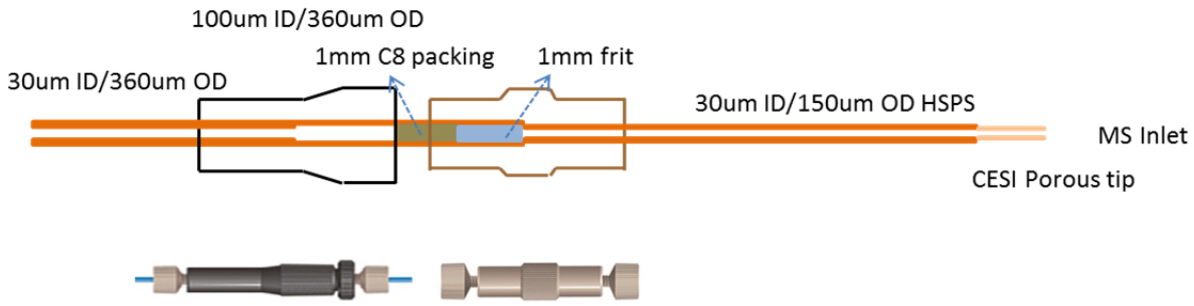
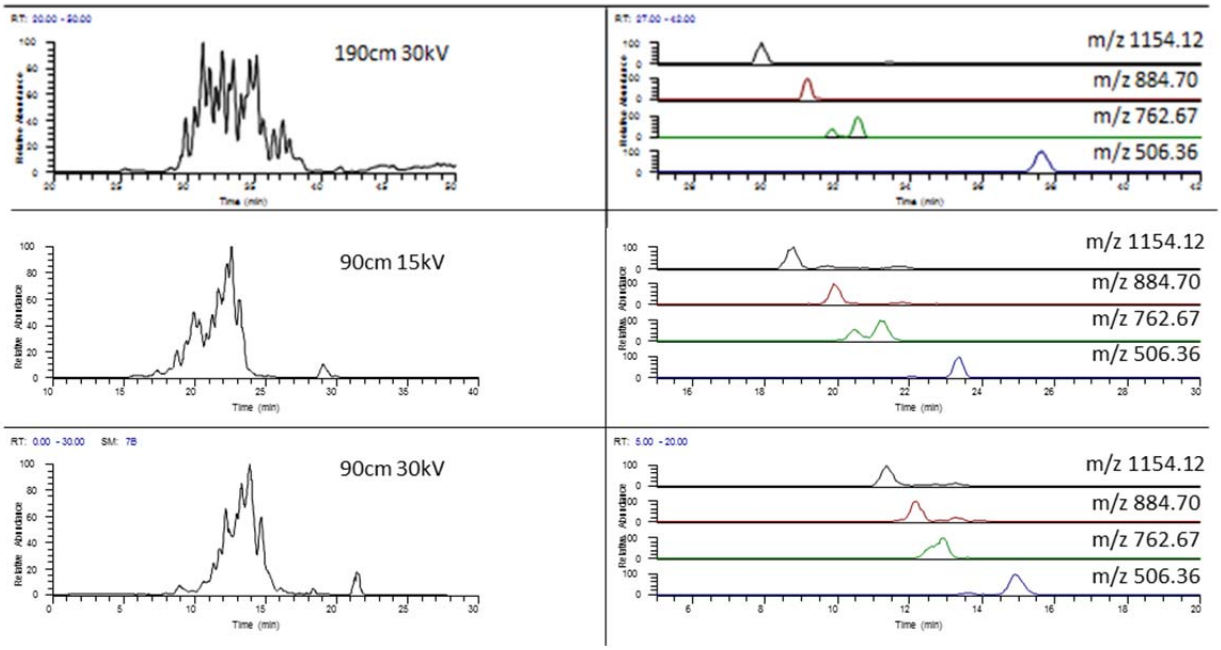
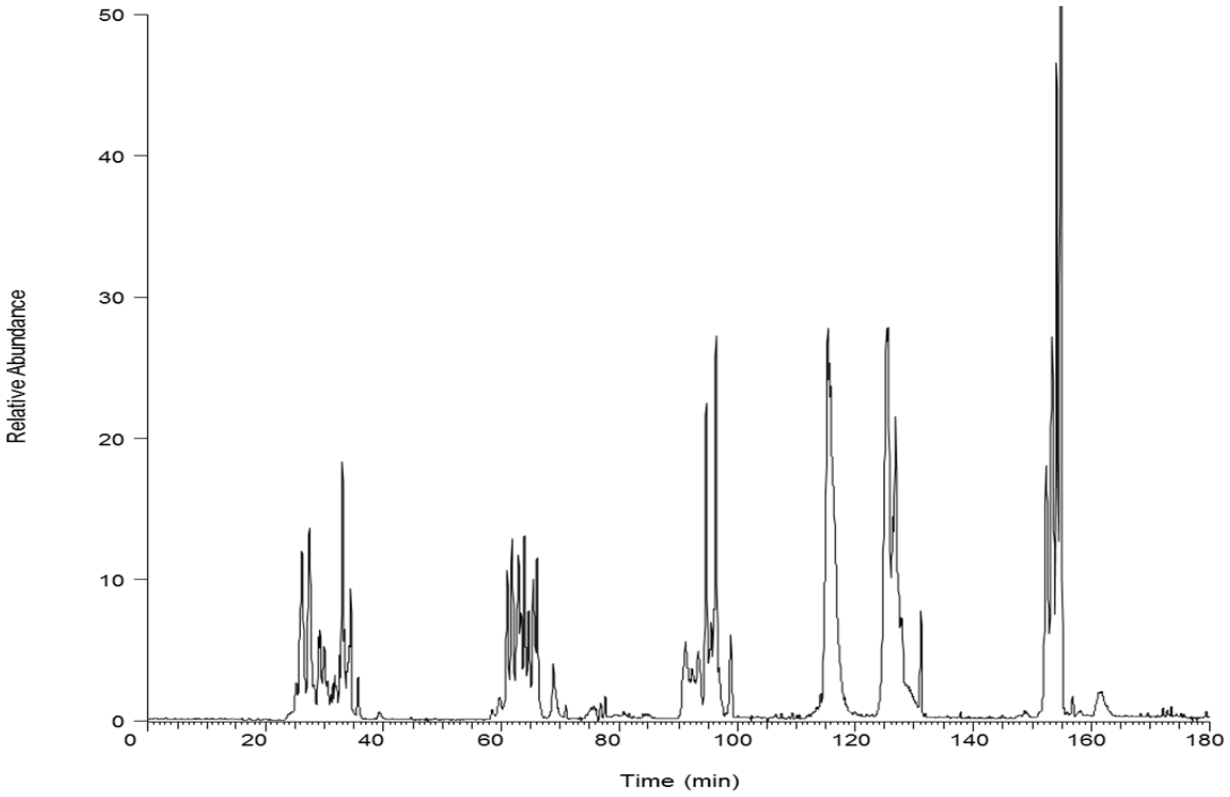
Supporting Information for *Improving the comprehensiveness and sensitivity of sheathless tITP-CE-MS/MS for proteomic analysis with solid-phase microextraction multistep elution*



