Multi-Dimensional Molecular Ion Glycopeptide Libraries for Targeted Analysis

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Introduction

Challenge:

- Glycopeptide Identification and glycan structure analysis is difficult.
- Preferred fragmentation is through the glycan resulting in product ion spectra rich in oxonium and sugar fragments but limited in y" and b ions.
- Identification is simply the presence of Y1, oxonium ions and the similarity of the calculated mass difference (precursor minus the peptide) compared to a library of glycan compositions formed by combinatorial rule.

Solution:

- Crowdsource data acquisition(multi-platform, multi-lab)
- Discover – identified & unidentified spectra
- Aggregate and cluster
- Find & validate molecular ion spectra (MIS) Targeted search and hole filling
- Validate multi-dimensional molecular ion array by fragment ions

Conclusions

- Product ion spectra generated using the same mechanism of fragmentation (collision cell) can be clustered into a single accurate MIS.
- Use of a lookup table of oxonium ions and delta mass differences limits spectral complexity of glycopeptides.
- Use of accurate MIS increase glycopeptide coverage in DIA data by ~30%.

Methods

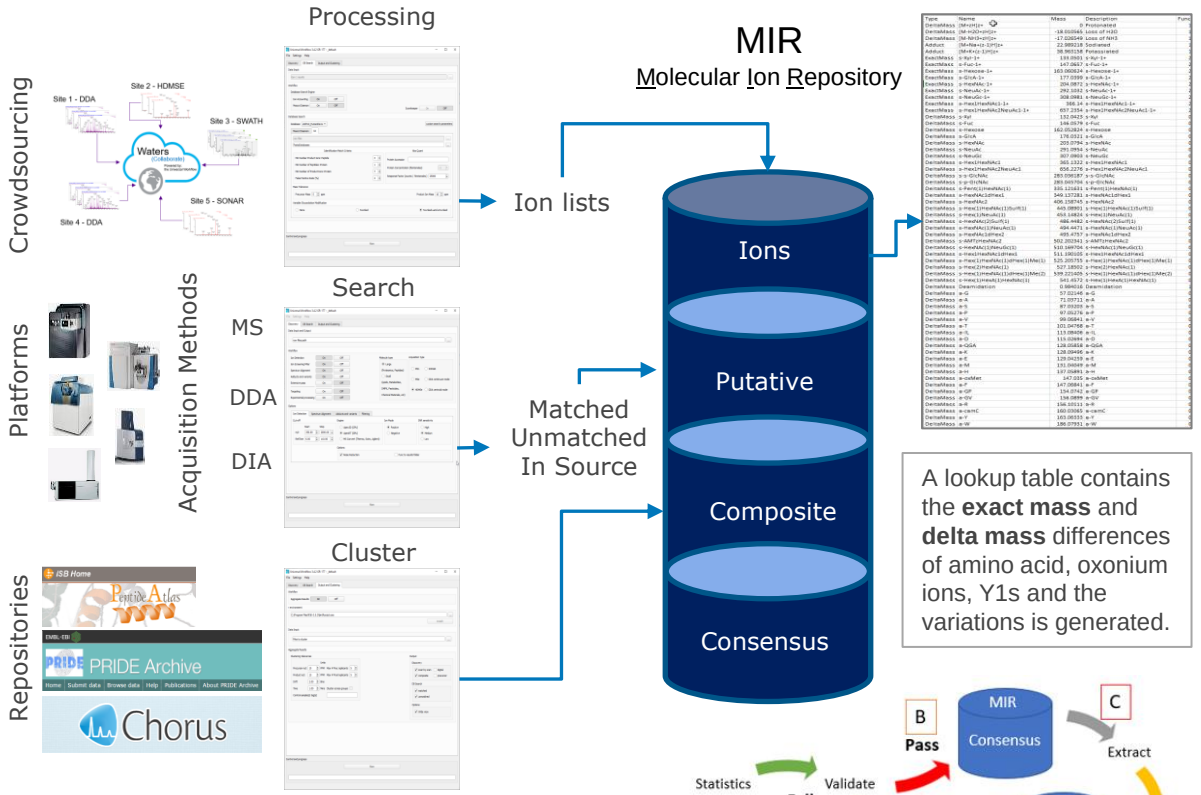

Figure 1. Experimental and Crowdsourced DDA & DIA files are processed into ion lists and product ion spectra. Precursor ions are grouped by charge, adducts, & in-source fragments. Spectra are searched with matched and unmatched spectra deposited in the Putative section of the MIR.

