Supporting material

Bottom-up proteomics of *E. coli* using dynamic pH junction preconcentration and CZE-ESI-MS/MS

Guijie Zhu, Liangliang Sun, Xiaojing Yan, Norman J. Dovichi*

Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556, USA

*Corresponding author. Tel.: +1 574 631 2778. E-mail address: ndovichi@nd.edu

Contents:

S- Figure 1. Normalization level *vs.* injection volume for the dilute BSA sample from Figure 1. The straight line is a least-squares fit of a line to the data between 0 and 260 nL injection volume.

ATEEQLK, m/z 409.93, +2, pI 4.5
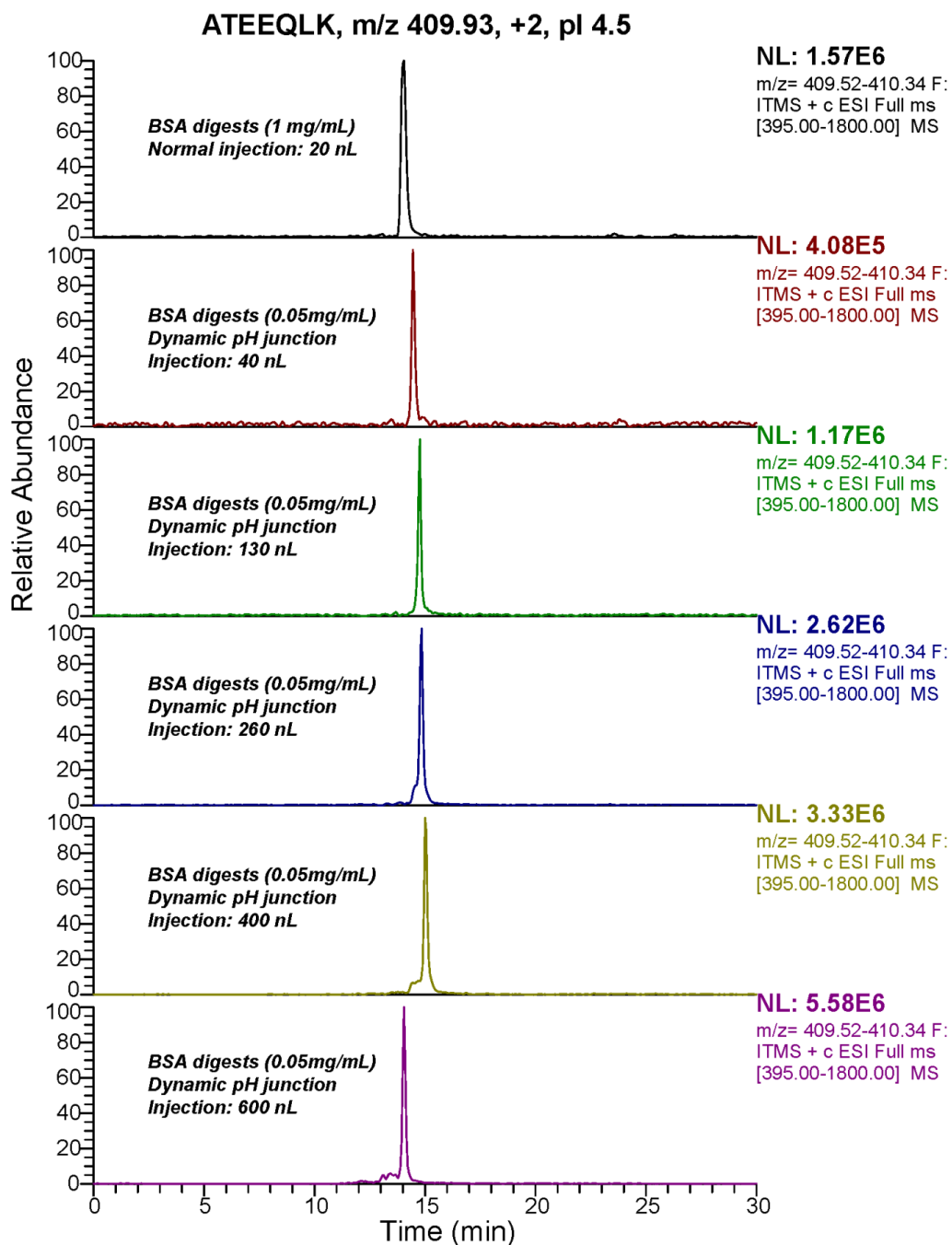
S-Figure 2 Extracted base peak electropherograms of a BSA peptide (ATEEQLK, m/z 409.93, +2, pI 4.5) from BSA digest results after analysis by CZE-MS/MS in different conditions.
