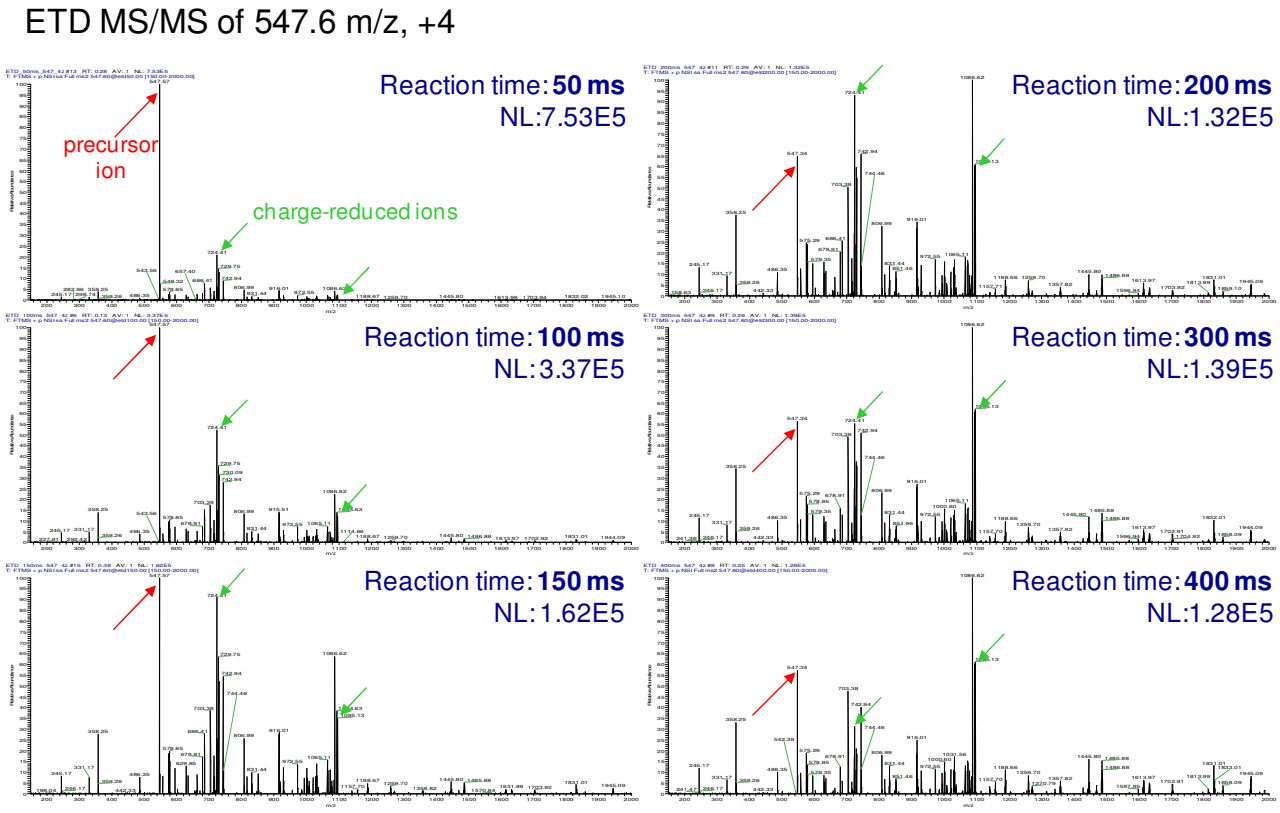
Supporting figures for "Effectiveness of CID, HCD, and ETD with FT MS/MS for degradomic-peptidomic analysis: evaluations from different peptide identification methods" by Yufeng Shen[1], Nikola Tolić[2], Fang Xie[1], Rui Zhao[2], Samuel O. Purvine[2], Athena A. Schepmoes[1], Ronald, J. Moore[1], Gordon A. Anderson[1], and Richard D. Smith[1,2]

