

# N-Linked Glycan Profiling for Monoclonal Antibodies with PEAKS AB® Software Platform using LC-MS/MS



Lin He,<sup>1</sup> Jonathan Krieger,<sup>2</sup> Paul Taylor,<sup>2</sup> Baozhen Shan<sup>1</sup>  
<sup>1</sup>Bioinformatics Solutions Inc., Waterloo, ON, Canada  
<sup>2</sup>Hospital for Sick Children, Toronto, ON, Canada

## Summary

N-linked glycosylation plays critical roles in functions of monoclonal antibodies (mAbs). Researchers have paid massive attention to the identification of glycan compositions and structures using different MS or MS/MS techniques. Compared to other fragmentation methods in MS/MS analysis, HCD seems to be an efficient way to assist the identification of intact glycopeptides from LC-MS/MS data derived from an mAb without deglycosylation. However, retrieving the identity and quantity information from the data is always a non-trivial problem. Here we demonstrated how to analyze an MS/MS data set with PEAKS AB® software platform to obtain qualitative and quantitative information of N-linked glycans in an mAb sample.

## Introduction

Monoclonal antibody (mAb) sequencing using multi-enzyme digestion and bottom-up LC-MS/MS has become more and more prevalent because of the emergence of reliable analytical techniques and platforms, e.g. PEAKS AB®. However, retrieving the primary structure of a mAb is only the first step of the comprehensive study. For example, qualitative and quantitative analysis of N-linked glycan forms is one of the critical tasks in biosimilar studies. Here we proposed a practical solution to profile N-linked glycan forms existing in mAb samples by analyzing multi-enzyme digested bottom-up LC-MS/MS data using our PEAKS AB® platform for antibody protein sequence retrieval and glycan form characterization.

## Methods

In-solution endoproteinase digestions of a mAb sample were performed for mAb sequencing analysis. The antibody was reduced, alkylated, and equally divided into 5 aliquots for 5 individual enzyme digestions: Asp N, chymotrypsin, elastase, trypsin, and pepsin following the manufacturer's instructions. After reversed phase cleanup of each digest, samples were analyzed by LC-MS/MS.

The purified peptides were analyzed on an Orbitrap mass spectrometer (Q-Exactive, Thermo Fisher Scientific) outfitted with a nanospray source and EASY-nLC 1000 system (Thermo Fisher Scientific). The instrument method consisted of one MS full scan followed by 10 data-dependent MS/MS scans at high resolution both in the Orbitrap mass analyzer. The dynamic exclusion was applied using a setting of 8 seconds. The raw data files were collected for data analysis.

## Results

The sequencing and N-linked glycan analysis were carried out in PEAKS AB® software installed on a 8-core desktop PC with Microsoft Windows 7. The raw files were loaded into PEAKS AB for antibody protein sequencing and PTM analysis thereafter. The heavy chain was identified with 100% coverage and 100% accuracy (I/L not distinguished) after a 1-hour data analysis. PTM Finder, an integrated module, was used to search for PTMs existing in the sample, including N-linked glycans. Fig. 1 shows the sequencing result around the N-linked glycosylation site in the heavy chain and all the reported N-linked glycans.

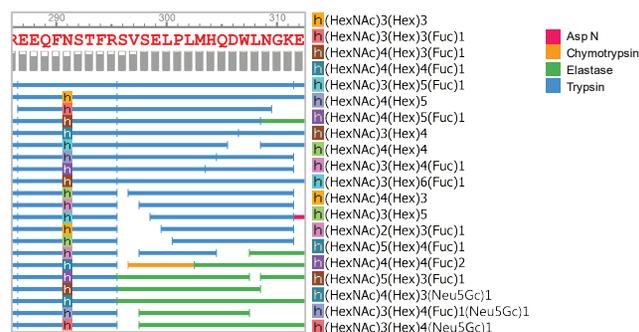


Figure 1. The protein sequence of the heavy chain was identified completely and all reported N-linked glycans were shown in this comprehensive peptide mapping view in PEAKS AB®.

Tuning the collision energy in HCD fragmentation helps generate high quality tandem mass spectra for intact glycopeptides. The glycan was fragmented to generate peak ladders but no peptide bond was broken, in contrast to the peptide without N-linked glycosylation as shown in Fig. 2.

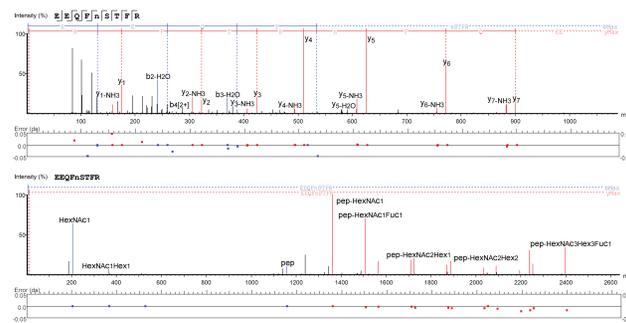


Figure 2. Comparison between the peptide-spectrum matches of the same tryptic peptide with and without N-linked glycan attached on the Asn. PEAKS AB® gives both peptide (top) and intact glycopeptide (bottom) identification with high confidence.

PEAKS AB® identified peptide features from LC-MS directly without the assistance of peptide identification. For intact glycopeptides reported in the result, PEAKS AB® easily assigned these features to reported glycopeptides and retrieved the extracted ion chromatogram (XIC) information (Fig. 3).

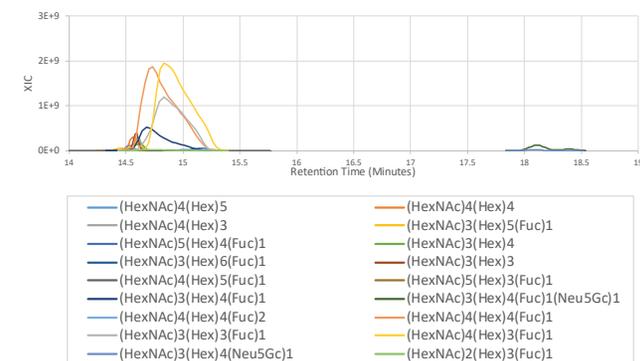


Figure 3. XIC of the tryptic peptide EEQFNSTFR with different N-linked glycan forms in PEAKS AB®

The N-linked glycan profiling (Fig. 4) was based on the XIC information extracted for the peptide with each glycan form.

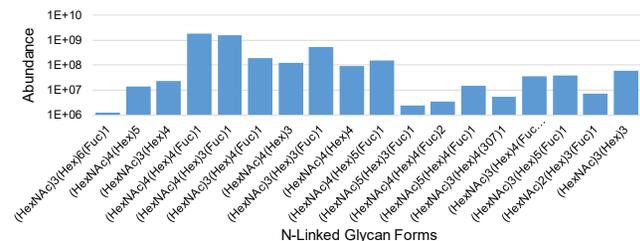


Figure 4. Profile of different N-linked glycan forms

## Conclusion

- PEAKS AB® offers complete de novo sequencing for antibody proteins, using reasonable computational resources.
- PEAKS AB® offers qualitative and quantitative analysis for N-linked glycans, as well as other possibly existing PTMs in the sample.