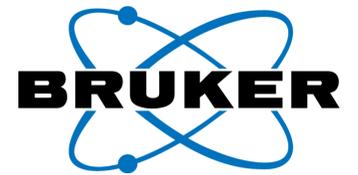


A Simple and Sensitive Method to Extract Glycan Profiles from Peptide Mapping Data



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Introduction

Characterization of therapeutic proteins such as monoclonal antibodies requires a broad range of analyses including the confirmation of the protein sequence as well as the identification of post-translational modifications such as glycosylation. A common workflow for sequence confirmation is the peptide mapping approach, in which tryptic peptides are separated by reversed phase chromatography and further analyzed using high-resolution MS/MS. Typically, N-linked glycans are then analyzed in a subsequent dedicated step after release and reducing end-labelling.

Here we describe a new method to determine glycan profiles directly from peptide maps using analytical LC and the new VIP-HESI ion source and fast MS/MS spectra acquisition using trapped ion mobility separation in a timsTOF Pro 2 instrument.

References

- 1: Hilliard M, Alley WR Jr, McManus CA, Yu YQ, Hallinan S, Gebler J, Rudd PM (2017). mAbs, DOI: 10.1080/19420862.2017.1377381
- 2: 1889617-lcms-188-glycopeptide-analysis-in-peptide-mapping-workflow-ebook.pdf

Methods

1. Tryptic digest of NISTmAb 8671 (Merck) was separated using an Elute UHPLC interfaced with a timsTOF Pro 2 via a VIP-HESI ion source (Bruker) and analysed by PASEF using the standard proteomics method
2. Raw data were processed using BioPharma Compass 2021b (BPC) by a standard peptide mapping method
3. Spectra were submitted to glycan search in BPC using parameters adapted to the tryptic Fc glycopeptides (2)
4. Glycan search results were filtered by score (>40), fragmentation coverage (>40%) and intensity coverage (>40%) providing glycan identification of highest certainty (Fig. 1)

Results

The peptide EEQYNSTYR (NIST Fc:296-304) carries the N-glycosylation site and is routinely obtained after trypsin digestion. Glycan searches of the glycopeptide MS/MS spectra were carried out with this peptide as mass tag – instead of 2-AA/2-AB or RapiFluor. The glycan search yielded 36 specific glycan compositions of different glycan classes, e.g., complex, hybrid, and high mannose structures (Tab 1). Compared to the results of a traditional analysis using released labeled glycans and HILIC separation (1), 27 out of 30 different compositions were found using our glycopeptide analysis method. The three missing compositions were h7n2, h7n3 and h8n5f1. The compositions h4n2, h4n3, h4n4f2, h5n3, h5n3g1, h5n4f2, h6n4g1 and h6n4f2 were additionally detected.