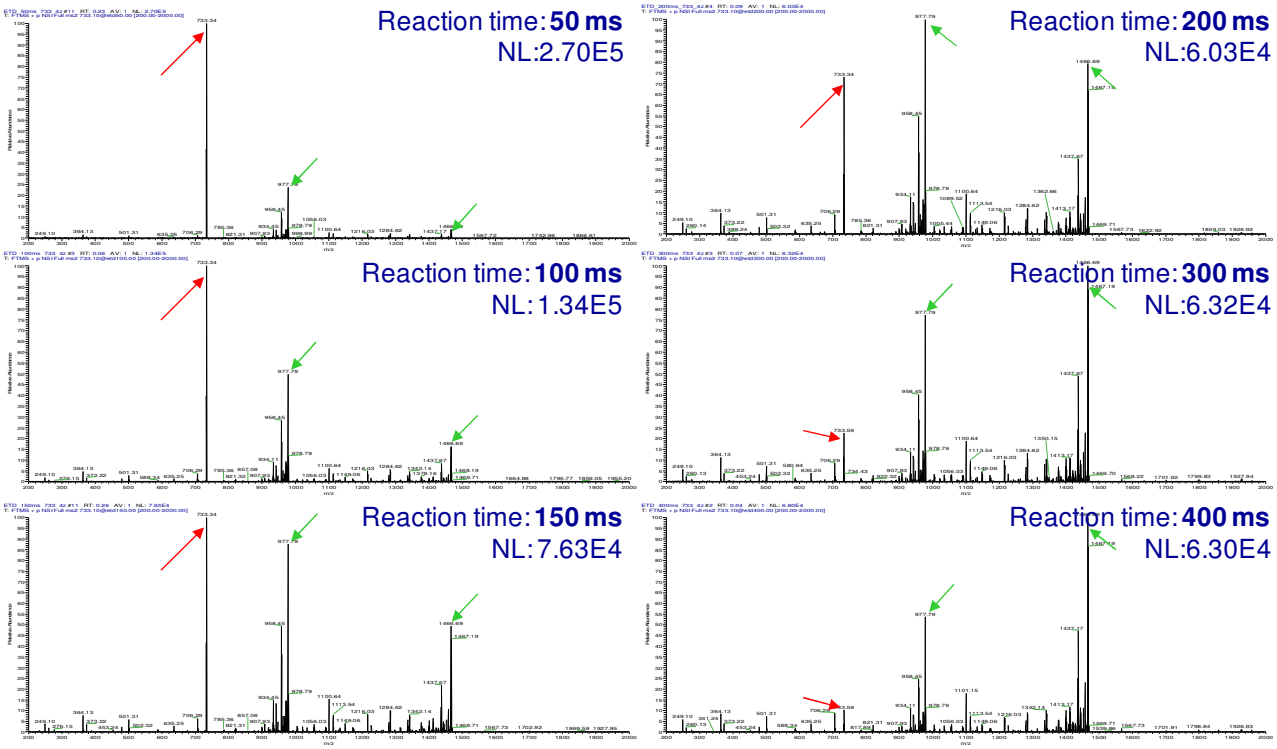
[1]Biological Sciences Division, and [2]Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA 99354

Supporting Figure 1. Optimization of ETD reaction time for fragmentation of CS 2 and 4 peptides.