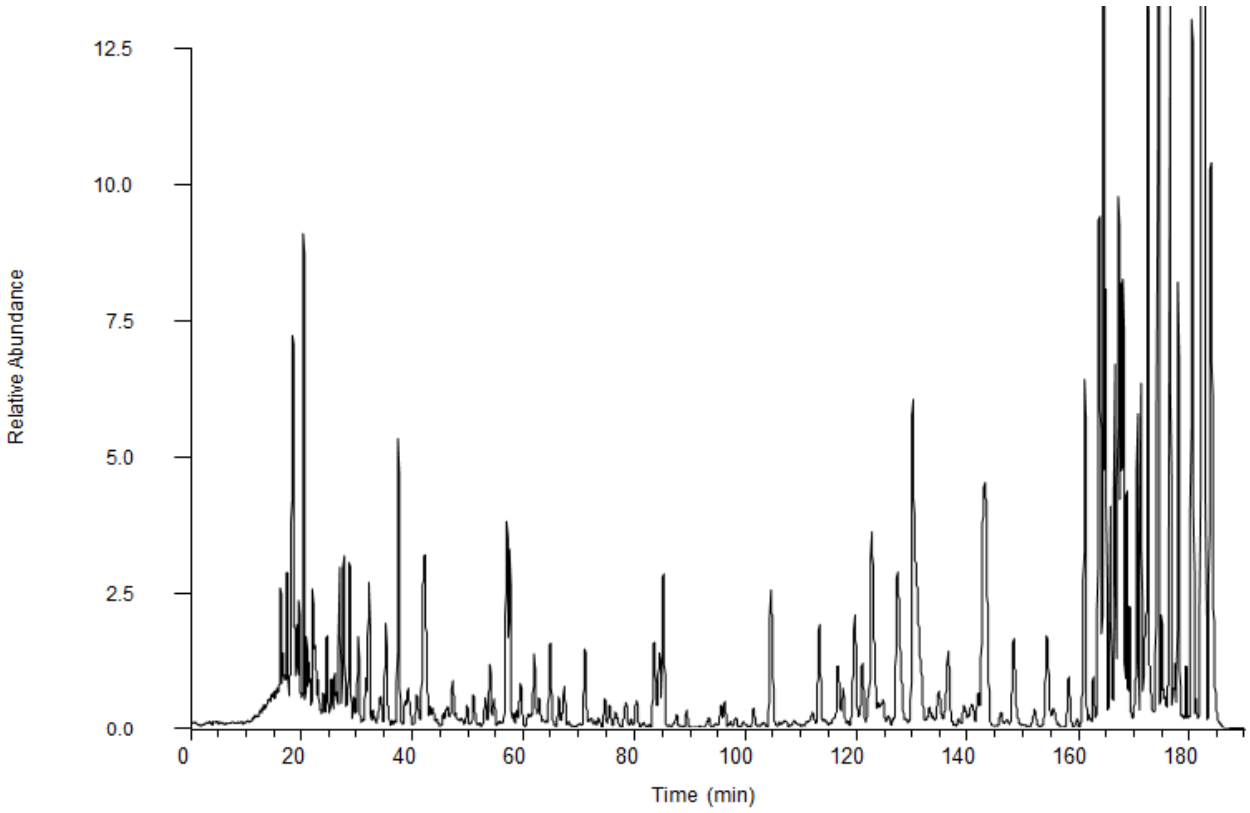
Supporting Figure 1: Schematic of the SPME-CE-MS/MS platform. The SPME column was sandwiched between two pieces of 30 μm ID capillaries, the frit end was connected to the HSPS through a zero dead volume union and the open end was connected to the inlet capillary with an Upchurch inline microfilter.



