

Complementarity of EThcD and HCD of Glycopeptides: Y ion Filtering for Increased Glycopeptide Identification



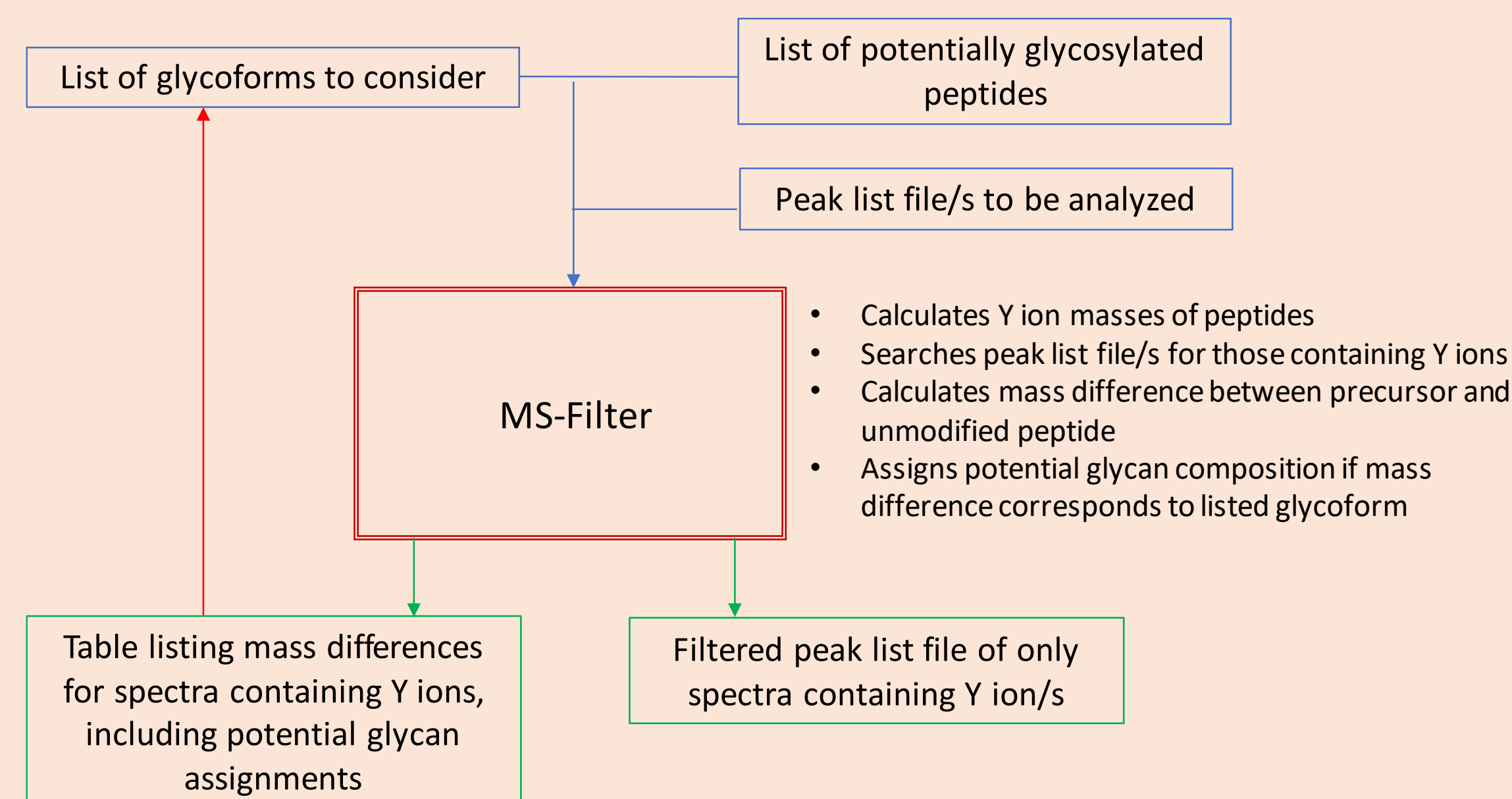
Robert J. Chalkley¹, Katalin F. Medzihradzsky^{1,2}, Adam Pap², Zsuzsanna Darula², Peter R. Baker¹

¹Mass Spectrometry Facility, University of California San Francisco, San Francisco, CA. ²Laboratory of Proteomics Research, Biological Research Centre, Szeged, Hungary

Introduction

This poster presents improvements to free web-based software (MS-Filter) for finding specific ions in peak list files. These changes are designed to assist with intact glycopeptide analysis. We present results using identifications from EThcD data to examine matched HCD data for the presence of Y ions that are diagnostic for a given glycosylated peptide, and using this strategy we demonstrate the identification of additional glycoforms in O-linked and N-linked glycopeptide datasets. In the case of O-linked glycopeptide the most prominent Y ions are Y0 and Y1, whereas for N-linked Y1 and Y2 are normally observed. If glycosylated peptides in a sample are known, these Y ions can be used to find other glycoforms.

Workflow for Glycopeptide Analysis using MS-Filter



Experimental

- Two datasets were analyzed
 - Urine dataset enriched for O-linked glycopeptides. Used ten glycopeptides to evaluate performance.
 - Human Glycoproteomics Initiative Study Dataset: enriched for glycopeptides using HILIC. Used to evaluate performance for N-linked glycopeptide analysis considering 260 glycopeptide sequences.
- Data was acquired on Orbitrap Fusion Lumos mass spectrometer with HCD and EThcD acquired on each precursor.
- EThcD data was searched using Protein Prospector to identify intact glycopeptides
- The list of identified glycosylated peptides was imported in MS-Filter to search for Y ions in matched HCD peak list files
- Parameters that were evaluated were:
 - Number of Y ions required to match
 - Number of most intense ions to consider for matching Y ions

O-Linked Glycopeptide Results

- Y0 and Y1 ions are mostly in the top 10 most intense ions in HCD spectra of O-glycopeptides
 - These ions are much more abundant than in N-glycopeptide spectra
- MS-Filter is able to detect more glycoforms than could be identified by database searching
 - Y ions are generally prominent even in poorer quality spectra
- Metal adduct spectra produce two Y ion series (see top right panel).