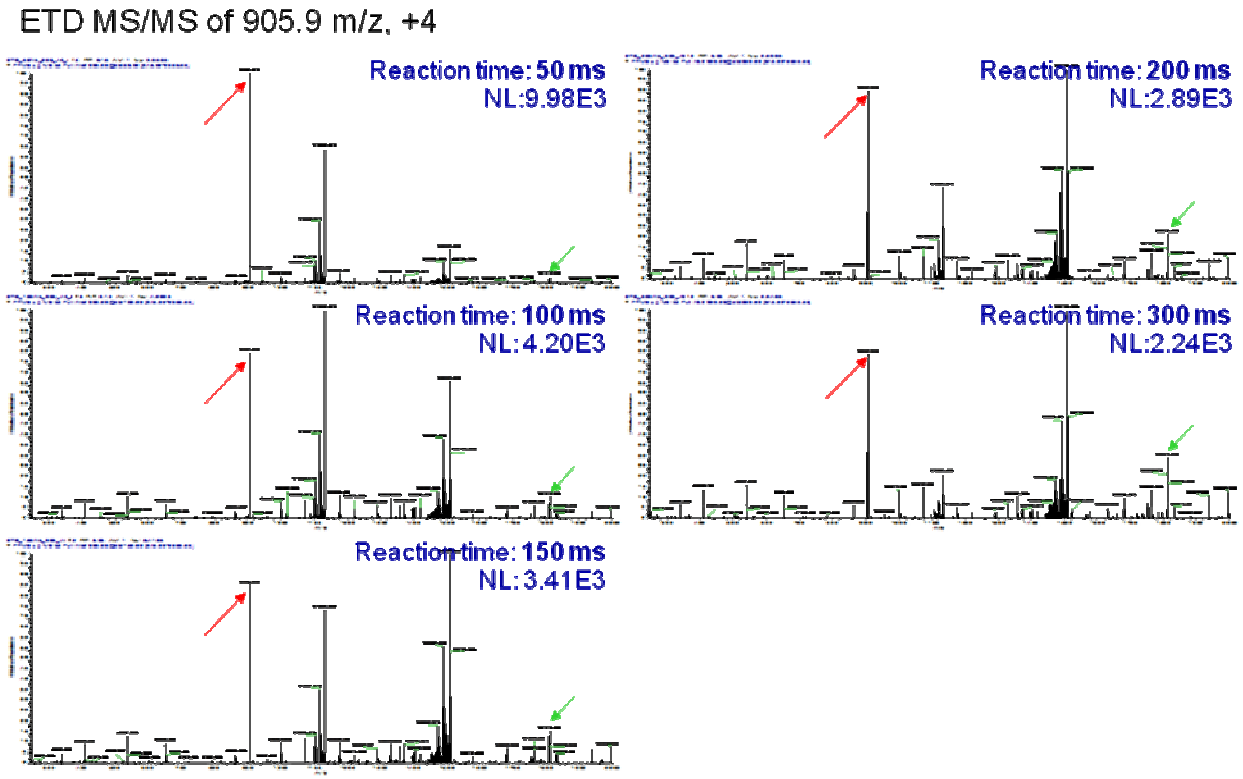


Continued.

ETD MS/MS of 733.1 m/z, +4

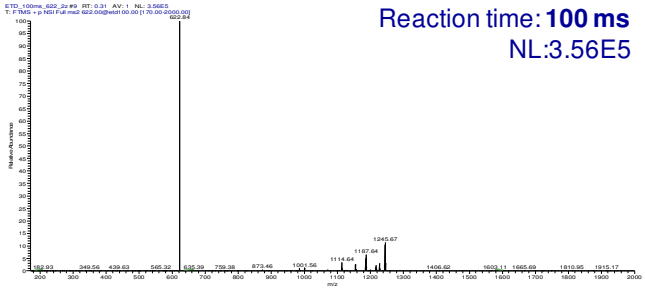


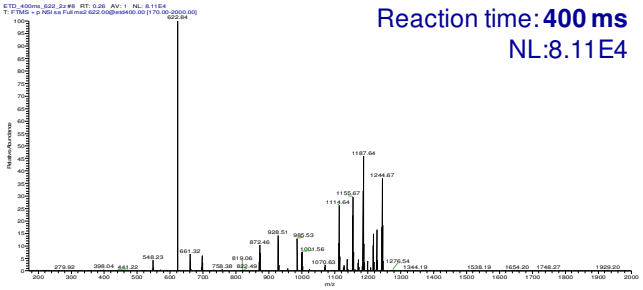
Continued.

