Global Serum Deglycosylation in High Throughput LC-MS Glycomics: Enhanced Data Clarity via Improved Enzyme Release, Reduction, NanoLC, and IMS-MS

Scott R. Kronewitter, Ioan Marginean, Erin S. Baker, Danny J. Orton, Gordon W. Slysz, Heather M. Brewer, Thomas L. Fillmore, Kevin L. Crowell, Brian L. LaMarche, Yehia M. Ibrahim, Matthew E. Monroe, Carrie D. Nicora, David G. Camp II, Gordon A. Anderson, Ronald J. Moore, Richard D. Smith
Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA

Pacific Northwest NATIONAL LABORATORY

Overview

- **Overcoming challenges related to glycomics sample processing, separations, and data processing.**
- **Application of a new LC-ESI-IMS-MS platform for analyzing enzymatically released glycans.**

Introduction

- Utilized expedited enzymatic release of N-glycans from glycoproteins for LC-MS-based analysis challenges downstream data analysis.
- Digested mixture included intermediate amino glycoforms and more stable aldehyde glycoforms (created after the amine on the intermediate is hydrolyzed), which both have ring anomers on the reducing end that manifest as two LC peaks, confounding data analysis.
- Sodium borohydride reduction eliminated intermediates by opening the reducing end ring glycoforms.
- Mixture analyzed using LC-ESI-(Velos Oribtrap) MS; LC-SPIN (subambient pressure ionization with nano-electrospray)-QTOF MS; and LC-ESI-IMS-MS.
- Increased enzyme release, reduction, and LC steps improved data quality of glycomics analyses.

CONTACT: Scott Kronewitter, Ph.D.
Biological Sciences Division, K8-98
Pacific Northwest National Laboratory
P.O. Box 999, Richland, WA 99352
E-mail: Scott.Kronewitter@pnnl.gov

Glycan sample processing pipeline

Serum Collection
- Commercial human serum (Sigma Aldrich)

Chemical Processing
- Protein denaturation
- Assisted PNGase F glycan release
  - 50 mM sodium phosphate, pH 5.5
  - Microwave reactor (CEM Corp)
  - 40 min, 20 W
- Ethanol precipitation

Automated Gilson Solid Phase Extraction
- C8 cartridge
- Graphatized carbon cartridge
- Gilson GX-274 liquid handler

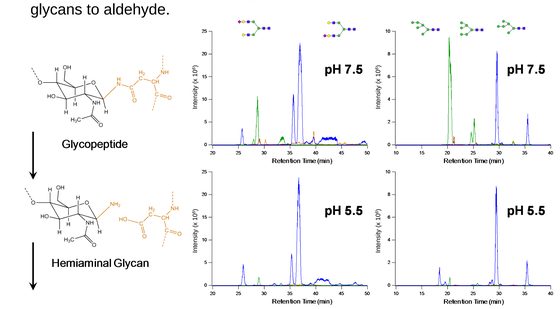
Liquid Chromatography-Mass Spectrometry
- Agilent pumps/Leap liquid handler
- 45 cm long, 75 µm i.d., 3 µm HyperCarb
- Gilson GX-274 liquid handler
- IMS-MS or LTQ-Orbitrap Velos

Data Processing
- External mass calibration
- Peak centroid and threshold
- Deisotoping with DeconTools
  - THRASH
  - Glycan Averagose

Glycan Profiling
- Theoretical glycan library
- Exact mass analysis
- Family relationships

Enzyme release

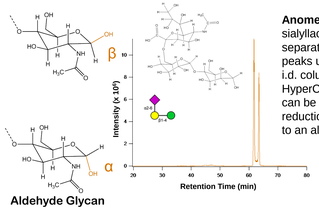
- **Challenge:** Rapid enzymatic reactions generate large amounts of N-glycan hemiaminal intermediates.
- **Solution:** Lowered pH of the digestion buffer to hydrolyze hemiaminal glycans to aldehyde.



Peak Consolidation: After low pH digestion and sodium borohydride reduction, intermediate hemiaminal (—), aldehyde (—), amino (—) glycans are converted to alditol (—) glycans.

Aldehyde reduction

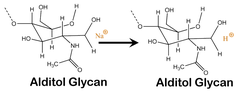
- **Challenge:** Ring anomers at the reducing end separate on graphitized carbon resulting in two LC peaks per glycan structure.
- **Solution:** Reduced the aldehyde into an alditol opening the ring that results in one LC peak per glycan.



