Glycan Scoring Incorporated into Protein Prospector Database Search Results

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Introduction

Several software tools are now available for large-scale analysis of intact glycopeptide spectra. In general, these tools perform well at reliably identifying the peptide portion of the glycopeptide, but are inconsistent at assigning the glycan component. When there are alternative glycan interpretations of similar mass, software needs to correctly consider all the possible compositions. It is necessary to score which fits best, and determine whether there is sufficient information to call a result reliable.

The first Human Glycoproteomics Initiative Study showed Protein Prospector to be a superior tool for glycopeptide identification, but at that time there was no glycan scoring. In this poster we present incorporated glycan scoring that allows reliable interpretation among possible glycan compositions.

The workflow makes use of MS-Filter, part of Protein Prospector and freely available on the web, which allows searching for spectra containing Y ions, then scoring peptide and glycan identifications.

1. Search for glycopeptides by database searching using e.g. Mascot-Tag.
2. Input list of potentially glycosylated peptide sequences to MS-Filter.
3. There is a direct link to do this from Batch-Tag.
4. Identify additional glycosylations and score between potential glycan assignments.
5. Unexplained mass shifts may correspond to modifications or glycosylations not considered.

We show in some cases there can be dramatic differences in the number of glycosylated spectra that can be identified in comparison to database searching. All data presented on this poster is HCD data from a published analysis of human SRB5.

Example Output Displayed in MS-Viewer

- MS-Filter

Peptide: Lisinopril, Fragment: 1062 to 1073

Glycan: HexHex4Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5Hex5H...