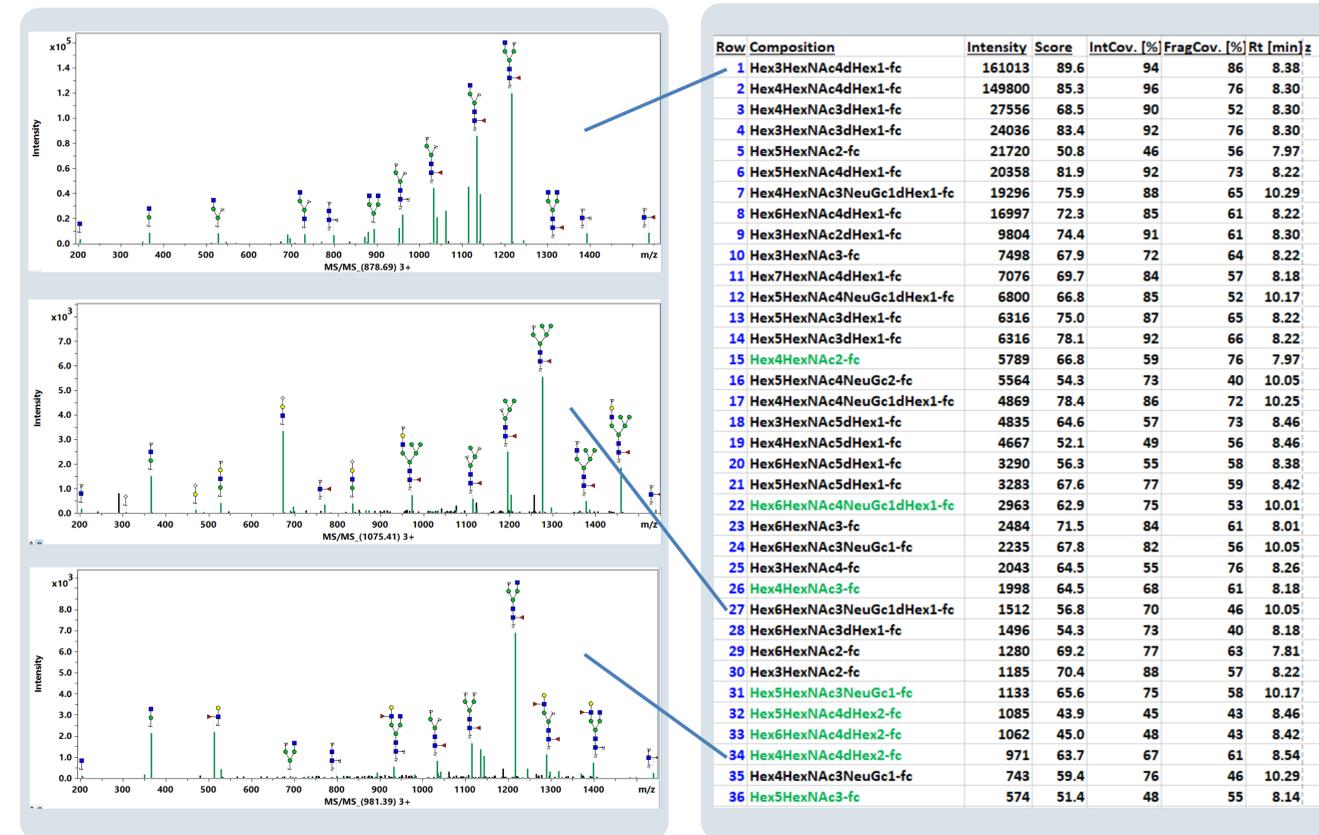


Fig. 1 MS/MS spectra of h3N4F1 (G0F, top), h6n3f1g1 (Man5G1FS hybrid, middle) and h4n4f2 (G1F2, bottom) showing the high spectra quality within a dynamic range of >300 (s. Tab 1). The di-fucosylated structure (bottom) was additionally identified compared to the standard method using released glycans and underlines the search result stringency for such a low abundant compound.

Tab. 1: Glycan search result showing 36 identified glycan compositions from the tryptic glycopeptides of NISTmAb derived from a typical RP-HPLC peptide mapping dataset and PASEF acquisition with the new VIP-HESI ion source. The compositions marked in green were previously not identified using released labeled glycan analysis (1).

Summary

The identification of 36 different glycan compositions using a PASEF peptide mapping dataset derived from RP-HPLC separation, timsTOF Pro 2 acquisition and Biopharma Compass software analysis correlates very well with previous studies using established labelled-glycan approaches. The PASEF technology was key to acquire high quality MS/MS spectra from co-eluting glycopeptides covering a dynamic range greater 300:1. A new ion source (VIP-HESI) together with analytical LC provided high sensitivity with high robustness and enables glycosylation analysis for non-experts.

Conclusions

- A new approach utilizing routine tryptic digest analysis was developed, capable of in-depth characterization of the complex glycan profile of NISTmAb
- The approach removes the usual requirement for dedicated glycan analysis protocols including labeling and LC conditions
- The tryptic NISTmAb peptide mass was used as reducing end mass tag in GlycoQuest glycan searches
- PASEF enabled fast and sensitive MS/MS spectra acquisition of glycopeptides with high dynamic range within a single chromatographic peak
- New VIP-HESI ion source provides high sensitivity for analytical flow analysis

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