Mass Spectrometry of Glycans and Glycoproteins

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Leaving the Glycan Attached

- Benefits – allows identification and heterogeneity to be characterized at individual glycosylation sites
- Disadvantages – much more difficult

MS/MS Analysis of Intact Glycopeptides

*Glycan oxonium ion

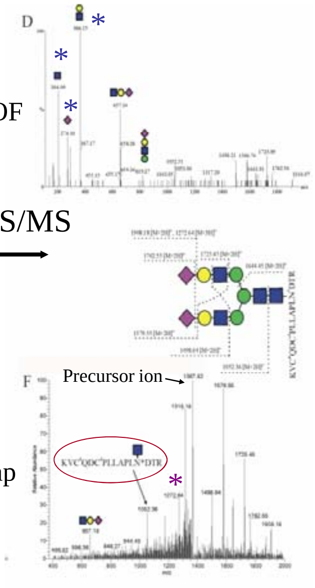
Q-TOF

LC-MS/MS

Glycopeptides →

Ion Trap

* Ion from neutral loss of a monosaccharide



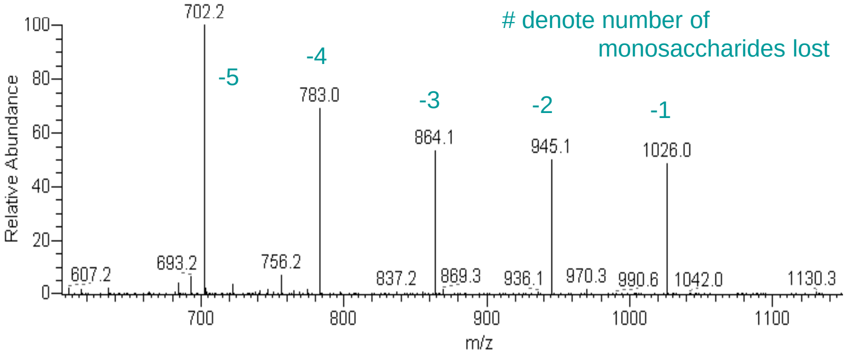
Fragmentation dominated by glycan

Typically cannot ID sites by MS/MS

With N-linked glycans GlcNAc-N linkage is stable, and can often be used to identify site, but often this is aided by MS^3

MS/MS Analysis of an Intact O-linked Glycopeptide from EPG 2

All fragment ions are from the loss of the glycan – no information on the peptide sequence or site of attachment



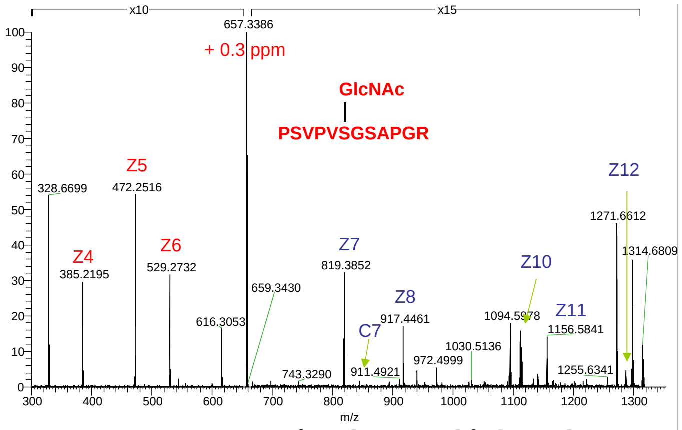
denote number of monosaccharides lost

* * * * *
40ATTCTFSGSEASK54

Sites identified by BEMAD

Electron Capture Dissociation (ECD)

leaves glycan attached allowing site identification



ECD Fragmentation of O-GlcNAc Modified Peptide.
Fragments in BLUE contain O-GlcNAc-Ser.