Supporting Figure 2: Effect of electric field on SPME-CE-MS/MS separation. The electropherograms of one step elution and CE separation of 16 protein mixture tryptic digests using 190 cm and 30 kV (158 V/cm), 90 cm and 15 kV (167 V/cm), and 90 cm and 30 kV (333 V/cm). The extracted ion electropherograms of 3 representative ions are shown from all three runs. Run conditions: 95 mM acetic acid (HOAc) - 5% methanol (MeOH) pH 3.1 was used as the background electrolyte. Peptides were eluted with 100 nL 95% MeOH - 50 mM NH_4Ac, pH 4.5. 15 or 30 kV was applied at the inlet of both 90 cm and 190 cm capillaries. MS/MS acquisition began upon application of the separation voltage.

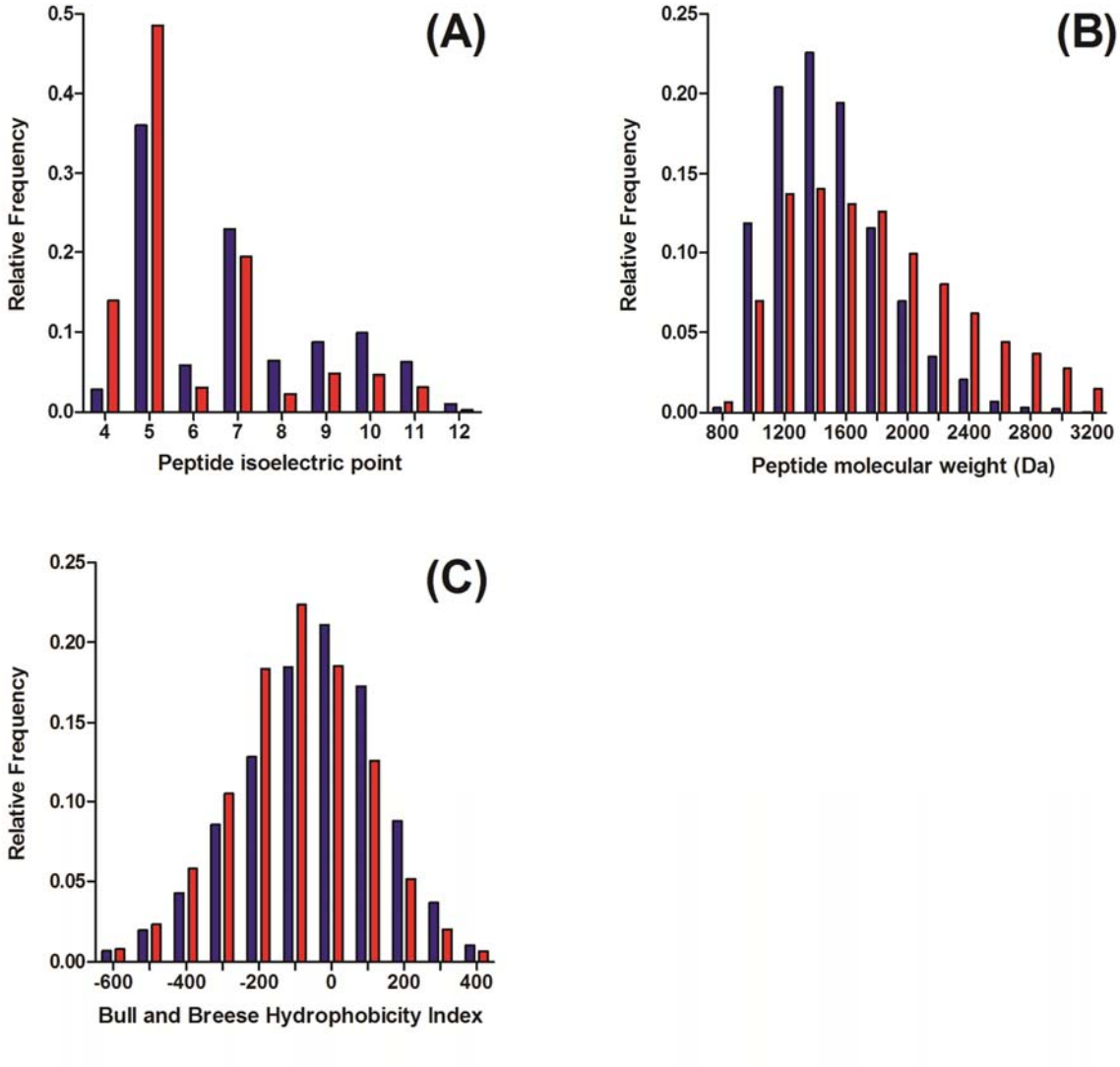


Supporting Figure 3: Base peak electropherogram for 5-step SPME multistep elution tITP-CE-MS/MS of 100 ng *Pfu* digest. The *Pfu* digest was first hydrodynamically loaded onto the SPME column followed by the electrokinetic elution by 5 increasing organic strength buffers (10%-10%, 20%-20%, 30%-30%, 40%-40% and 48%-48% MeOH-IPA-50mM NH4Ac, pH 4.5) at 15kV for 2.5min, followed by 35min CE separation at 30kV. The multistep electrokinetic elution and CE separation was repeated until the last organic buffer was injected and separation finished.