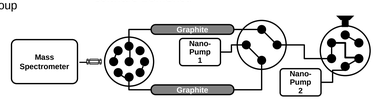
Anomer separation: α2-6 sialyllactose ring anomers are separated into two well defined peaks using 45 cm long, 75 µm i.d. column packed with 3 µm HyperCarb particles. This split can be removed through reduction of the aldehyde group to an alditol.

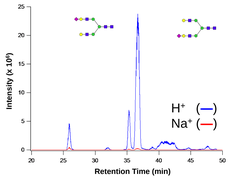
Desalting

- **Challenge:** Glycans are often detected in multiple forms involving sodium and proton cations.
- **Solution:** Solid phase desalting was performed on cartridge during sample preparation and on column prior to LC separation.

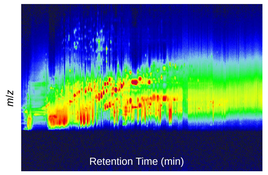


On Column LC Desalting: Multiple column systems can be used for additional desalting. This system was automated using in-house software LCMSnet.

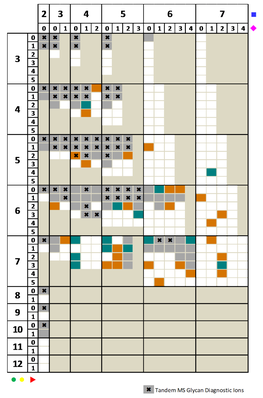




LC-IMS-MS application



LC-IMS-MS Dataset: 128 glycan compositions and hundreds of isomer peaks were detected within 10 ppm mass error from a 120 min LC gradient using a 45 cm graphite capillary column.

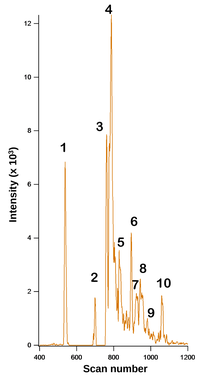


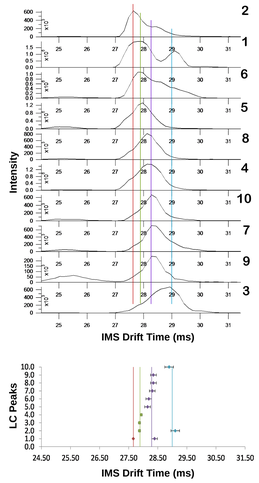
Glycan Library Coverage: Matrix depicting compositions of all the glycans detected. Columns correspond to the number of HexNAc monosaccharides and rows, to the number of Hex monosaccharides.

The detailed axes within each group depicts degree of fucosylation (y-axis) and sialylation (x-axis) present.

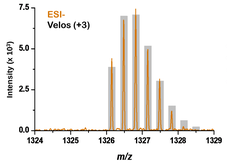
Gray: Common glycans
Orange: SPIN-IMS
Green: IMS only
Tan: Outside of Library

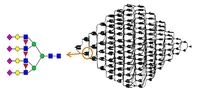
Drift Times: The extracted ion chromatogram for 967.85 depicts several well resolved peaks. Drift profiles for each peak are shown and the drift times calculated.

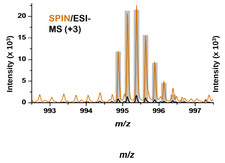


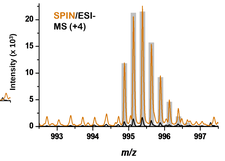


Data quality









Large Glycans: Spectra centered around the $Hex_7HexNAc_6Fuc_3Neu5Ac_4$ glycan. Spectra are shown from two ionization sources (SPIN and ESI) and multiple detectors (Agilent Q-TOF and Thermo LTQ-Orbitrap Velos). The sample and LC system were held constant. This glycan represents one of the largest predicted by the theoretical retrosynthetic glycan library network.

Conclusions

- Peak consolidation improved data quality by removing redundant peaks. Low pH glycan release incorporated deglycosylation and deamidation in one step.
- Two stage desalting eliminated sodiated forms of the glycans.
- Glycan isomers separated using LC-IMS-MS prove information beyond that obtained using LC-MS alone.

Acknowledgements

Portions of this research were funded by NIH (grants R24 RR016354-06A2 and U54 GM62119) and DOE/BER. Research was performed using capabilities developed under the support of the NIH (5P41RR018522-10 and 8 P41 GM103493-10). Significant portions of the work were performed in the Environmental Molecular Science Laboratory, a DOE/BER national scientific user facility at Pacific Northwest National Laboratory (PNNL) in Richland, Washington. PNNL is operated for the DOE by Battelle under contract DE-AC05-76RLO-1830.

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