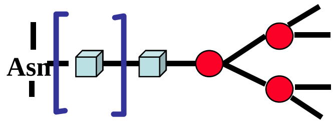
Glycopeptide Identification

mass spec experiments can be used to selectively identify glycopeptides

- In source CID – skimmer induced fragmentation
- Precursor ion scans
- Constant neutral loss scans

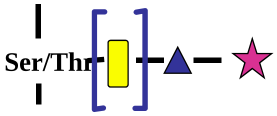
Class Specific Glycopeptide CAD Fragment Ions

***N-linked* carbohydrate**



HexNAc$^+$, *m/z* = 204

***O*-linked carbohydrate**

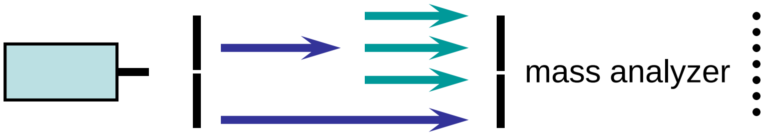


HexNAc$^+$, *m/z* = 204

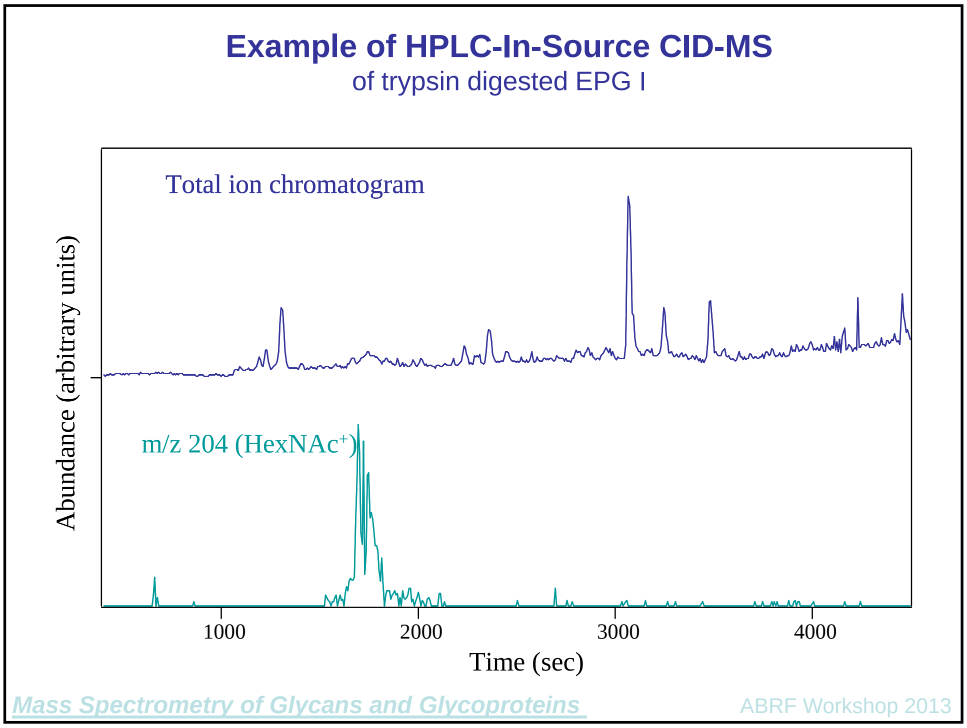
- □ **N-Acetyl glucosamine**
- ● **Mannose**
- ▲ **Galactose**
- ▯ **N-Acetyl galacosamine**
- ★ **NeuAc**

In-source Collision Induced Dissociation (CID)