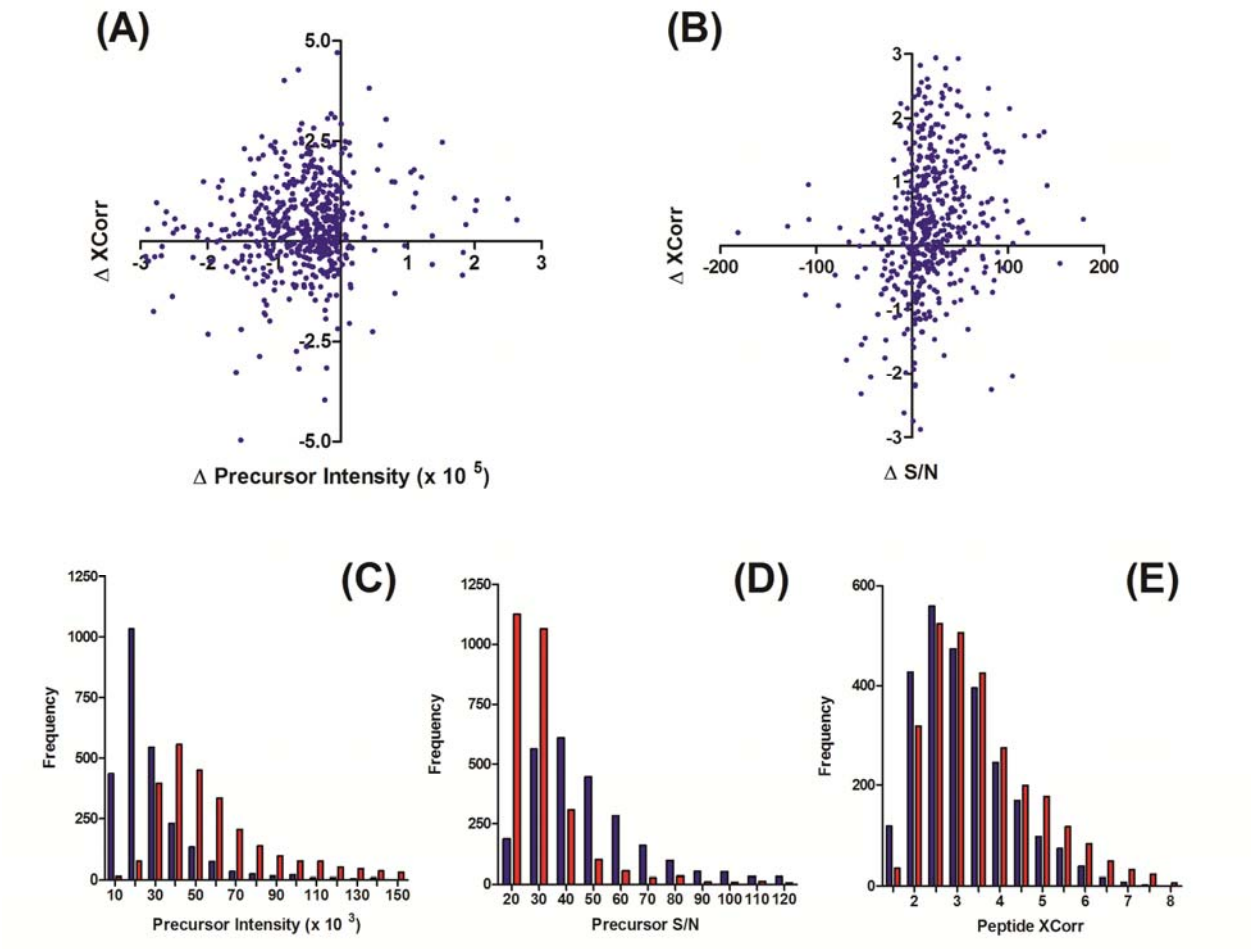


Supporting Figure 4: Base peak chromatogram for nLC-MS/MS of 100 ng *Pfu* digest.

Supporting Figure 5: Venn diagrams of identified (A) peptides and (B) proteins combined from duplicate analysis of 5, 25, 50, and 100 ng *Pfu* digests using SPME-tITP-CE (blue) and nLC (red). Identified peptides and proteins for these Venn diagrams are listed in the Supporting Information Excel file.



