A chemoenzymatic method for simultaneous profiling of N and O-glycans in one-pot

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Background

- Protein drugs, such as Fc-fusion proteins are often modified by complex glycans with heterogeneity
- Glycosylation can impact safety, stability and efficacy of protein therapeutics, therefore in-depth characterization of N-and O-glycans is critical
- Analysis of protein glycosylation poses major challenges
- No generic method for release of all glycans is available
- Poor ionization of neutral carbohydrates requires derivatization such as permethylation or labeling to enhance detectability of glycans during mass spectrometric analysis

Methodology

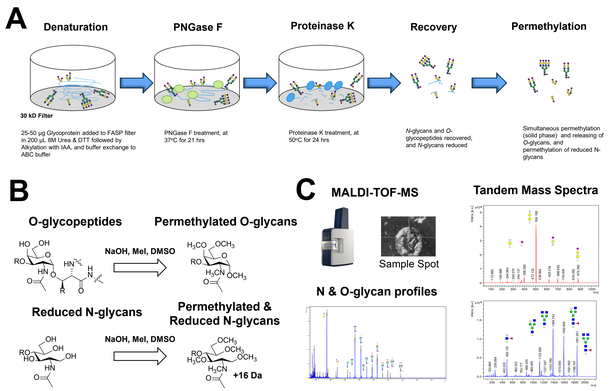

Figure 1. Workflow of the one-pot method for simultaneously analysis of N-, and O-glycans of glycoproteins
A. Isolation of N-glycans and O-glycopeptides in tandem by FASP filter. **B**. Differentiation of permethylated N and O-glycans by unique reducing ends. **C**. Mass Spectrometry analysis of N and O-glycans in one pot by MALDI-TOF/MS.

Disclaimer

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Results

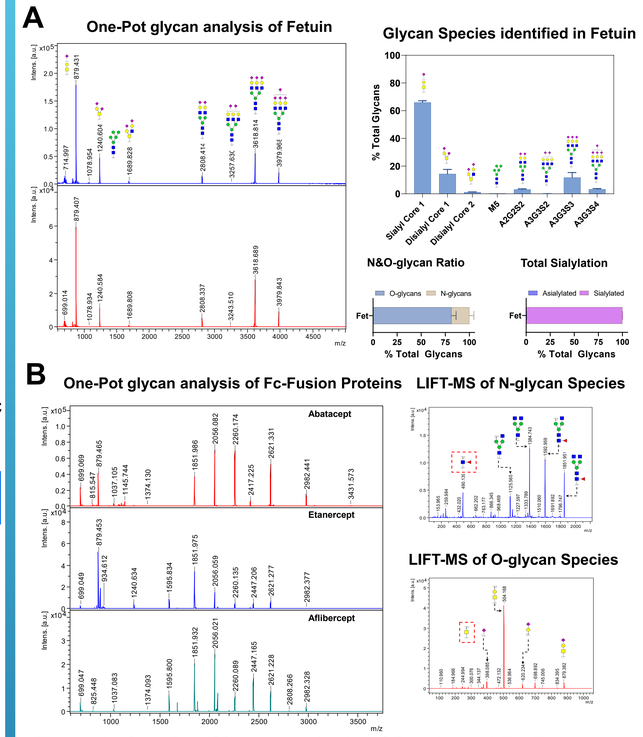

Figure 2. One Pot Glycan analysis of Fetuin and Fc-Fusion Proteins
A. One-pot N and O-glycan profiling of a model glycoprotein, Fetuin
B. Glycan profiling of Fc-Fusion Proteins Abatacept, Etanercept, and Aflibercept and LIFT-MS analysis for differentiation of N- and O-glycans by unique reducing end fragments

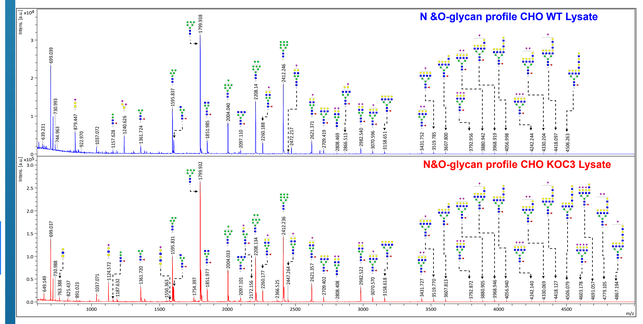

Figure 3. Simultaneous N &O-glycan profiling of WT and KOC3 Cellular Glycoproteins
N- and O-glycans from WT and KOC3 (Cosmc-KO and C3GnT expressing) glycoengineering CHO cell lines were prepared with One-pot methodology..

Results (Continued)

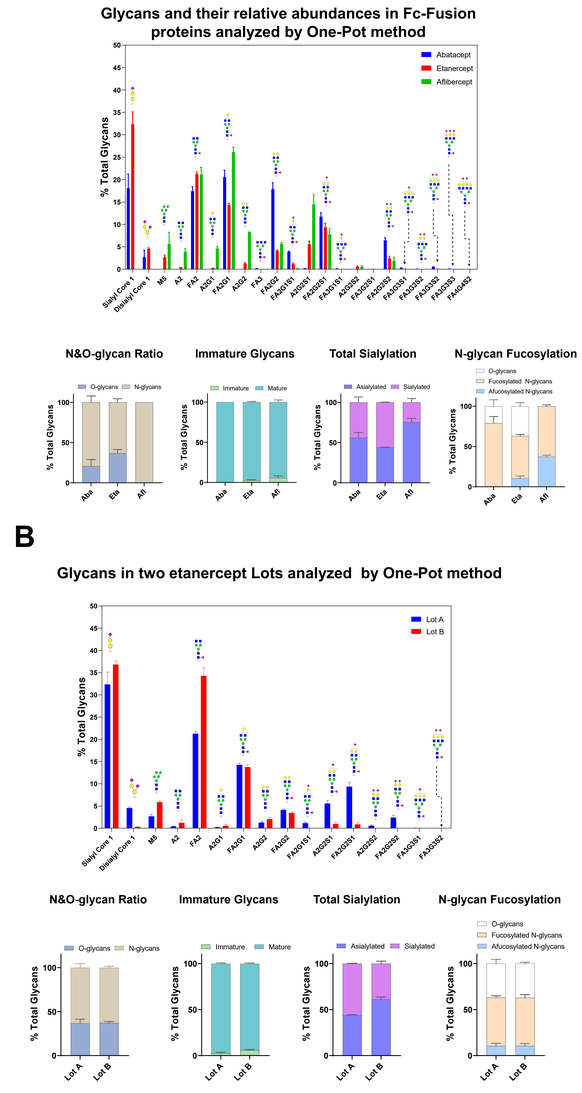

Figure 4. Summary and Reproducibility of One Pot Glycomics Method
A. Summary of unique glycan species and their relative abundances in Fc-Fusion proteins Abatacept, Etanercept, and Aflibercept (Data from 3 independent preparations)
B. Summary of one-Pot glycan analysis of two different lots of etanercept

Summary

- We developed a reproducible method for simultaneously profiling N- and O-glycans from purified proteins and cellular proteins from cell lysates in One-Pot.
- Relative abundances of unique N-and O-glycan species were determined consistently from three independent preparations of Fetuin and Fc-Fusion Proteins
- N- and O-glycans were differentiated by tandem mass spectra based on unique mass shifts (+16 Da) associated by reduced N-glycan core fragments.
- We evaluated our One-Pot method with O-glycoengineering CHO cell lines and identified unique Core 3 O-glycans in C3KO CHO Cells

References

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