offset = 120 V for m/z < 500

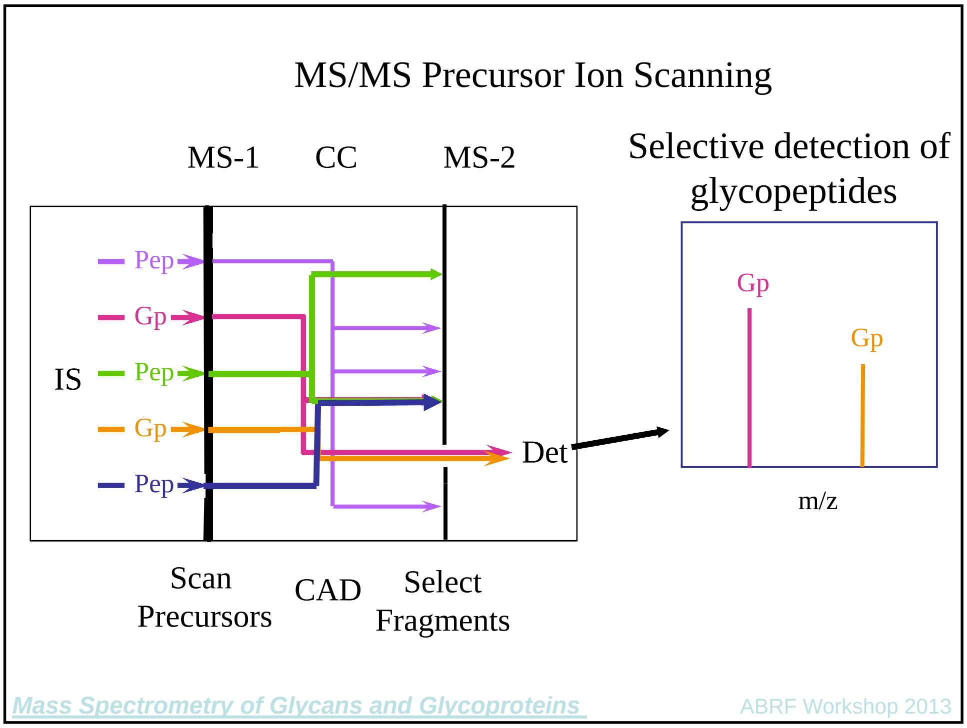


offset = 30 V for m/z > 500



Example of HPLC-In-Source CID-MS

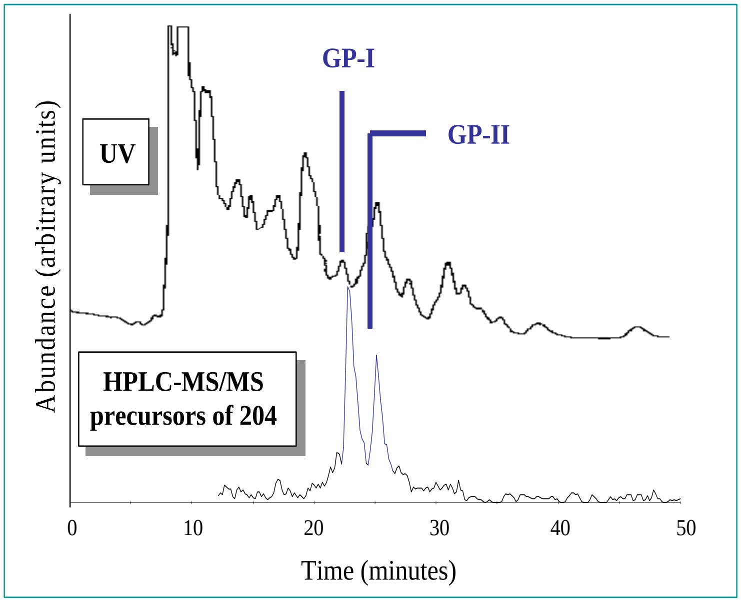
of trypsin digested EPG I



MS/MS Precursor Ion Scanning

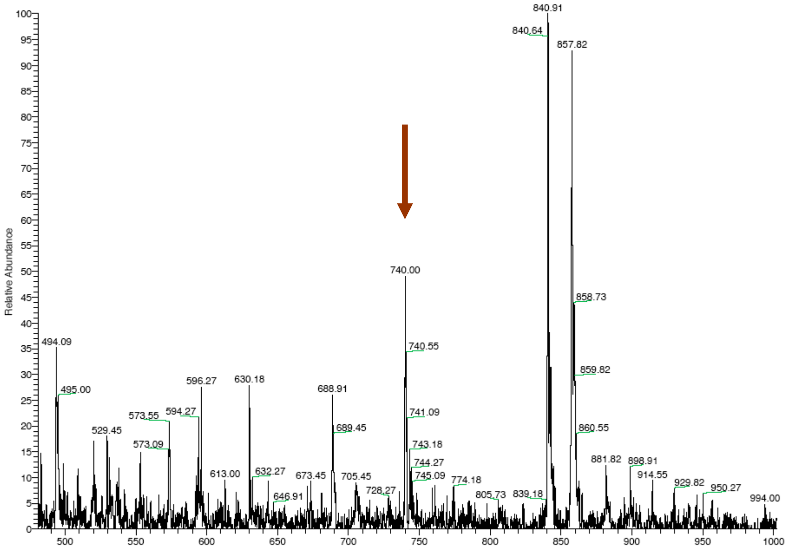
Example of HPLC-Precursor Ion Scanning-MS