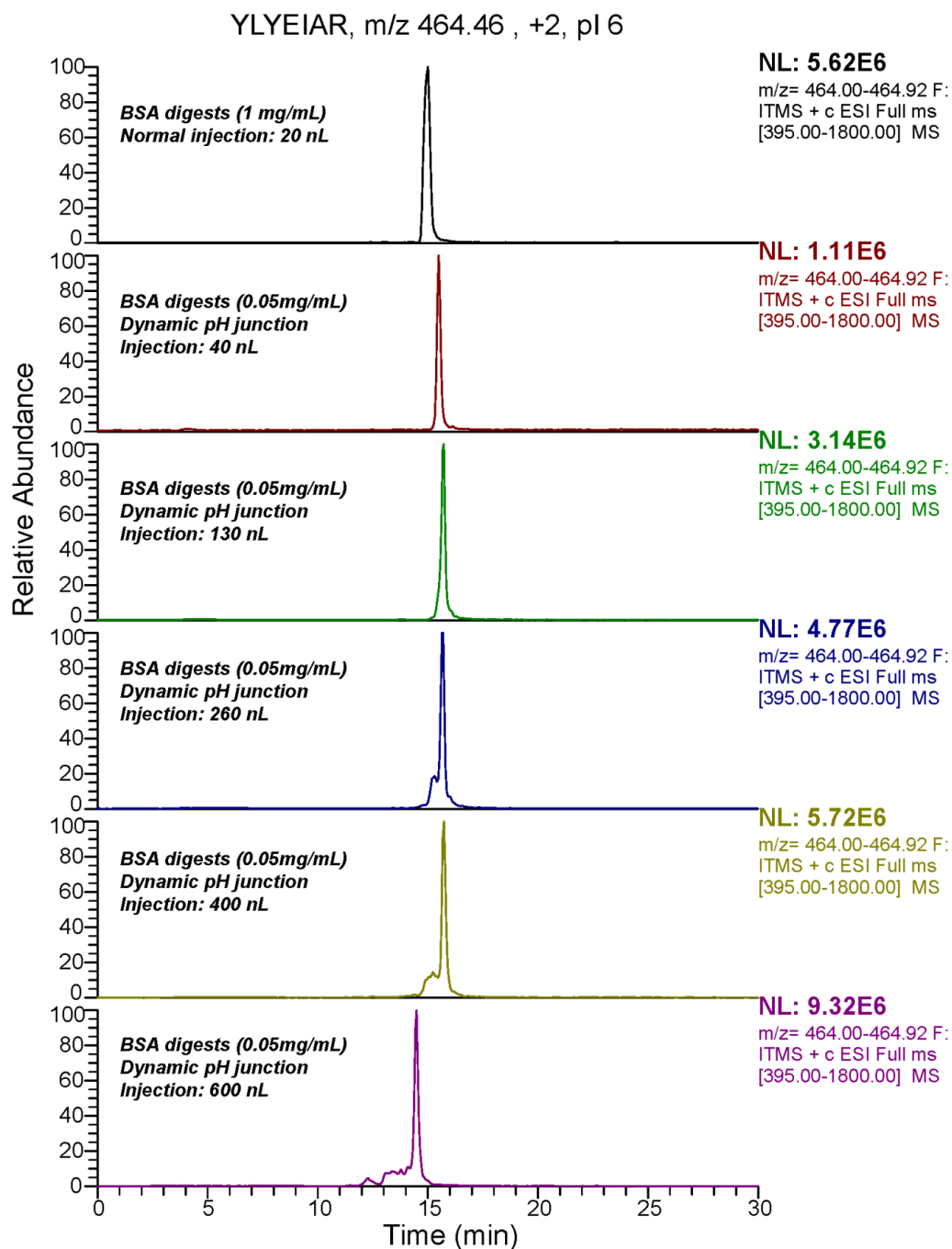
S-Figure 3 Extracted base peak electropherograms of a BSA peptide (YLYEIAR, m/z 464.46, +2, pI 6) from BSA digest results after analysis by CZE-MS/MS in different conditions.