Supporting Figure 6: Histograms of (A) isoelectric point, (B) molecular weight, and (C) Bull and Breese hydrophobicity index of peptides identified from 5, 25, 50 and 100 ng duplicate runs of *Pfu* using both SPME-tITP-CE(blue) and nLC(red).



Supporting Figure 7: Comparison of peptide precursor intensity, signal-to-noise ratio (S/N), and XCorr from duplicate analysis of 100 ng of *Pfu* tryptic digests using 5-step SPME-tITP-CE-MS/MS and nLC-MS/MS. Correlation plots of changes (CE values – nLC values) in (A) precursor intensity and (B) S/N to changes in XCorr values of peptides identified in both CE and nLC experiments. Distributions of peptide (C) precursor intensities, (D) S/N ratios and (E) XCorr values were plotted for CE (blue) and nLC (red) experiments. Noise was calculated by averaging the peak intensities from the lowest 10% of peaks from the corresponding precursor MS scan. S/N ratios were calculated by dividing peptide precursor intensities by the noise within each scan.

Supporting Table 1: Description of MS RAW files.

We have provided all analyzed RAW data files for this work at: http://fields.scripps.edu/published/SPME-CE2012/

The file structure is the same as figure structures where .RAW files are placed in the appropriately named folder of the figure annotation (i.e. data for Figures 1A & B is in folder structure Figure1/AB/). For some consecutive CE runs we continuously acquired MS data files and later parsed them into their corresponding runs at the .MS2 file level. The following table describes the time windows in the .RAW files for which their data was extracted.

Figure descriptions	Website link structures	.RAW file names	Time windows analyzed
Figure 1A and 1B: 90 cm capillary	Figure1 AB	110520_CEMS_16PM_95MeOH_50NH4Ac_30kV_90cm.raw	17 – 37 min
Figure 1C and 1D: 190 cm capillary	Figure 1 CD	110520_CEMS_16PM_95MeOH_50NH4Ac_30kV_190cm.raw	33 – 83 min
Figure 2: Direct Injection Replicates	Figure 2 DCE Replicate1 Replicate2	 120802_CEMS_16PM_DCE_1.raw 120802_CEMS_16PM_DCE_2.raw	Entire run/file
Figure 2: 95% MeOH Replicates	Figure 2 95%MeOH Replicate1 Replicate2 Replicate3	110606_CEMS_16PM_95MeOH_50NH4Ac_30kV_190cm.raw 110705_CEMS_16PM_95MeOH_50NH4Ac_30kV_190cm.raw 110706_CEMS_16PM_95MeOH_50NH4Ac_30kV_190cm.raw	Entire run/file
Figure 2: 85% MeOH – 10% IPA Replicates	Figure 2 85%MeOH-10%IPA Replicate1 Replicate2 Replicate3	110713_CEMS_16PM_85MeOH_10IPA_50NH4Ac_30kV_190cm.raw 110715_CEMS_16PM_85MeOH_10IPA_50NH4Ac_30kV_190cm.raw 110721_CEMS_16PM_85MeOH_10IPA_50NH4Ac_30kV_190cm.raw	Entire run/file

