Title:

Peptide-first Glycopeptide Search: Combining MSFragger Glyco Search with Glycan FDR Control in PTM-Shepherd

Abstract:

Advances in methods for enrichment and mass spectrometric analysis of intact glycopeptides are increasingly producing large-scale, high-quality glycoproteomics datasets, but confidently annotating both peptide and glycan identities in the resulting spectra remains challenging. We have developed a "peptide-first" glyco search strategy using the mass offset search of MSFragger to identify glycopeptides as the combination of a peptide sequence and a glycan mass. This approach takes advantage of glycan fragmentation and the indexed search of MSFragger to greatly improve the sensitivity of glycopeptide spectrum matching in CID/HCD data. We have recently introduced a module in the post-search annotation tool PTM-Shepherd to convert the glycan mass to a specific glycan composition and perform glycan composition-specific FDR estimation. Matching the peptide sequence first greatly reduces the number of possible glycans being considered in glycan matching, and, along with the use of both Y- and oxonium ions from the spectrum, allows our method to achieve sensitive and robust glycan assignment in the presence of entrapment glycans known not to be present in the sample. Combined with tools for quantitation, we now have a complete pipeline in the Fragpipe computational environment for analysis of glycopeptide tandem MS data.

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