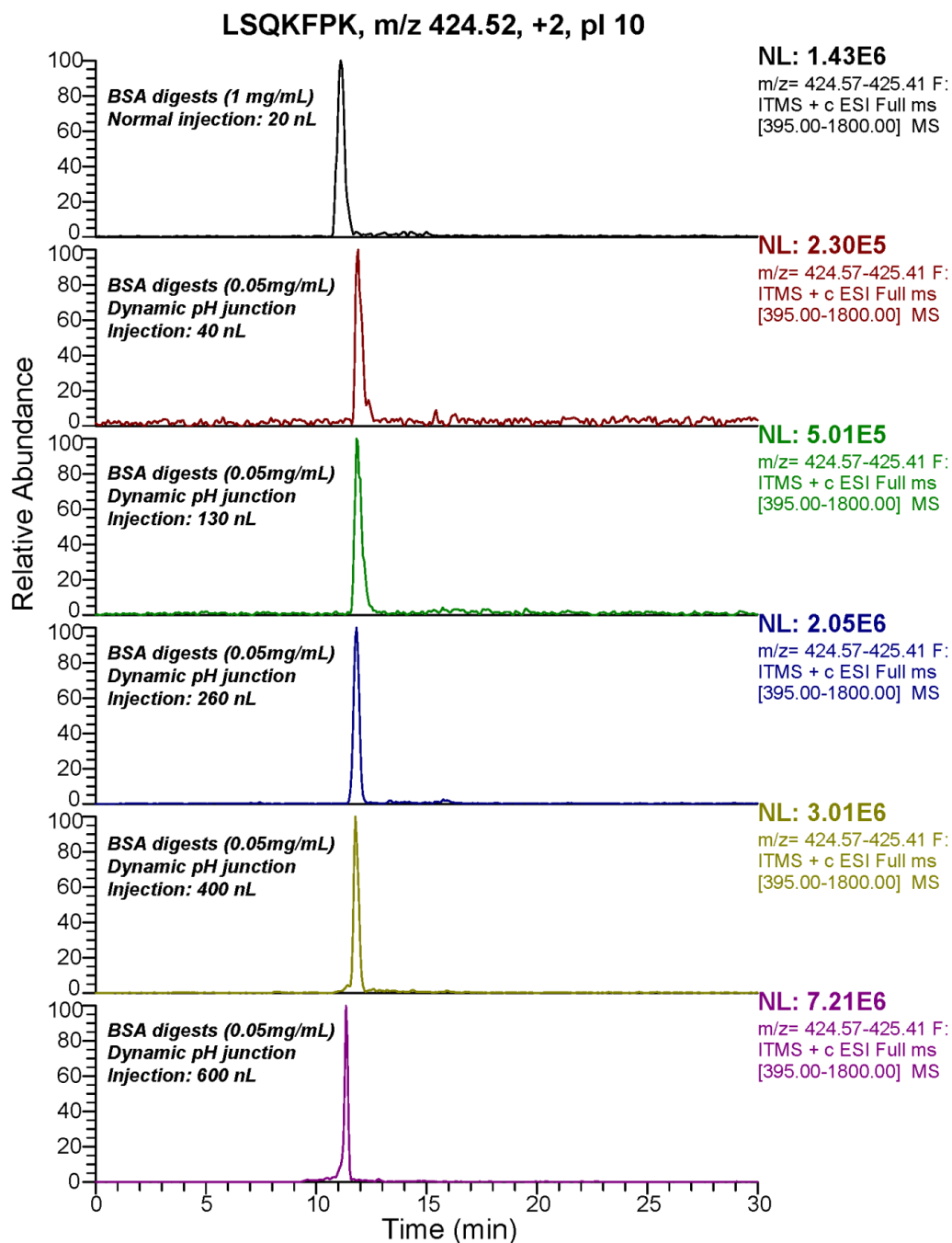
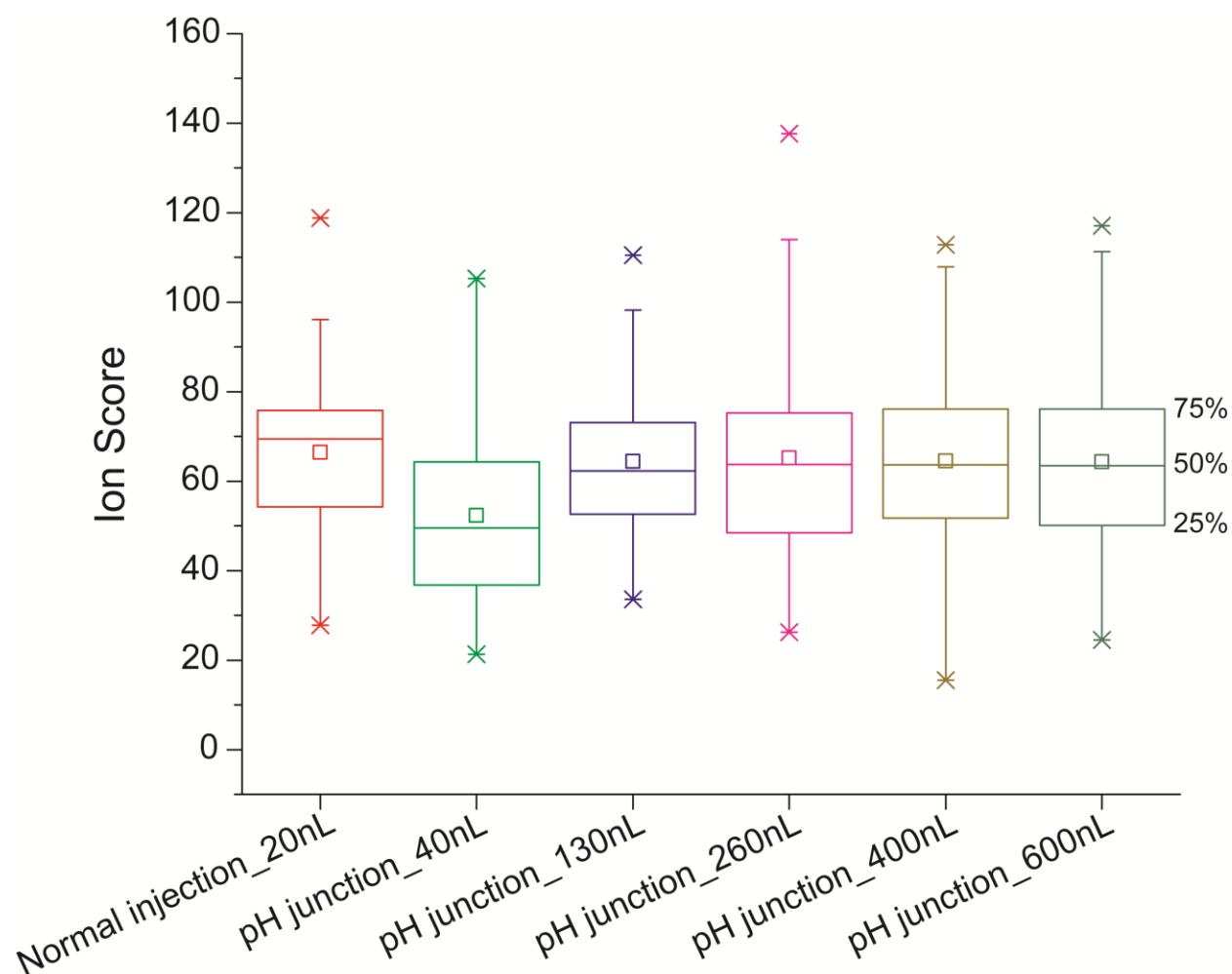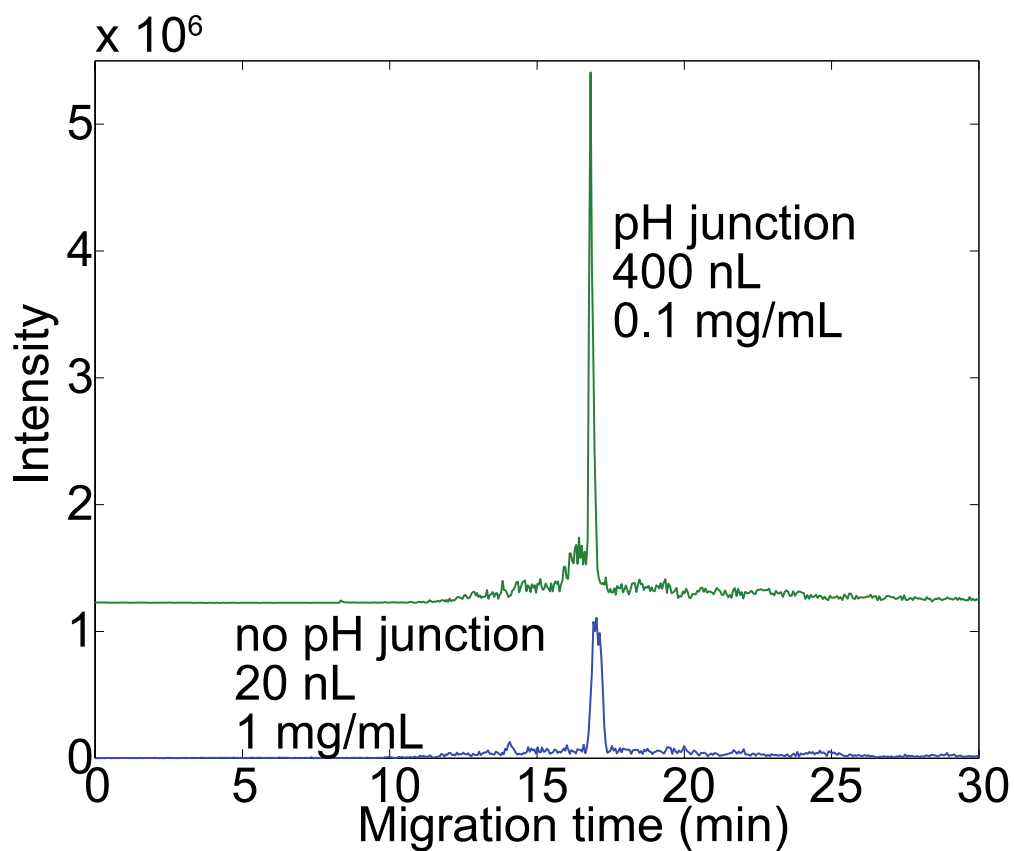**S-Figure 4** Extracted base peak electropherograms of a BSA peptide (LSQKFPK, m/z 424.52, +2, pI 10) from BSA digest results after analysis by CZE-MS/MS in different conditions.

S-Figure 5 Box charts of peptide ion scores from CZE-ESI-MS/MS analysis of BSA digests in different conditions. For normal injection, 1 mg/mL of BSA digest in 