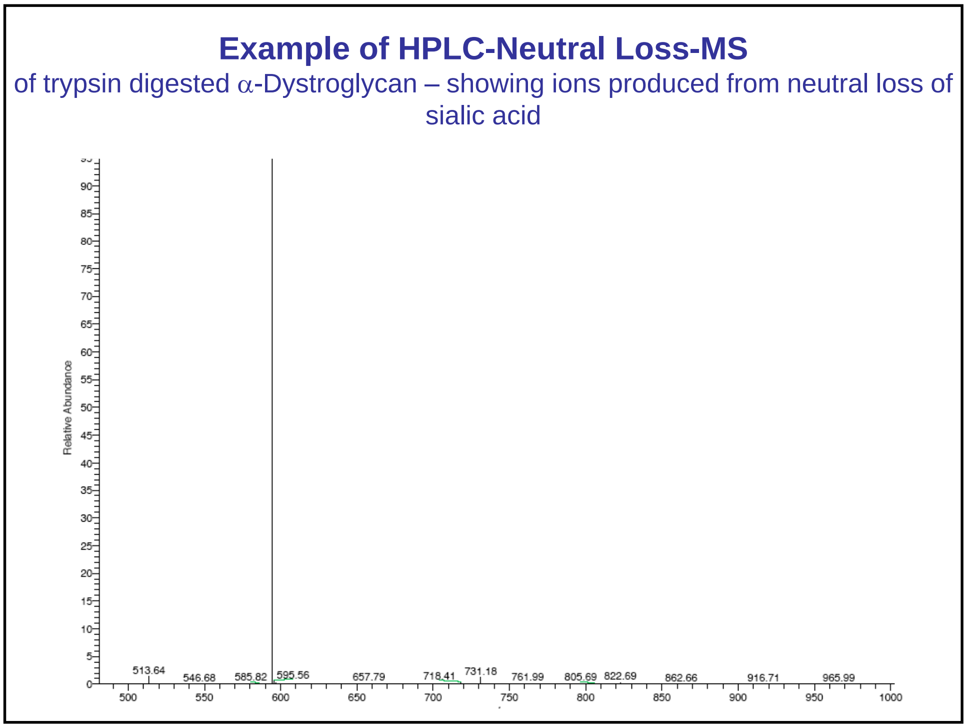
of trypsin digested Canine Serum Transferrin



Example of HPLC-Neutral Loss-MS

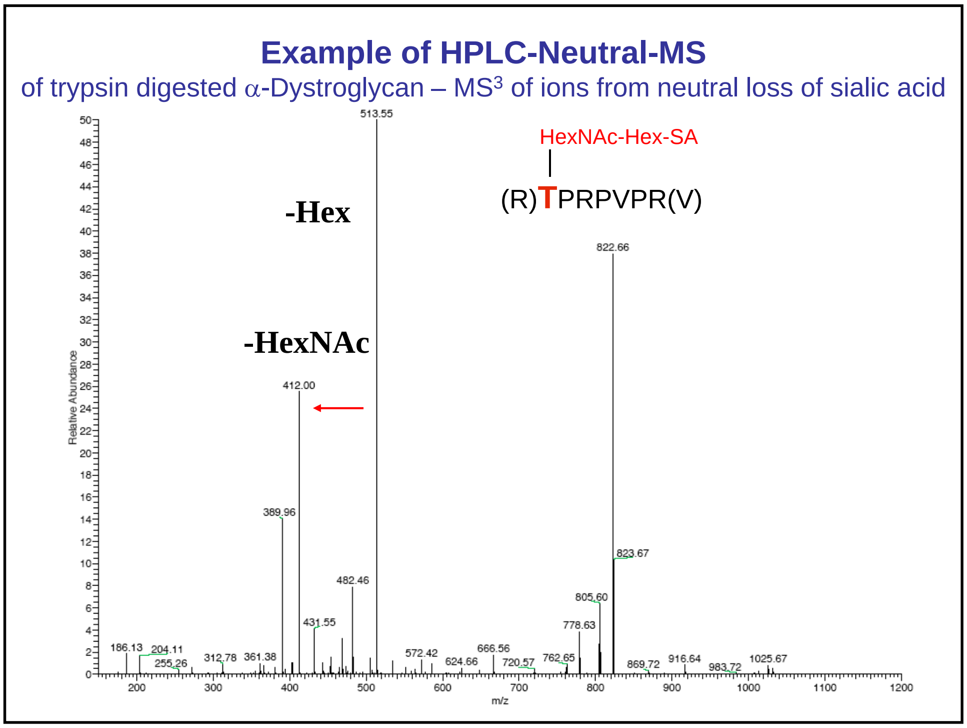
of trypsin digested α-Dystroglycan – full mass spectrum of LC peak