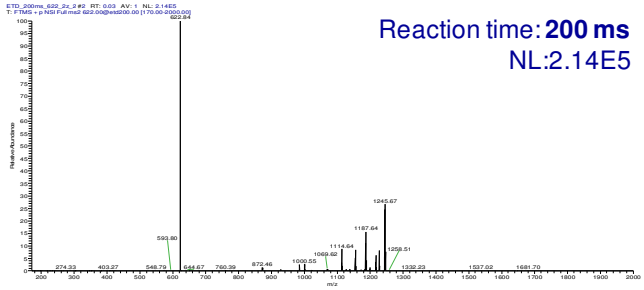

ETD MS/MS of 905.9 m/z, +4
Reaction time: 50 ms
NL:9.98E3
Reaction time: 200 ms
NL:2.89E3
Reaction time: 100 ms
NL:4.20E3
Reaction time: 300 ms
NL:2.24E3
Reaction time: 150 ms
NL:3.41E3

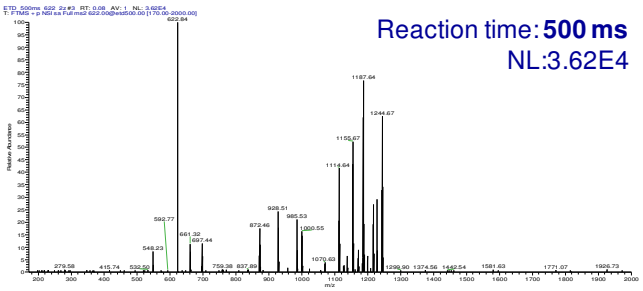
Continued.