Figure 2: 48% MeOH – 48% IPA Replicate 1 & 2	Figure2 48%MeOH-48%IPA Replicate1 Replicate2	110727_CEMS_16PM_48MeOH_48IPA_50NH4Ac_30kV_190cm.raw 110801_CEMS_16PM_48MeOH_48IPA_50NH4Ac_30kV_190cm.raw	Entire run/file
Figure 2: 48% MeOH – 48% IPA Replicate 3	Figure2 48%MeOH-48%IPA Replicate3	110803_CEMS_16PM_48MeOH_48IPA_50NH4Ac_30kV_190cm.raw	40 – 100 min
Figure 3: 10 kV elution; 50 mM BGE; 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 5 min elution times	Figure3 50mM_BGE_10kV	111017_CEMS_16PM_48MeOH_48IPA_95mMBGE_10kV_190cm.raw	1 min: 0 – 70 min 1.5: 105 – 175 2: 220 – 290 2.5: 340 – 410 3: 450 – 520 3.5: 560 – 630 4: 670 – 740 4.5: 780 – 850 5: 890 – 960
Figure 3: 15 kV elution; 50 mM BGE; 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 5 min elution times	Figure3 50mM_BGE_15kV	111017_CEMS_16PM_48MeOH_48IPA_95mMBGE_15kV_190cm.raw	1 min: 0 – 70 min 1.5: 105 – 175 2: 220 – 290 2.5: 320 – 390 3: 430 – 500 3.5: 540 – 610 4: 880 – 950 4.5: 770 – 840 5: 655 – 725
Figure 3: 10 kV elution; 95 mM BGE; 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 5 min elution times	Figure3 95mM_BGE_10kV	111017_CEMS_16PM_48MeOH_48IPA_50mMBGE_10kV_190cm.raw	0.5 min: 0 – 70 min 1: 110 – 180 1.5: 220 – 290 2: 330 – 400 2.5: 440 – 510 3: 550 – 620 3.5: 660 – 730 5: 770 – 840

Figure 3: 15 kV elution; 95 mM BGE; 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 5 min elution times	Figure3 95mM_BGE_15kV	111017_CEMS_16PM_48MeOH_48IPA_50mMBGE_15kV_190cm.raw	0.5 min: 40 – 110 min 1: 147 – 217 1.5: 260 – 330 2: 370 – 440 2.5: 480 – 550 3: 595 – 665 3.5: 710 – 780 5: 820 – 890
Figure 4: 15 kV elution for 2.5 min with;0, 25, 50, 75, and 100mM NH_4OAc	Figure4	111025_CEMS_16PM_48MeOH_48IPA_30kV_190cm_tITP.raw	0 mM: 520 – 580 min 25: 620 – 680 50: 720 – 780 75: 820 – 880 100: 920 – 980
Figure5: Replicate 1 of *Pfu* dilution series (5, 25, 50, 100 ng starting mass)	Figure5 CE Replicate1	111026_CE_Successive_5_25_50_100 ng_Pfu_180min.raw	5 ng: 0 – 190 min 25: 220 – 410 50: 435 – 625 100: 655 – 845
Figure5: Replicate 2 of *Pfu* dilution series (5, 25, 50, 100 ng starting mass)	Figure5 CE Replicate2	111027_CE_Successive_5_25_50_100 ng_Pfu_180min.raw	5 ng: 20 – 220 min 25: 240 – 440 50: 460 – 660 100: 680 – 880
Figure 5: Replicates 1 and 2 of *Pfu* dilution series (5, 25, 50, 100 ng starting mass) from nLC analysis	Figure5 nLC 5ng Replicate1 Replicate2 25ng Replicate1 Replicate2 50ng Replicate1 Replicate2 100ng	 111006_nLC_5ng_Pfu_180min.raw 120118_nLC_5ng_Pfu_180min.raw 120111_nLC_25ng_Pfu_180min.raw 120118_nLC_25ng_Pfu_180min.raw 120113_nLC_50ng_Pfu_180min.raw 120119_nLC_50ng_Pfu_180min.raw	Entire run/file

	Replicate1 Replicate2	120118_nLC_100ng_Pfu_180min.raw 120113_nLC_100ng_Pfu_180min.raw	
Figure 5: Replicate 1 and 2 of 5 ng *Pfu* with direct injection CE	Figure 5 DCE Replicate1 Replicate2	 120718_DCE_5ng_Pfu_1.raw 120718_DCE_5ng_Pfu_2.raw	Entire run/file
Supporting Figure 2:	SupportingFigure2 190cm30kV 90cm15kV 90cm30kV	 120628_CEMS_16PM_95MeOH_50NH4Ac_30kV_190cm.raw 120628_CEMS_16PM_95MeOH_50NH4Ac_15kV_90cm.raw 120628_CEMS_16PM_95MeOH_50NH4Ac_30kV_90cm.raw	Entire run/file