Example of HPLC-Neutral Loss-MS

of trypsin digested α-Dystroglycan – showing ions produced from neutral loss of sialic acid



Example of HPLC-Neutral-MS

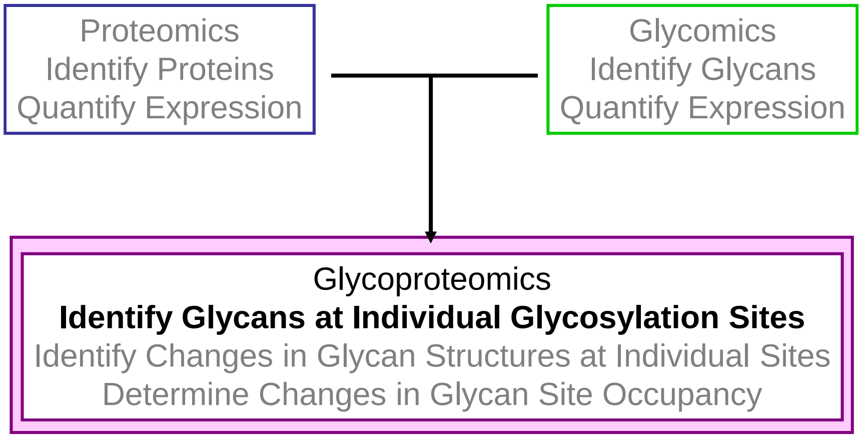
of trypsin digested α-Dystroglycan – MS^3 of ions from neutral loss of sialic acid

What to do?

Depends on what kind of instrument you have

- In source CID
  - can be done on any ESI instrument
  - gives information from both peptides and glycopeptides
  - least selective
  - Does not identify glycopeptides just where they elute
- Neutral loss
  - Can only be done on MS/MS instruments, best performed on those with fast scan rates
  - gives information from both peptides and glycopeptides
  - Requires software to analyze data
  - Moderate selectivity (can have 4 terminal sugars and multiple charge states – lots of potential neutral losses)
  - Misses large glycopeptides which typically don't fragment by neutral loss of glycan (also lose a charge)
- Precursor scanning
  - Can only be done on QqQ instruments on LC time scale
  - Only provides information (*m/z* values) on glycopeptides
  - Most selective

Glycoproteomics: Identification



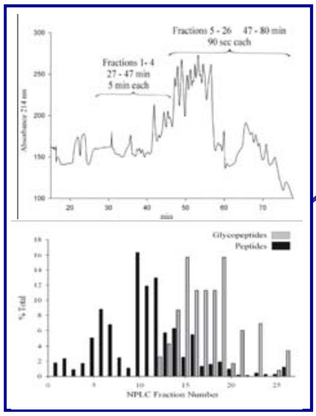
Proteomics
Identify Proteins
Quantify Expression

Glycomics
Identify Glycans
Quantify Expression

Glycoproteomics
Identify Glycans at Individual Glycosylation Sites
Identify Changes in Glycan Structures at Individual Sites
Determine Changes in Glycan Site Occupancy