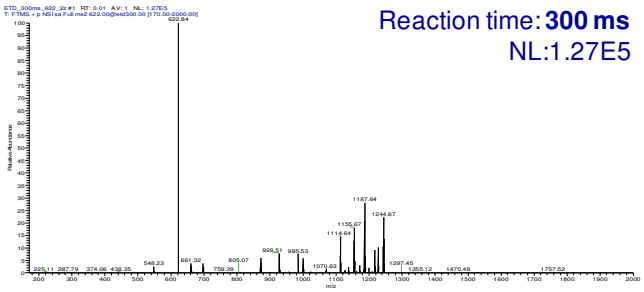
ETD MS/MS of 622.0 m/z, +2

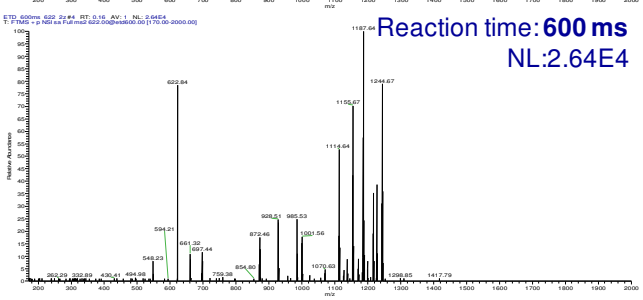






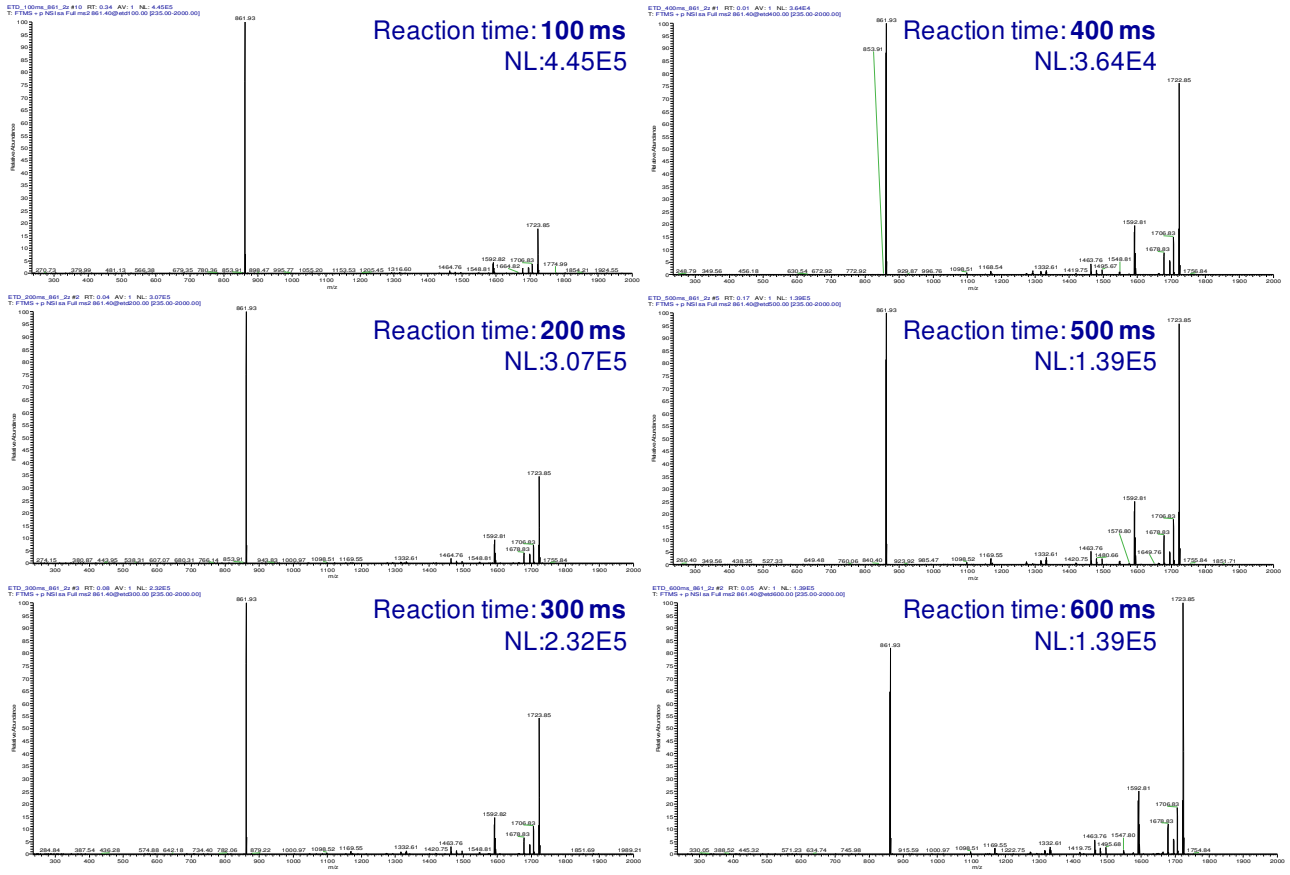






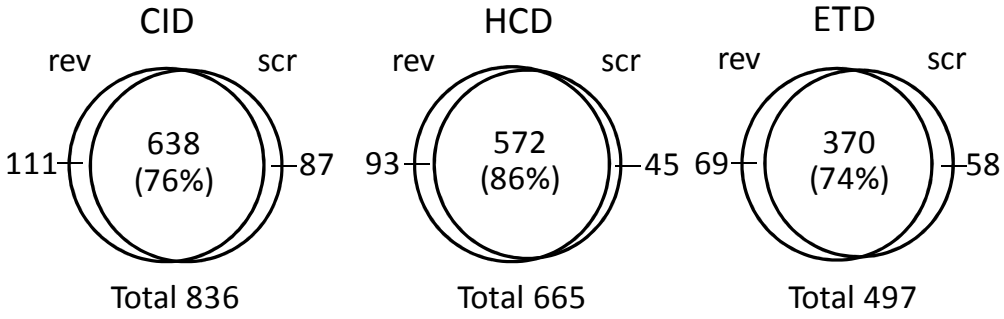
Continued.

