Towards the automated monoclonal antibody sequencing and characterization from LC-MS/MS data

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PEAKS® AB
Antibody Characterization Software

Overview

Purpose: To develop an automated software tool to accelerate the identification and characterization of monoclonal antibodies (mAbs) from LC-MS/MS data.

Methods: A novel algorithm is proposed to assemble de novo sequencing tags from LC-MS/MS data. After acquiring the correct sequence, we can apply PEAKS functions to characterize the antibody.

Results: A software package, PEAKS AB Software, is provided to fulfill the requirements of mAb sequencing, validation, and characterization.

Introduction

Monoclonal antibodies have been emerging as one of the most active areas of biologic drug therapies. Before clinical use, it is a regulatory requirement to comprehensively verify an antibody. The regulatory standards require extensive protein sequencing, including assessment and identification of numerous molecular entities and closely related variants. It is also fundamental to provide detailed characterization of the existing PTMs. These necessary requirements put in place are creating a strong demand for reliable, fast, and efficient solutions for antibody characterization. It has been proven that current LC-MS/MS technologies can provide an accurate and sensitive solution for both mAb sequencing and characterization. However, no integrated pipeline is readily used to transfer LC-MS/MS data to mAb knowledges for researchers. We propose PEAK AB Software which starts from a novel algorithm to construct the mAb sequence from de novo sequencing results. The constructed sequences are validated and characterized based on our leading software packages and expertise accumulated for years from our PEAKS AB Service [1].

Sample Preparation

The mAb sample is prepared using a standardized procedure for LC-MS/MS analysis. The heavy and light chains of the mAb are separated by SDS-PAGE. Each chain is reduced, alkylated, and then digested using multiple enzymes. The digested peptides are extracted and subjected to LC-MS/MS analysis and the resultant raw data files are collected for further mAb sequencing and characterization.

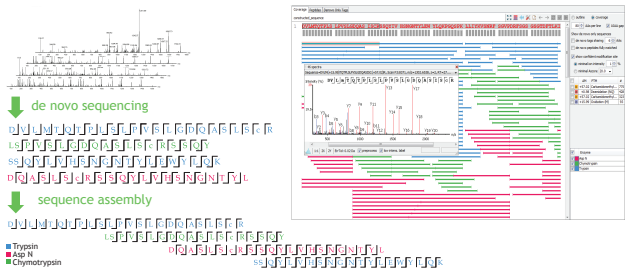

Figure 1. Antibody protein sequencing using PEAKS AB Software.

Antibody Protein Sequencing

After directly loading the raw data files and pre-processing each tandem mass spectrum, PEAKS AB Software generates de novo sequencing peptides from the tandem mass spectra and then assemble these peptides into antibody sequences using a novel algorithm. The sequences and its supporting peptide-spectrum matches (PSMs) are then presented into a protein coverage view for users' evaluation (Fig. 1).

PTM and Variant Analysis

Based on the PEAKS PTM function integrated in PEAKS AB software, possible PTMs and sequence vaiants existing in the mAb sample are identified and reported in a user-friendly graphic interface (Fig. 2). PEAKS AB Software also provides an improved PTM profiling function to visualize the quantities of PTMs and sequence variants. For each modified amino acid, PEAKS AB Software shows the relative amount of the modified (red) and unmodified (blue) version of the related fully digested peptide. Furthermore, the quantitative analysis result of PTMs and variants can be easily exported into figures or spreadsheets for reports or further analyses.

PEAKS AB Software also provides the ability to examine each PSM visually. For each modified peptide, one of its unmodified counterparts is show simultaneously for users to analyze the supporting ions directly and easily (Fig. 2).

Figure 2. Qualitative and quantitative analysis of PTMs and sequence variants using PEAKS AB Software.

Sequence Validation

PEAKS AB Software provides comprehensive validation of a given antibody protein sequence using LC-MS/MS data to help customers on their product quality control. The LC-MS/MS data from the sample, which is supposed to mainly contain the antibody with the give protein sequence, is loaded into PEAKS AB Software and a protein sequence will be reported accordingly. Comparing the given (reference) and the calculated (constructed) protein sequences using the protein coverage views and the annotated chromatograms, customers can easily evaluate the quality of their antibody products (Fig. 3).

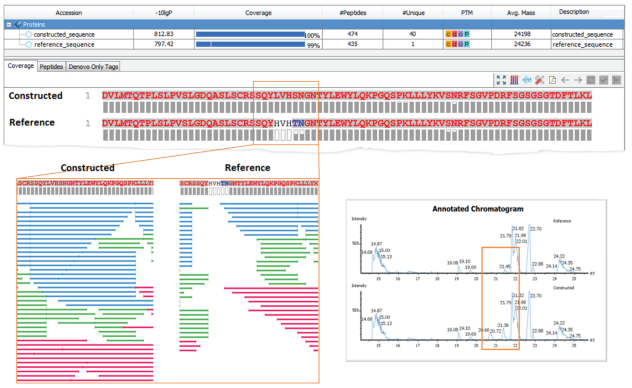

Figure 3. Sequence validation using PEAKS AB Software with a given antibody protein sequence.

Using the calculated intensity for each peptide, PEAKS AB Software is able to display the sequence coverage as an intensity-based map to help further validating the constructed protein sequence from the antibody product (Fig. 4).

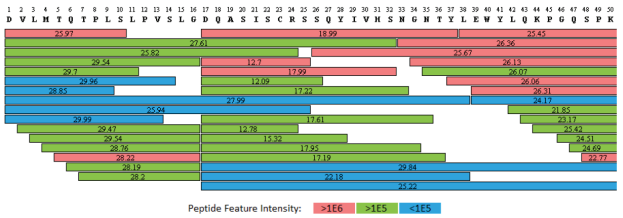

Figure 4. Sequence coverage of the constructed antibody protein sequence from the sample.

Conclusion

After providing PEAKS AB Service for years we integrated our accumulated experiences and expertise into the new PEAKS series software, PEAKS AB Software. This new software concentrates on the mAb sequencing and characterization and provides powerful capacity to make LC-MS/MS based antibody studies easier.

Reference

[1] B. Shan & L. Xin. Integrating de novo sequencing and database search for monoclonal antibody sequencing. ABRF 2013.