Simplify the Problem

Complex mix of
Peptides & Glycopeptides

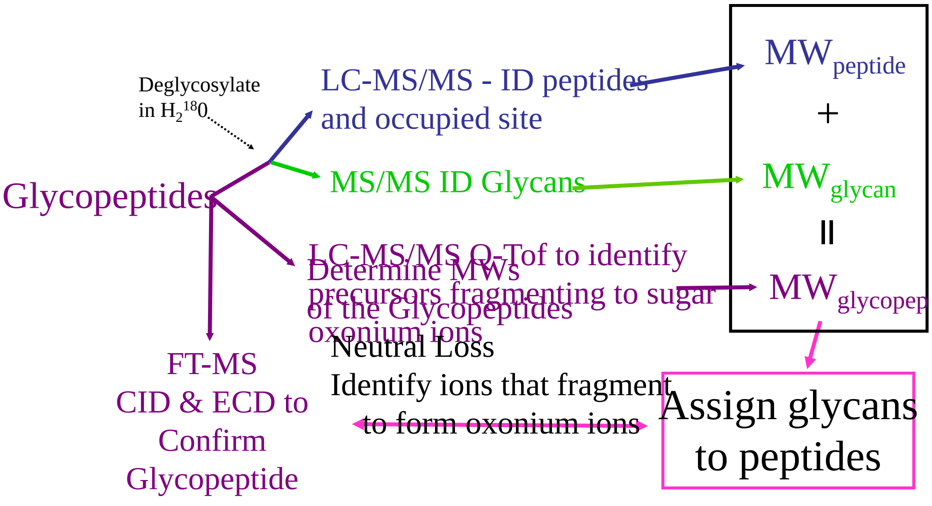


Normal Phase or HILIC
Chromatography
Not specific to glycan structure

Glycopeptides

[1]J.A. Atwood III, T. Minning, F. Ludolf, G. Alvarez-Manilla, D.B. Weatherly, R. Tarleton, R. Orlando, "Identification of N-Linked Glycopeptides from *Trypanosoma Cruzi* Trypomastigotes Using Lectin Affinity and Stable Isotope Labeling," *J. Proteome Res*, **2006**, *5* (12), 3376.
[2]Atwood, J.A. III, Luo, Z. Weatherly, B., Boyes, B., Orlando, R. "A novel glycoproteomic approach for the complete characterization of glycopeptides from complex biological mixtures." *Glycobiology* **2006**, *16*, 2006, 1138.

Characterizing Intact Glycopeptides



Deglycosylate
in $H_2{}^{18}0$

LC-MS/MS - ID peptides
and occupied site

$MW_{peptide}$
+

Glycopeptides

MS/MS ID Glycans

MW_{glycan}
=

LC-MS/MS Q-Tof to identify
precursors fragmenting to sugar
oxonium ions

Determine MWs
of the Glycopeptides

$MW_{glycopep}$

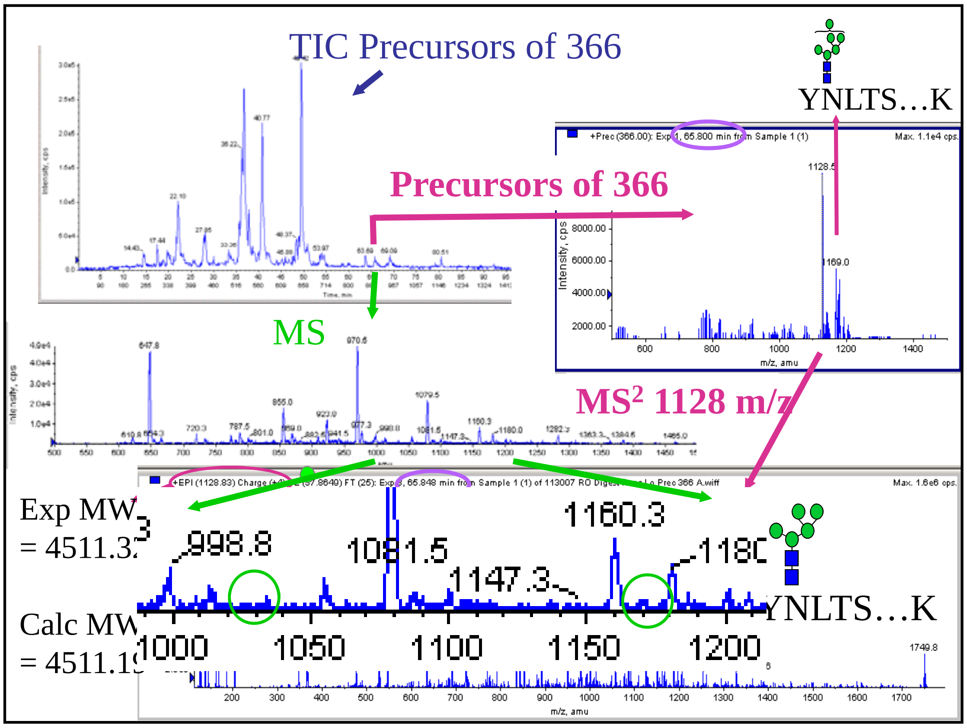
Neutral Loss
Identify ions that fragment
to form oxonium ions