0.1%(v/v) FA was used as the sample; for dynamic pH junction experiments, 0.05 mg/mL BSA digests in 10 mM ammonium acetate (pH ~6.5) was used as the sample. The squares (□) in the box charts represent the mean values of the ion scores.

S-Figure 6 Selected ion electropherograms for the peptide ALQTAGKSDVMVVGFDGTPDGEKAVNDGK (+3) using conventional injection (blue) and a 400 nL pH junction injection (green). Curves are offset for clarity.
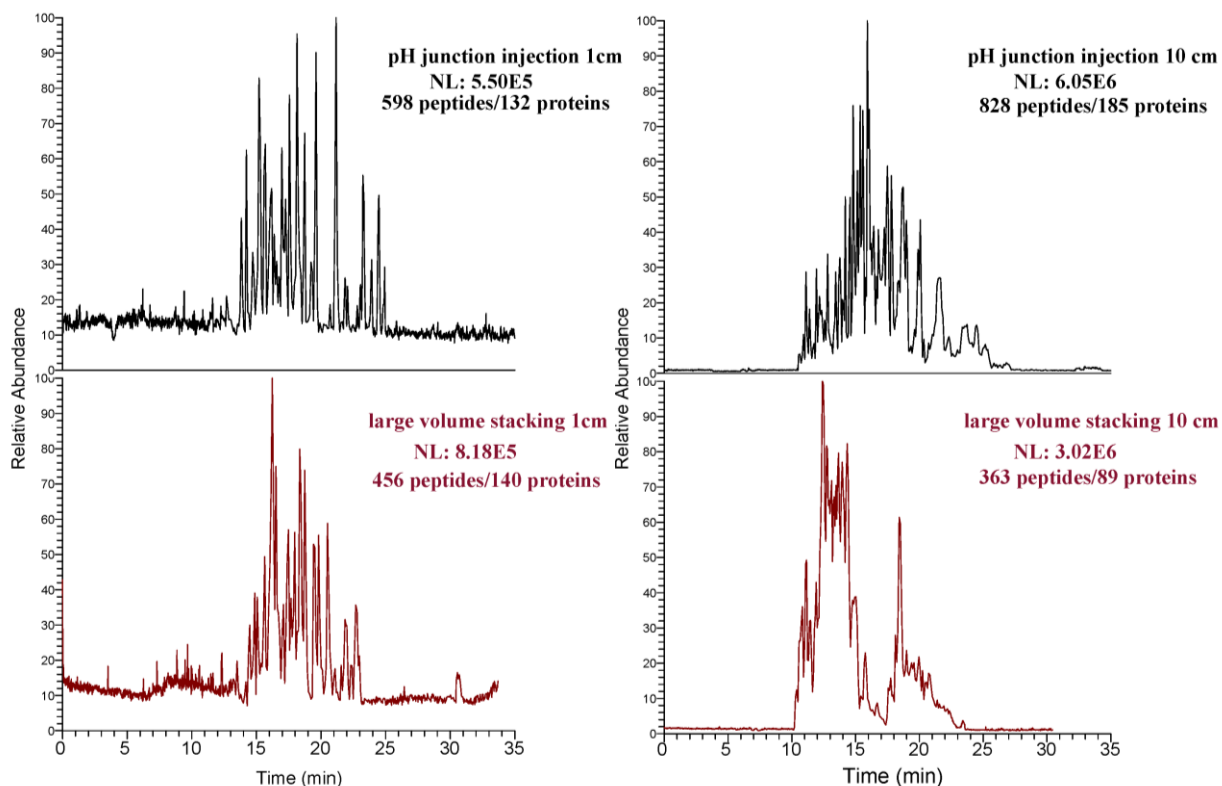
