COMPARISON OF GLYCOPEPTIDE AND RELEASED GLYCAN ABUNDANCES FOR IgG-BASED THERAPEUTIC ANTIBODIES

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INTRODUCTION

Glycosylation is a post-translational modification (PTM) in the heavy chain of therapeutic monoclonal antibodies (mAb) that can affect the immune response function, making proper glycoform quantification highly important.

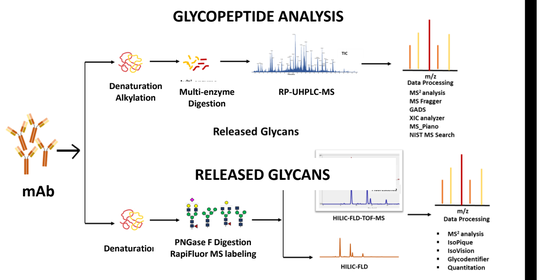
OBJECTIVES

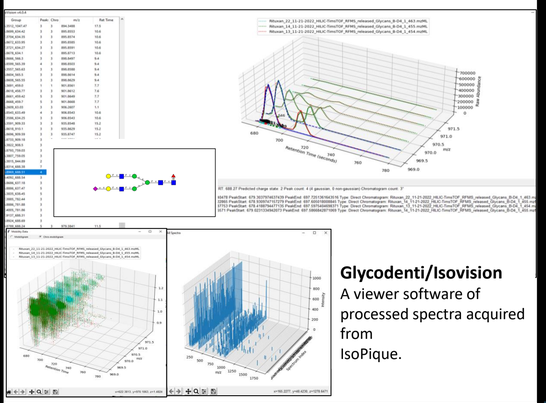
This work compares glycan abundances derived from glycopeptide analysis and peptide modifications to those from fluorescent detection of labeled, released glycans from mAbs.

MATERIALS & METHODS

Brand	Name	Structure	Organism
[a]RM 8671	NISTmAb	Humanized IgG1κ	Murine
Remicade	Infliximab	Chimeric human murine IgG1	Sp2/0-Ag14
Repatha	Evolocumab	Human IgG2	CHO
Rituxan	Rituximab	Chimeric IgG1	CHO

[a]RM=Reference Material
[b]CHO=Chinese Hamster Ovary cells

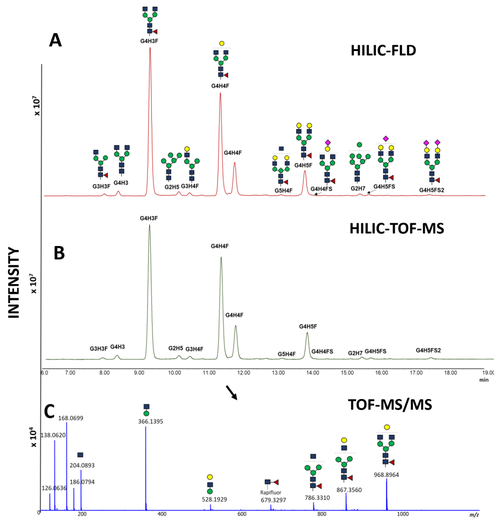




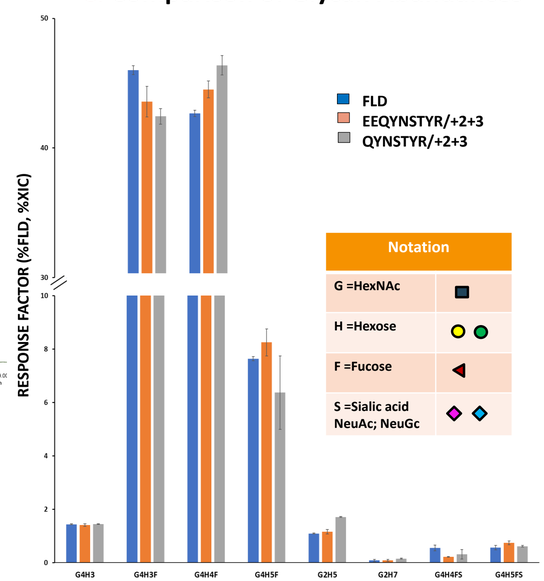
Glycodenti/Isovision
A viewer software of processed spectra acquired from IsoPique.