ETD MS/MS of 861.4 m/z, +2



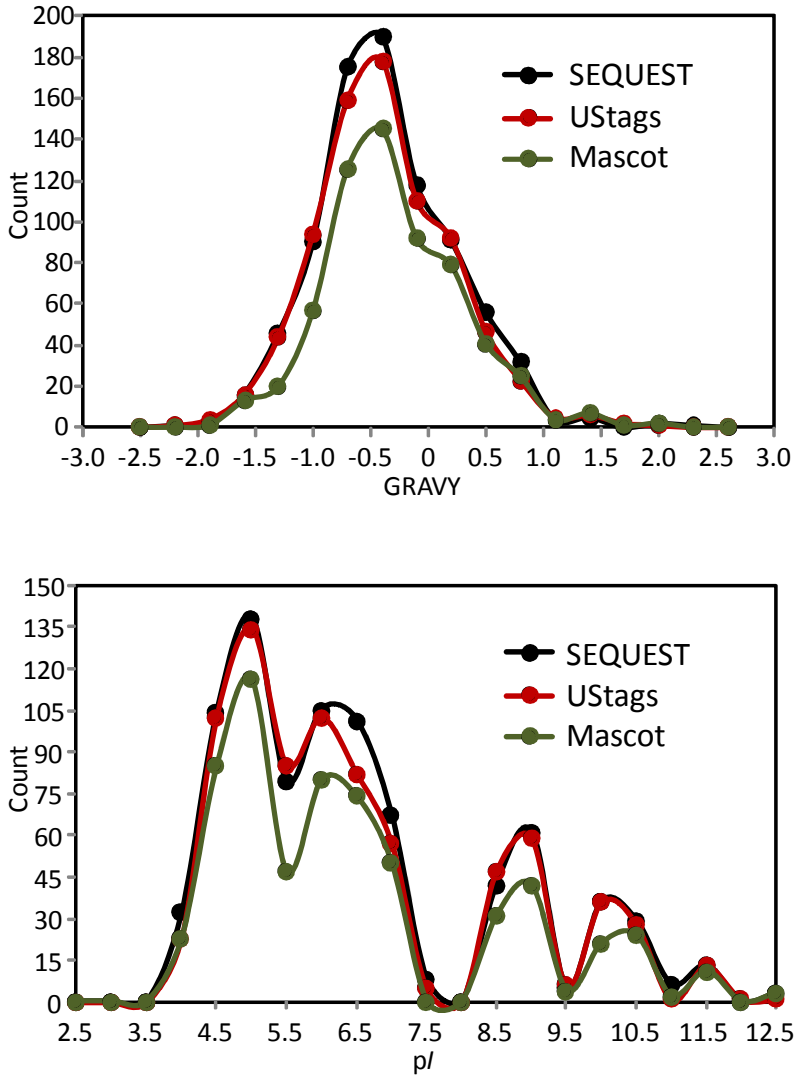
Conditions: the optimization was performed on a LTQ-Orbitrap Velos mass spectrometer (Thermo Fisher Scientific, San Jose, CA) with the instrument default CS 2 and with the supplementary activation enabled; the BSA Glu-C digest was used for this optimization.

Supporting Figure 2. The peptide overlaps between the CID, HCD, and ETD SEQUEST peptide subsets obtained with use of reverse and scrambled decoy databases for peptide identification.