Assign glycans
to peptides

FT-MS
CID & ECD to
Confirm
Glycopeptide
assignments

P.M. Angel, J. Lim, L. Wells, C. Bergmann, R. Orlando,"A Potential Pitfall in ^{18}O Based N-Linked Glycosylation Site Mapping," *Rapid Commun. in Mass Spectrom.*, **2006**, *in press.*

Atwood, J.A. III, Luo, Z. Weatherly, B., Boyes, B., Orlando, R. "A novel glycoproteomic approach for the complete characterization of glycopeptides from complex biological mixtures." *Glycobiology* **2006**,*16*, 2006, 1138.



TIC Precursors of 366

YNLTS…K

Precursors of 366

MS

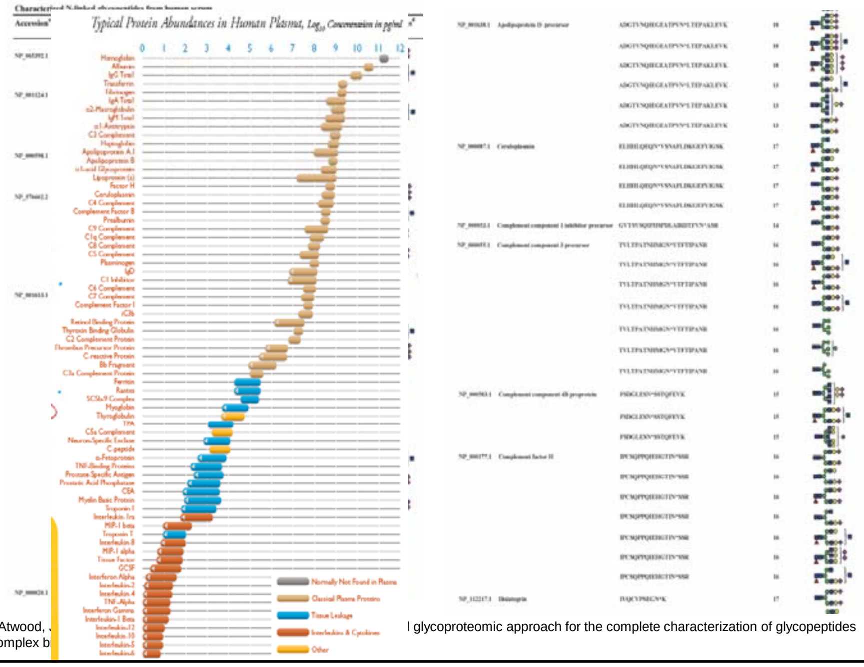
MS² 1128 m/z

Exp MW = 4511.3

Calc MW = 4511.1

YNLTS…K

Partial list of glycopeptides characterized from human serum



From: Atwood, J … l glycoproteomic approach for the complete characterization of glycopeptides from complex b

Acknowledgements

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- Standard protocols for much of the chemistry presented here are available from
  - Parastoo Azadi - azadi@ccrc.uga.edu
- We provide a range of glycan related services
- We also offer more detailed training

http://www.ccrc.uga.edu/home.html

Utilize our knowledge and services