RESULTS & DISCUSSION

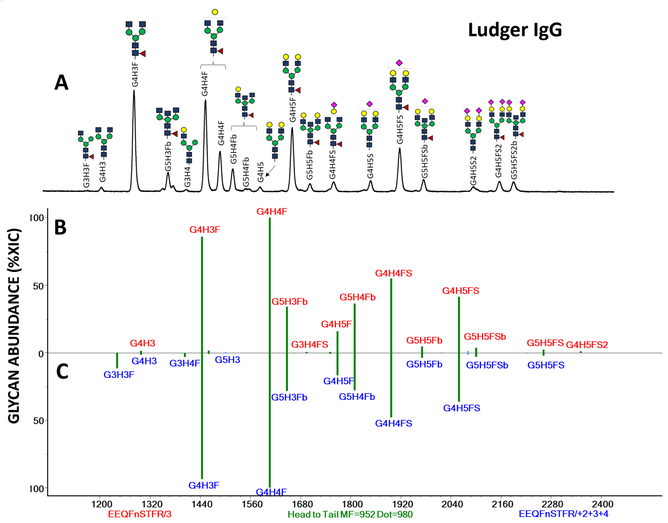
A. Released Glycans from Rituximab



C. Comparison of Glycan Abundances

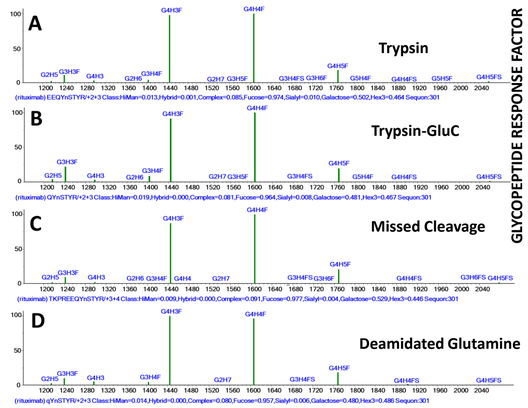


D. Application to other monoclonal antibodies

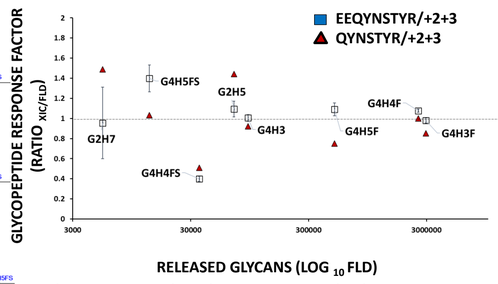


We report the identification and quantification of the glycosylation pattern of mAbs by bottom-up proteomics with released glycan determinations. Glycopeptide abundances (XIC) were compared to fluorescence intensities of the corresponding released and Rapifluor-labeled glycans [1]. A strong linear correlation between the N-glycan abundance levels obtained after RFMS labeling and protein digestion was found over a dynamic range of 500.

B. GADS of Modified Peptides

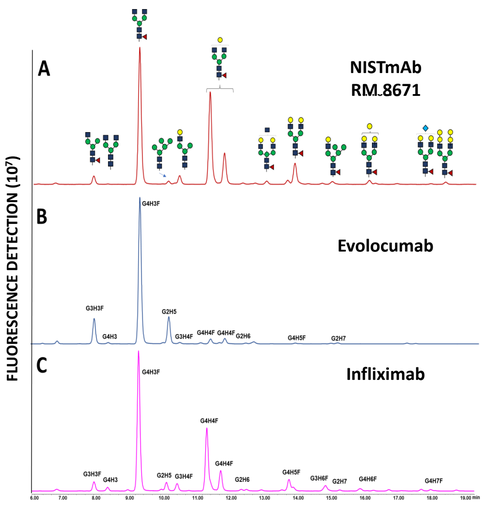


(A) R.EEQYNSTYR.V; Charge states +2 & +3, Number of Spectra (nSpec) ≥ 50 (B) R.EQYNSTYR.V; Charge states +2 & +3, nSpec ≥ 90 (C) R.TKPREEQYNSTYR.V; Charge states +3 & +4, nSpec ≥ 70 (D) E.qYNSTYR.V; Charge states +2 & +3, nSpec ≥ 40.



The principal glycans are high mannose, galactosylated, and fucosylated and can be used for quality control measurements of mAbs from multiple sources or expression systems.

The MS-based values showed increased amounts of G3H3F (G0F-N) in the lower charge state peptides, due to in-source fragmentation of the higher charge-state glycopeptides. Hence, G3H3F was excluded from the analysis. After this exclusion, the range of variation was ≤5% across all data points in therapeutic mAbs.



CONCLUSIONS

Excellent linearity was observed between glycan abundances of glycopeptides and glycan fluorescence from labeled, released glycans. Relative responses of MS and FLD were virtually identical for the fifteen glycopeptides and fifteen fluorescently labeled and released N-glycans examined. This finding demonstrates that relative abundances of glycopeptides measured by mass spectrometry can provide accurate values for relative abundances of the various glycoforms found in proteomics experiments and support the quantitative significance of 'Glycan Abundance Distribution Spectra' (GADS) [2].

REFERENCES

[1] Lauber et.al, *Analytical Chemistry* 2015
[2] Remoroza et al., *J. Proteome Research* 2021