**S-Figure 7** Base peak electropherograms of *E. coli* digests (0.1 mg/mL) analyzed by dynamic pH junction and large volume stacking based CZE-ESI-MS/MS with 1 cm and 10 cm injection length. For dynamic pH junction injection, *E. coli* digests were dissolved in 10mM ammonium acetate (pH 6). For large volume stacking injection, the sample was dissolved in 0.05 formic acid with 50% ACN. Running buffer was 0.1% formic acid(pH 2.6).
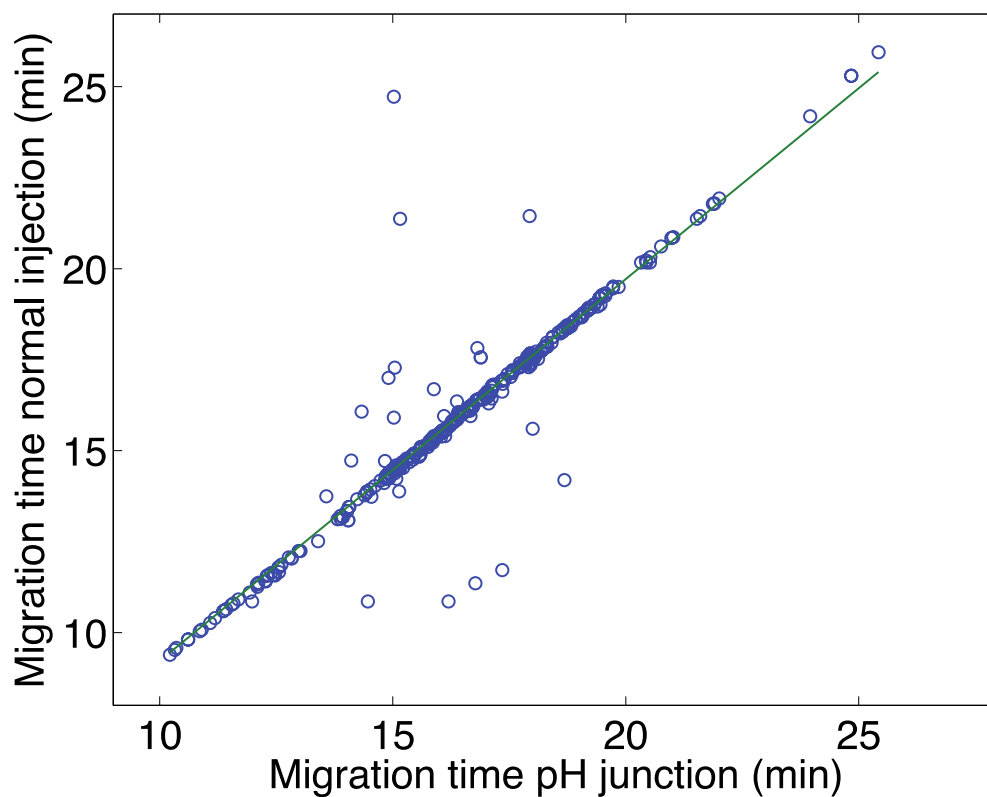
S-Figure 8 Plot of migration time for 1-cm (20 nL) pH junction and normal injection of a 0.1 mg/mL *E. coli* tryptic digest. Migration time was estimated from the results of an unsupervised nonlinear least-squares fit of a Gaussian function to the selected ion electropherogram. The result of an unweight fit of a straight line to the migration times is shown as the green solid line. Slope = 1.05 ± 0.02, r = 0.94, N = 307 points.