Search	Top15	Top10	Top7	Top5
#Results	145	122	84	52
Suggested Glycans	61	57	46	30
Unique Glycan Assignments	37	33	25	15
Unique Peptide Sequences	10	9	7	7

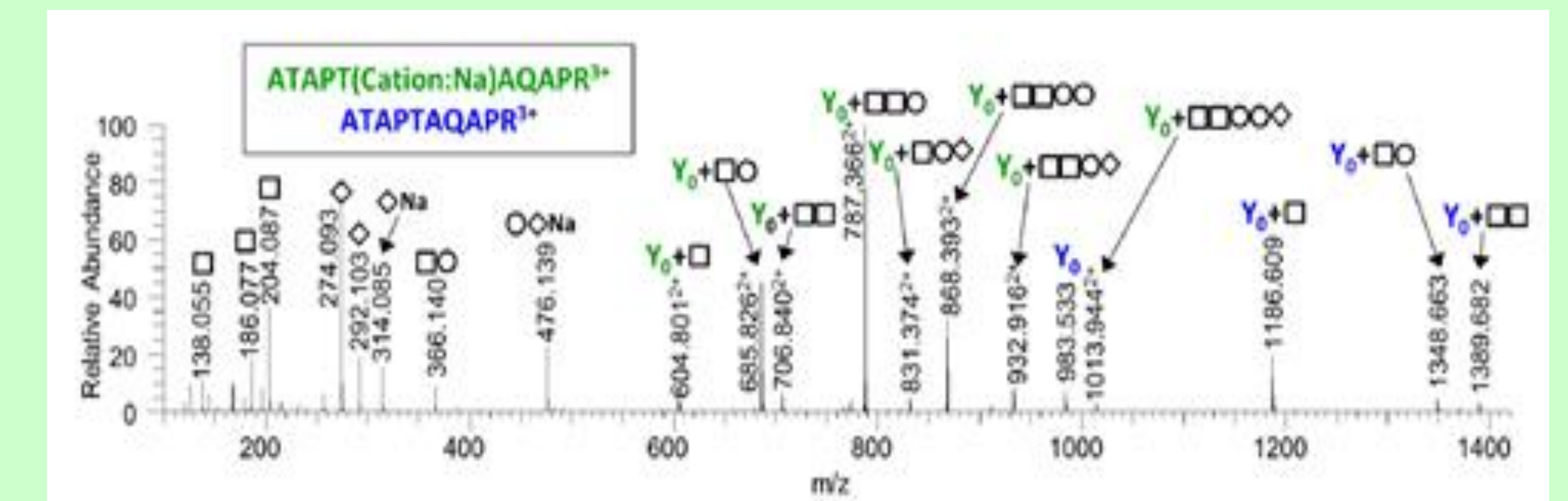
N-Linked Glycopeptide Results

- Requiring only Y1 mass leads to many false positives, even if only ten most intense peaks are considered
 - For N-linked requiring two Y ions is necessary for any reliability.
- Much larger problem with false positives in this analysis than in O-linked data
 - Combination of Y ions not being as intense as in O-linked data and considering more peptides.
- At any threshold the overlap with the EThcD identifications is never more than 60%
 - HCD provides complementary information.
 - EThcD much better at identifying spectra of metal adduct species
- Metal adduct spectra can produce unmodified and shifted Y ions (see top right panel)
 - Considering both ion series leads to significantly more identifications

Search	40_Y1	30_Y1	20_Y1	10_Y1	40_2	30_2	20_2	10_2	30_2_Cation:K
#Results	9394	7630	5566	3262	1878	1489	900	427	1840
#Spectra	4861	4362	3701	2723	1571	1328	857	424	1568
Suggested Glycans	1174	1080	918	728	539	470	384	244	680
Unique Suggested Glycans	802	720	598	457	335	294	242	154	392
Unique Peptide Sequences	244	242	234	214	147	130	107	70	171
Overlap with EThcD results	154	148	135	113	82	72	65	48	128

Metal Adduct Glycopeptide Identifications

- Both expected Y ions and those shifted by the mass of metal adducts (.e.g sodium) were observed.
 - Some peptides only gave one or the other
 - No obvious relationship between sugar and whether it formed metal adduct Y ions



Conclusions

- MS-Filter analysis of HCD data was beneficial for both O-linked and N-linked glycopeptide analysis
- For O-linked data:
 - Y0 and Y1 mostly present in ten most intense peaks in spectrum
 - Filtering led to several identifications not found by database searching of corresponding EThcD data
- For N-linked data:
 - Y1 and Y2 need to be considered in at least top 30 peaks in order to not lose significant results
 - Was able to rescue results from corresponding EThcD data that were correct but below the 1% FDR threshold.
- Metal adducts decrease Y ion intensity/detection and can form two sets of Y ions

Hyperlinks:

[Protein Prospector website](#)

[MS-Filter](#)

[Manuscript associated with this work](#)

[Link to MS-Viewer for O-linked Glycopeptide Results](#)

[Link to MS-Viewer for N-linked Glycopeptide Results](#)

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