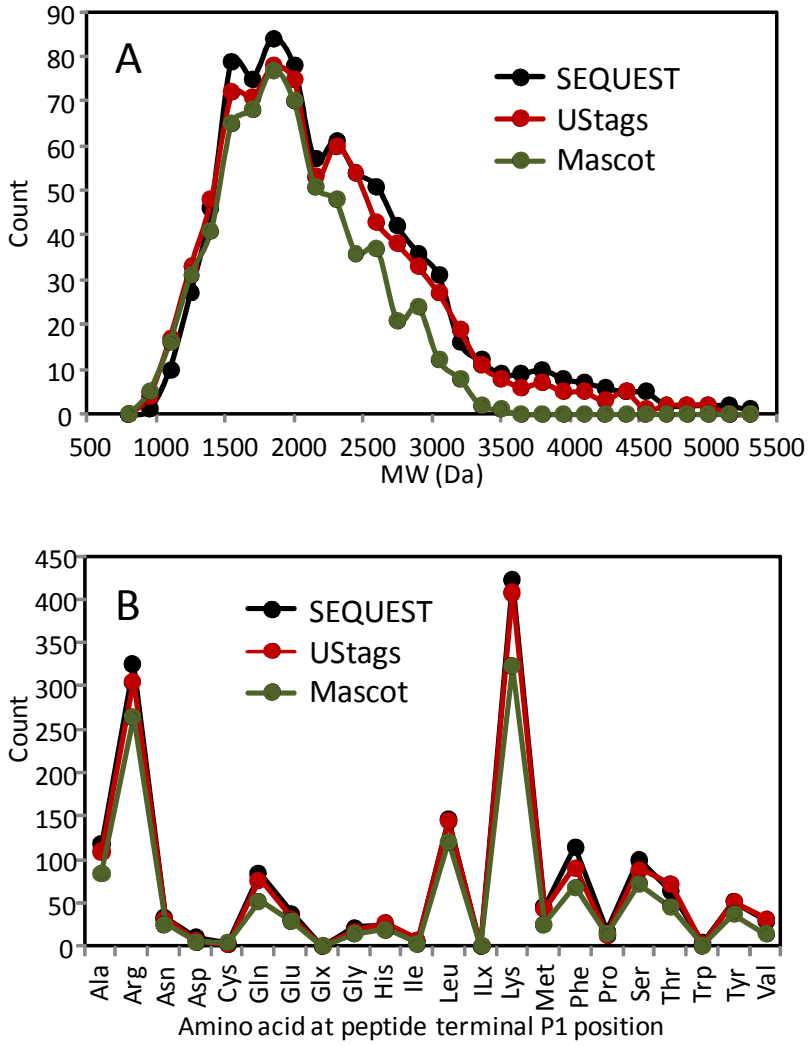
Conditions: mass tolerances of [5Da, 0.05Da] were used for the database search and all identifications were based upon 1% FDR. “rev” and “scr” represent the reverse database and scrambled database were used for peptide identification, respectively.

Supporting Figure 3. The GRAVY and p*I* distributions of peptides identified from the SEQUEST, Mascot, and UStags peptide identification methods.



