PEAKS AB – A Reliable Workflow for Monoclonal Antibody Characterization Using LC-MS/MS

PEAKS®
Complete Software for Proteomics

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Overview

Purpose: To propose a reliable workflow for monoclonal antibody characterization
Methods: Combining the latest LC-MS/MS technology & PEAKS solutions
Results: An LC-MS/MS based workflow for monoclonal antibody characterization

Introduction

The development of reliable workflows using liquid chromatography-tandem mass spectrometry (LC-MS/MS) for antibody characterization has become an active research area. Furthering beyond the identification of the primary sequence, researchers are also interested in post-translational modifications (PTMs), degradation analysis, and variant characterization. To fulfill these requirements, we propose PEAKS AB to provide a reliable workflow for monoclonal antibody characterization from the purified antibody sample to MS/MS data analysis.

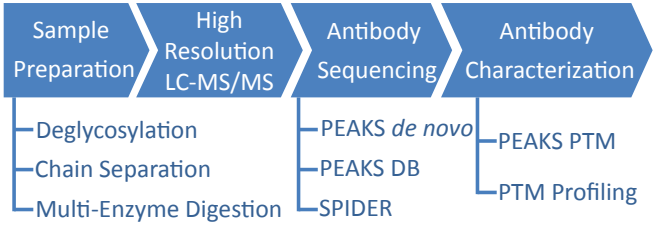

Figure 1. Workflow of monoclonal antibody characterization.

Methods

The PEAKS AB workflow (Figure 1) includes:

- Sample preparation: deglycosylation, heavy & light chain separation, and multi-enzyme digestion
- High-resolution LC-MS/MS
- PEAKS-based antibody sequencing: PEAKS de novo, PEAKS DB, SPIDER
- PEAKS-based PTM identification tools: PEAKS PTM, PTM Profiling, Peptide Mapping

Result

This workflow takes advantage of the latest LC-MS/MS technology, and both the existing modules and new released features of our leading proteomics software, PEAKS 7.5. In our experiment, a human monoclonal antibody sample was separated into the light and heavy chains by SDS-PAGE, reduced with DTT, alkylated with iodoacetamide, and digested with six different enzymes (Asp N, chymotrypsin, Glu C, Lys C, proteinase K, and trypsin). Peptide mixtures were subjected to a Thermo Easy-nLC UPLC system and then a Thermo Q-Exactive Orbitrap mass spectrometer. MS data was collected at a 70K FWHM resolution and MS/MS data at a 17.5K FWHM resolution. From the light chain, six mass spectra files, which contained 52,033 MS scans and 46,576 MS/MS spectra in total, were collected and then analyzed using PEAKS 7.5.

All MS/MS spectra were searched against the UniProt database (Release 2015-08). The parent and fragment error tolerance were set as 10 ppm and 0.02 Da, respectively. We allowed non-specific cleavage at one end of the peptide and considered at most three missed cleavages. Carbamidomethylation on Cys was set as a fixed PTM. Oxidation on Met, and deamidation on Asn and Gln, were set as variable PTMs. At most three variable PTMs were allowed per peptide. With 0.1% of false discovery rate (FDR) at peptide-spectrum match (PSM) level, majority of the constant region sequence of the light chain Ig kappa could be found in the current database with 100% coverage. However, the variable domains containing CDRs could not be identified in the database. The best matching protein covered 61% of the light chain. Homology search using SPIDER was then carried out to match de novo only sequences to database sequences and helped to find the amino acid substitutions. Iteratively revising the target protein sequence according to SPIDER results and searching the MS/MS data against the new sequence, we finally identified the full sequence of the target light chain, where every amino acid was mapped within more than 20 peptides and the confidence level is more than 95% (Figure 2). In Figure 2, each blue bar denotes a peptide. Multiple PSMs which correspond to the same peptide sequence are listed together in the same blue bar.

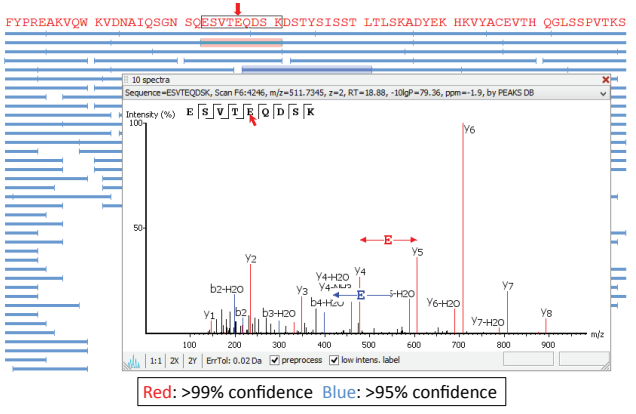

Figure 2. Full sequence coverage and validation at the amino acid level based on direct mass fragment evidence.

We applied PEAKS PTM on the identified antibody sequence for PTM identifications. In total, 14 PTMs were reported as frequently (detected in > 50 PSMs) observed ones in the antibody sample. Figure 3 shows an example of the deamidation PTM profiling result. In Figure 3, each bar represents a modification site on the antibody sequence, where the position of the modification is indicated in the x-axis. The blue and the red sections show the percentages of accumulated quantities of unmodified and modified peptides in the spectral data, respectively.

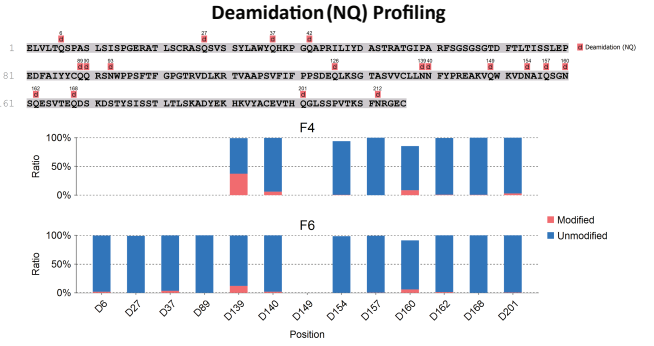

Figure 3.An example of quantitative analysis of PTMs.

PEAKS AB further provides the ability to determine the identity of Leu and Ile in the final antibody sequence. By exploiting enzyme cleavage preferences [2] and database frequencies, we are able to distinguish these two amino acids. Figure 4 shows an example of how Leu and Ile were differentiated using the chymotrypsin cleavage preference.

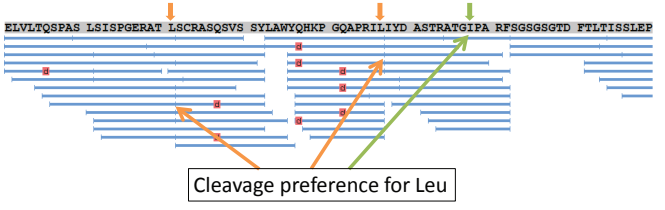

Figure 4. Identification of Leu and Ile based on enzyme cleavage preference.

Conclusion

PEAKS AB provides a reliable workflow for comprehensively characterizing monoclonal antibodies. Taking advantage of both the latest LC-MS/MS technology and features presented in the PEAKS software package, the quality of the antibody characterization is guaranteed.

References

[1] B. Shan & L. Xin. Integrating de novo sequencing and database search for monoclonal antibody sequencing. ABRF 2013.
[2] C. Poston, et al. A quantitative tool to distinguish isobaric leucine and isoleucine residues for mass spectrometry-based de novo monoclonal antibody sequencing. J. Am. Soc. Mass Spectrom. 25.7 (2014): 1228-1236.

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