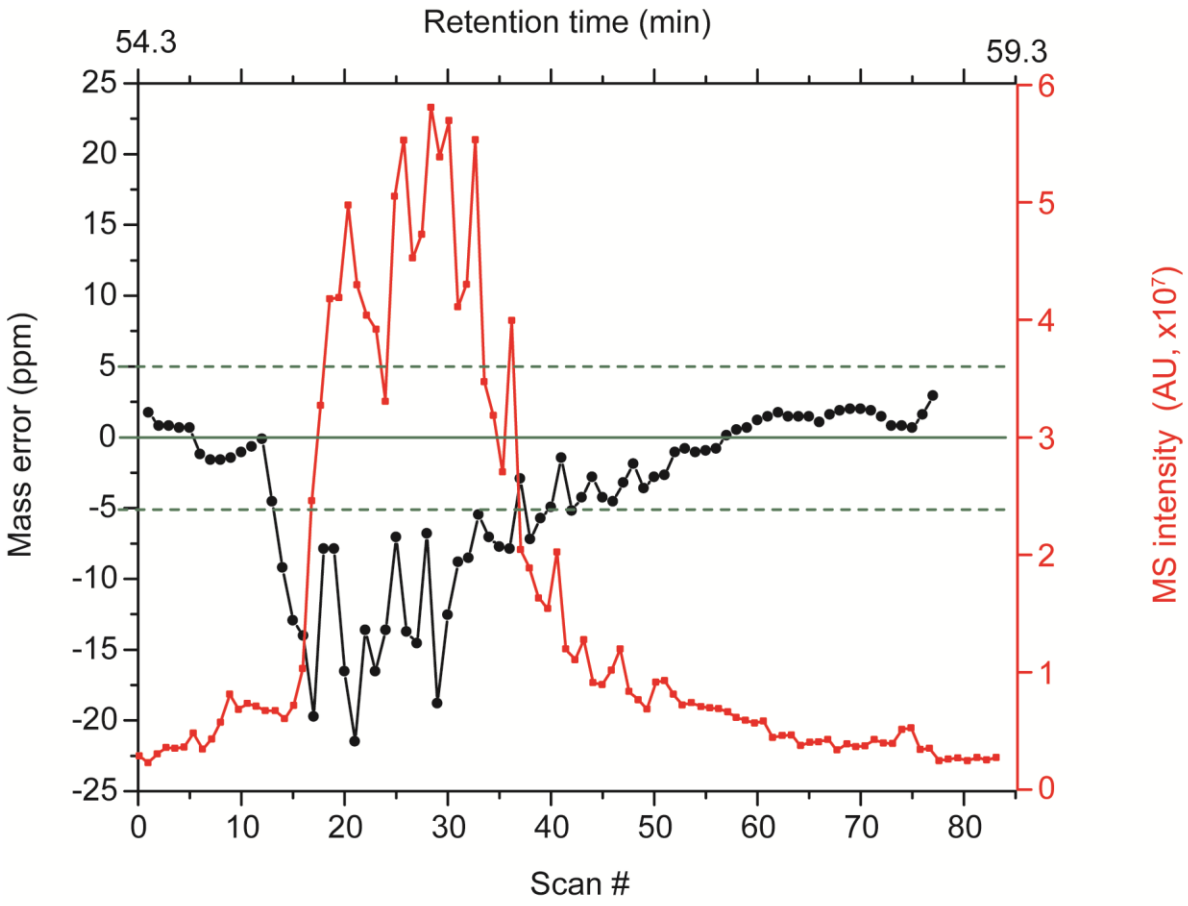
Supplementary Figure 5.



Supplementary Figure 6.



Supplementary Figure 7.



SUPPLEMENTARY MATERIALS

(provided as separate files)

Supplementary Table 1. Optical absorbance determination of individual peptide concentration in stock solutions.

Supplementary Table 2. MS signal recorded for each target under direct infusion in neat solvent

Supplementary Table 3. AIM signal recorded for each target under 1D and 2D.

Supplementary Table 4. PRM signal recorded for each target under 1D and 2D.

Supplementary Methods. Detailed description and operational parameters of the automated 2D chromatography system.