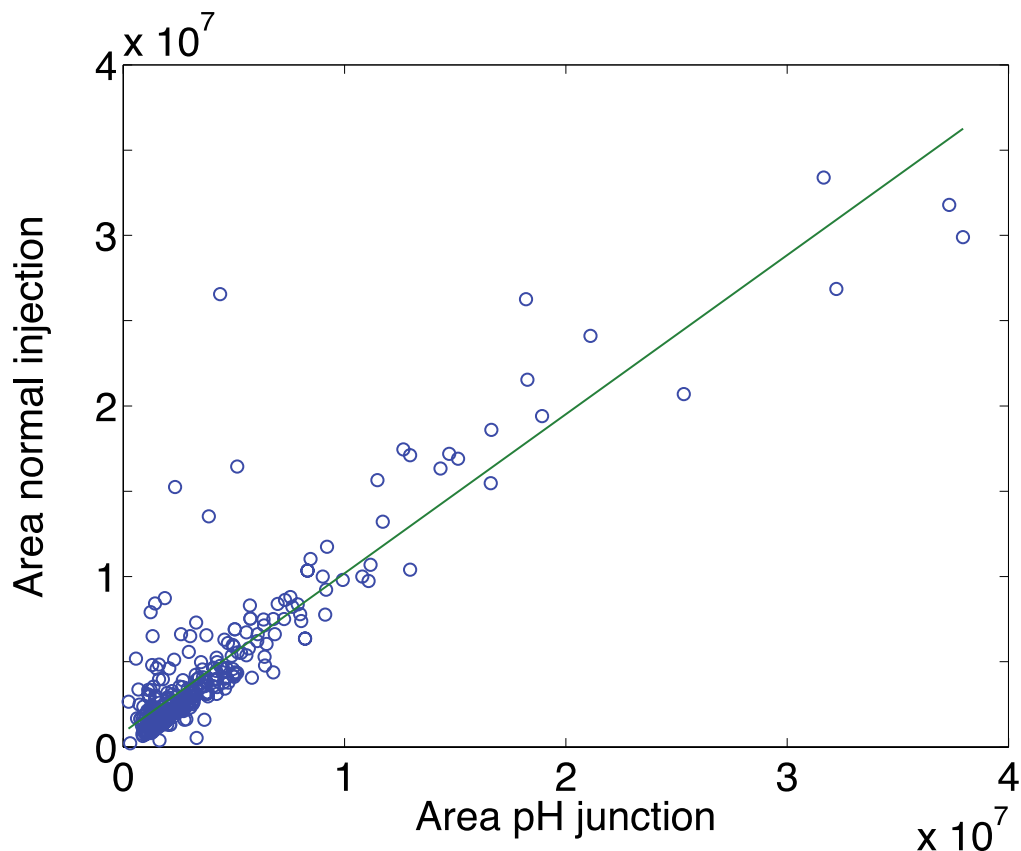
S-Figure 9 Plot of peak area for 1-cm (20 nL) pH junction and normal injection of a 0.1 mg/mL *E. coli* tryptic digest. Peak area was estimated as the product of peak amplitude and peak width as estimated from a nonlinear least-squares fit of a Gaussian function to each selected ion electropherogram. The result of an unweight fit of a straight line to the migration times is shown as the green solid line. Slope = 0.93 ± 0.03, r = 0.90, N = 307 points.