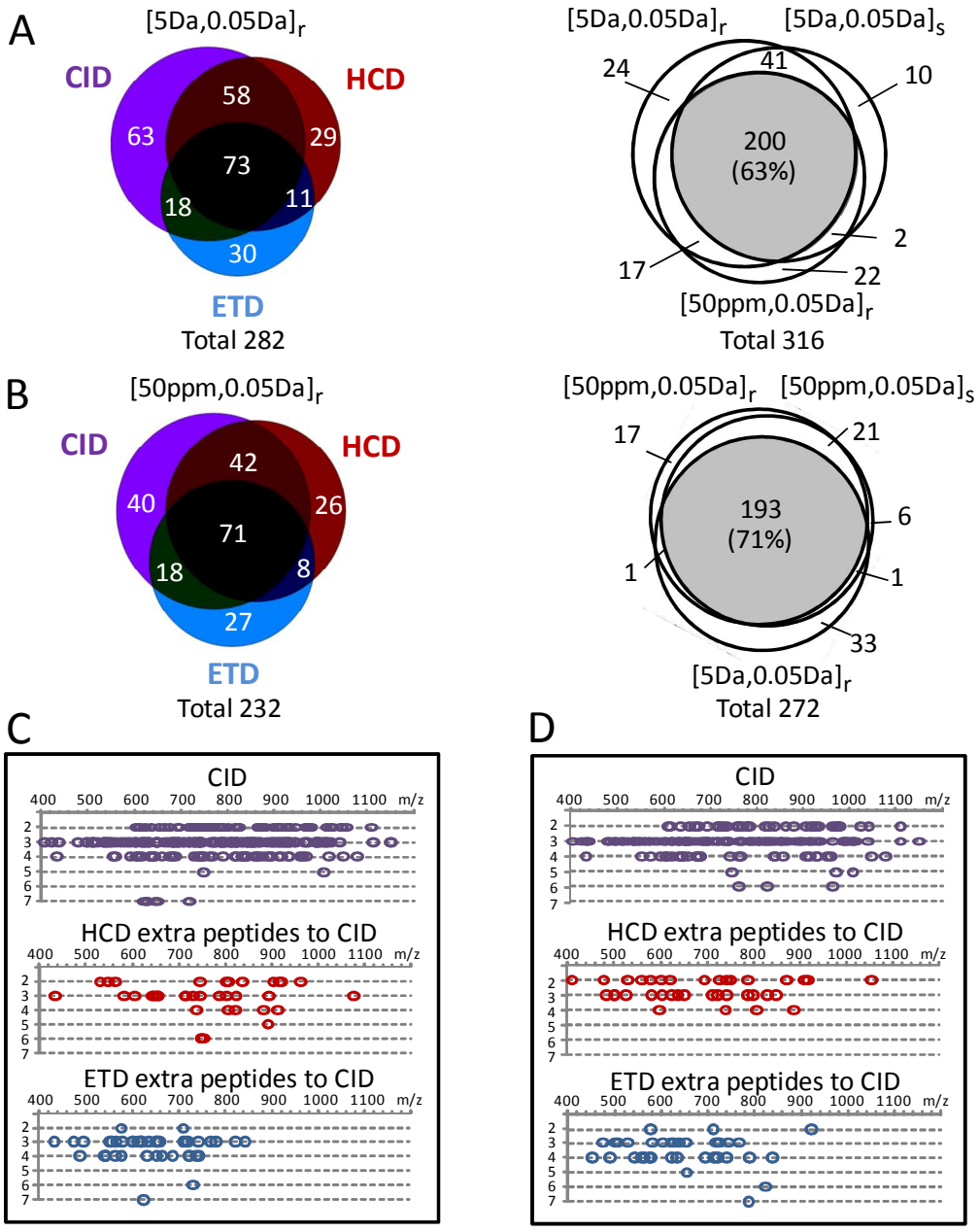
Conditions: the peptide dataset $[5Da, 0.05Da]_{F,r}$ from 0% FDR SEQUEST, $[5Da, 0.05Da]_{U,r}$ from UStags, and $[50ppm, 0.05Da]_{F,r}$ from 1% FDR Mascot methods were used for this examination.

Supporting Figure 4. Distributions of the MW and amino acid at peptide P1 position for peptides identified from the SEQUEST, Mascot, and UStags peptide identification methods.



Conditions: the peptide dataset $[5Da, 0.05Da]_{F,r}$ from 0% FDR SEQUEST, $[5Da, 0.05Da]_{U,r}$ from UStags, and $[50ppm, 0.05Da]_{F,r}$ from 1% FDR Mascot methods were used for this examination.

Supporting Figure 5. The contributions of CID, HCD, and ETD and variances of peptide subsets containing with Arg and Lys at two P1 positions of peptide termini.



Conditions: peptides were identified on 2% FDR level with use of "trypsin rule" for database search and peptide validation; other conditions were the same as described in the text. (A) SEQUEST peptides and their overlaps, (B) Mascot peptides and their overlaps, (C) SEQUEST $[5Da,0.05Da]_r$ peptide distributions, and (D) Mascot $[50ppm,0.05Da]_r$ peptide distributions.