# Supplementary Materials

MS2 spectra were separated by fragmentation method (37810 HCD spectra, 25734 ETD spectra, and 33586 CID spectra) and searched with MS-GFDB v6439. Spectra were identified at 1% spectrum-level FDR against the refseq human database (version 2011.0301). The following parameters were set for all searches: 7 ppm precursor mass tolerance, carbamidomethylation (+57 Da) Cysteine protecting group, 1 allowed 13C, Tryptic digest, High-res LTQ, and 1 allowed non-enzymatic termini. Fragmentation ID was also set appropriately for each search (CID, HCD, or ETD). FDR P-values were calculated by MS-GFDB using the target-decoy approach. Furthermore, all searches were conducted allowing for the following variable post-translational modifications: oxidation of methionine, N-terminal pyroglutamate formation, deamidation of asparagine, and deamidation of aspartic acid.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Unique Peptide Breaks | | | | |
| Prec. Charge | 2 | 3 | 4 | 5 | All |
| # Unique Pep. | 4361 | 2873 | 712 | 87 | 8037 |
| CID | 4.1 | 2.5 | 3.3 | 3.7 | 3.4 |
| HCD | 4.3 | 6.1 | 4.7 | 4.6 | 5.0 |
| ETD | 9.3 | 24.9 | 32.9 | 27.7 | 18.8 |
| CID/HCD | 20.7 | 14.6 | 11.5 | 12.5 | 17.0 |
| CID/ETD | 5.7 | 5.4 | 4.3 | 2.6 | 5.3 |
| HCD/ETD | 7.3 | 10.1 | 5.6 | 3.1 | 8.1 |
| CID/HCD/ETD | 41.8 | 23.3 | 13.1 | 6.9 | 30.2 |

Table SM-1: Peptide break statistics for combinations of alternative fragmentation modes - Peptide breaks unique to a particular fragmentation mode or combinations of fragmentation modes were counted over all unique peptides identified by all three fragmentation modes (i.e. each break could only be counted in one of the 7 rows per precursor charge). In CID and HCD spectra, the presence of breaks was indicated by the presence of *b* or *y* ions. For ETD, *c*, *z*°, or *z*°+H ions indicated the presence of a peptide break. Multiply charged ions (up to the spectrum’s precursor charge) were also considered in each spectrum. Prior to this analysis, peak filtering was applied all CID, HCD, and ETD spectra such that each peak was retained only if its intensity was ranked 5th or higher over all neighboring peaks in a ±56 Da radius. If a peptide was identified by more than one CID, HCD, or ETD spectrum, a single representative spectrum was randomly chosen for each fragmentation mode.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | CID |  | HCD |  | ETD |  | Union |  | Intersection |
| All Charges | 14327 |  | 17385 |  | 10813 |  | 23362 |  | 8037 |
| Charge 2 | 9068 |  | 10629 |  | 5583 |  | 12456 |  | 4361 |
| Charge 3 | 5282 |  | 6854 |  | 4708 |  | 8387 |  | 2873 |
| Charge 4 | 1306 |  | 1534 |  | 1305 |  | 2109 |  | 712 |
| Charge 5 | 196 |  | 201 |  | 244 |  | 359 |  | 87 |
| Charge 6 | 13 |  | 20 |  | 38 |  | 46 |  | 4 |
| Charge 7 | 1 |  | 1 |  | 5 |  | 5 |  | 0 |

Table 2: Unique PSMs for alternative fragmentation modes - The left three columns detail the number of unique PSMs per precursor charge state and per fragmentation method. Remaining columns count the number of unique peptides identified by at least one of the three fragmentation methods (Union) and the number of unique peptides identified by all three fragmentation methods (Intersection).