A lookup table contains the **exact mass** and **delta mass** differences of amino acid, oxonium ions, Y1s and the variations is generated.

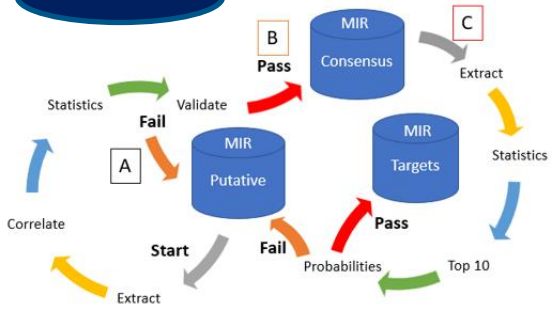

Figure 2. Spectra are extracted for correlation (A). Validated spectra are clustered into composite spectra (B). Composite spectra from multiple sample set are clustered (C) Those exceeding the minimum similarity score create the Consensus/Target spectrum.

Results

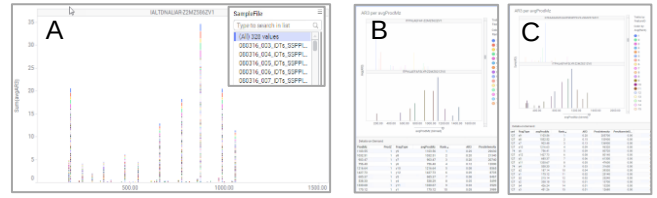

Figure 3. 328 samples (A), 78 Xevo SONAR (B), 250 QE DDA (C). Comparison of composite MIS 98.2% similarity.

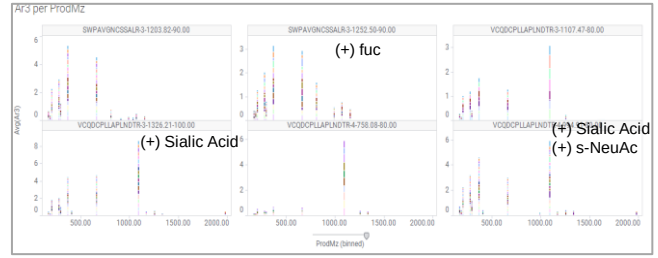

Figure 4. Illustrated are clustered product ion spectra reflecting multiple charge states and Glyco forms of the same glycopeptides from Hemoglobin & Fetuin.

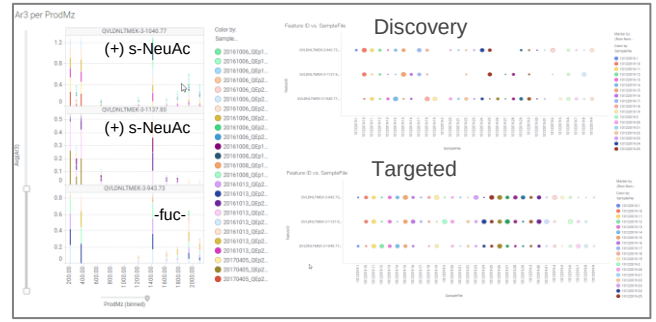

Figure 5. Targets are queried against the MSMS product ions validating group membership and identifying missing glycopeptides from the initial search (hole filling). The identification of a glycopeptide in 68 samples (Discovery) to 87 samples (Targeted) increase of ~27%.