

Supplemental Figure 1

The number of unique peptide identifications from a soluble fraction of a *C. elegans* lysate is plotted as a function of normalized collision energy. A precursor scan was followed by 10 MS/MS scans where the collisional energy was stepped by a value of 3 in each MS/MS scan (20 CI to 46 CI). Each scan was directed at the same precursor. Data were extracted according to collisional energy from the raw file and searched separately via Sequest percolator pipeline. Each point represents the average of three separate runs and error bars correspond to the standard deviation.

Supplemental Figure 2

In this experiment both RE-CID and fHCD were performed in the same run. One precursor scan was followed by alternating RE-CID and fHCD scans. This effectively allowed another direct comparison of the efficiency of RE-CID and fHCD as each pair of MS/MS events were directed at the same precursor. A histogram displays the number of scans as a function of total number of ions (bins = 400). Similar efficiencies were observed to the direct infusion data.

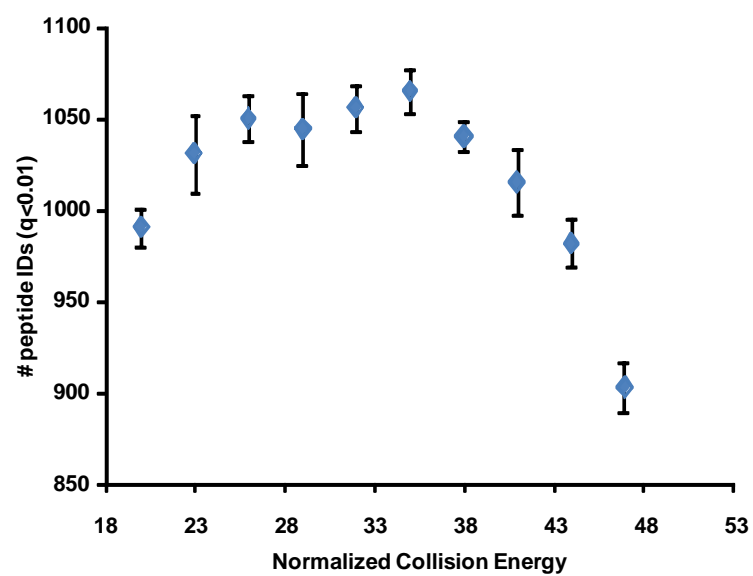
Supplemental Figure 3

A bar plot comparing total number of scans using Rapid vs. Normal scan rates for fHCD and RE-CID. The LC-MS run was 90 minutes. It was observed on average that runs employing CID took more scans than fHCD – presumably due to the doubling of IT for the fHCD events (**Figure 2**).

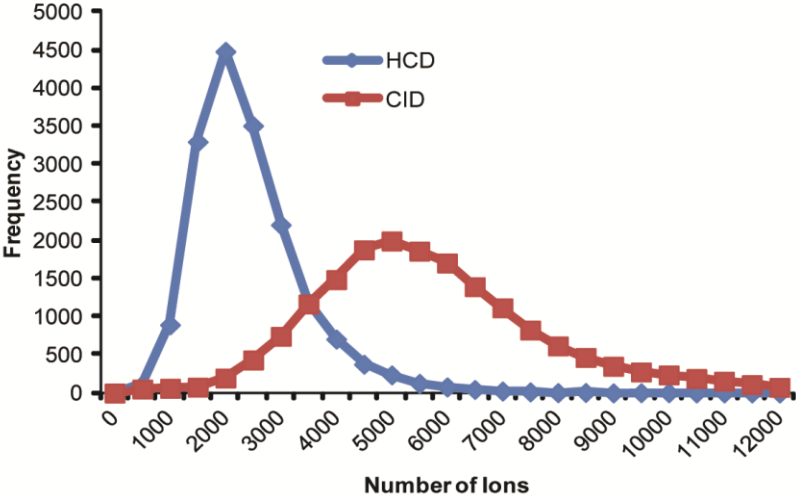
Supplemental Figure 4

A) A comparison of spectra resulting from the dissociation of a glycosylated peptide (O-GlcNAc) by RE-CID and fHCD (mirrored axis). RE-CID shows predominately the charge loss of the GlcNAc moiety and the resulting singly charged unmodified peptide. The majority of peaks in the fHCD spectrum correspond to several fragment ions without the modification. **B)** As a result, the XCorrs for this peptide for both RE-CID and fHCD were relatively low. However, statistically, fHCD yielded on average a higher score ($p < 0.05$). In addition several peaks in the low molecular weight range of fHCD have been previously identified as indicative of a GlcNAc modified peptide (asterisk). These peaks were not observed in the RE-CID due to the low molecular weight cut-off.

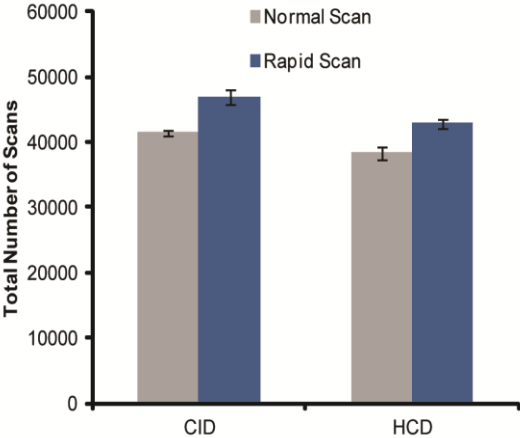
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4

