Towards the Comprehensive Mass Spectrometry-based Biopharmaceutical Analysis using PEAKS MAPS

Lin He, Yi Liu, Weiping Sun, Di Wang, Wen Zhang, Tom Yang, Baozhen Shan

Bioinformatics Solutions Inc.

Overview

Purpose: To develop an automated bioinformatic workflow to accelerate biopharmaceutical analysis using LC-MS Multi-Attribute Method.

Methods: Intact mass, database search, and *de novo* sequencing were utilized for primary structure characterization of antibodies.

Results: A software suite, PEAKS MAPS (or Multi-Attribute Pharma Suite), was developed to fulfill the thorough requirements of antibody protein sequencing and characterization from LC-MS Multi-Attribute data.

Introduction

High-resolution mass spectrometry (MS) is widely used for the characterization of therapeutic proteins, such as monoclonal antibodies (mAbs), in both the discovery and development stages. More specifically, MS is a highly effective technique to measure the molecular weight of intact proteins, retrieve amino acid sequence information, perform peptide mapping analysis, and identify post-translational modifications (PTMs) and sequence variants. Further in-depth studies involve disulfide bond linkage analysis and glycan profiling. However, the higher performance of emerging instruments outstrips the capacity of the data analysis. A fully equipped analytical pipeline handling the aforementioned tasks is highly demanded by both industrial and academic communities. Here we propose a novel software suite, PEAKS MAPS, which is composed of three major components, including PEAKS Intact ID, PEAKS BioValidate and PEAKS AB, to readily translate experimental LC-MS data to comprehensive knowledge.

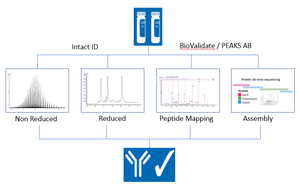


Figure 1. Workflow of Multi-Attribute Methods

Intact Biotherapeutic Mass Analysis by PEAKS Intact ID

The intact, non-digested, NIST mAb was analyzed by LC-MS using size exclusion chromatography (SEC) coupled to a Bruker Daltonics (Bremen, Germany) maXis Q-TOF mass spectrometer. Using the PEAKS Intact ID module, we could accurately deconvolute the intact mass spectrum and annotate the deconvoluted peaks with theoretical masses calculated from the input protein sequences (Figure 2). Further intact mass analysis of subunits of the NIST mAb generated from IdeS digestion was performed on the same mass spectrometer. Table 1 lists the intact masses deconvoluted from the LC-MS data.

Mass	Relative Abundance (%)	RT Range	Modification	Matched Mass Error (ppm)
Isotope Groups				
148037.52	56.26	7.44	2*Gln->Pyro-Glu 2*Lys-Trunc 2*A2G0F 16(S-S)	4.7
148200.64	100	7.44	2*Gln->Pyro-Glu 2*Lys-Trunc 1*A2G1F 1*A2G0F 16*(S-S)	-1.9
148362.86	84.5	7.44	2*Gln->Pyro-Glu 2*Lys-Trunc 2*A2G1F 16*(S-S)	-2.4
148523.75	38.22	7.44	2*Gln->Pyro-Glu 2*Lys-Trunc 1*A2G1F 1*A2G2F 16*(S-S)	6.0

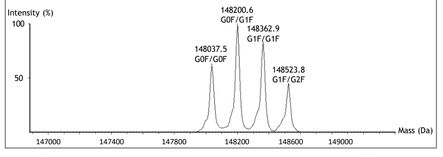
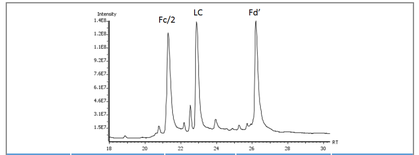


Figure 2. Deconvoluted masses of the intact NIST mAb



Subunit	Modification	Mw. Ref	Mw. Sample	ΔMw (ppm)
Fc/2	G0F	25235.98	25235.52	-18.2
	G1F	25398.12	25397.59	-20.9
	G2F	25560.26	25559.89	-14.5
LC		23127.51	23127.35	-6.9
Fd'	pQ	25688.86	25687.94	-35.8

Table 1. Deconvoluted intact masses of NIST mAb LC-MS data

Automated Comparative Peptide Mapping by PEAKS BioValidate

The PEAKS BioValidate component of PEAKS MAPS is a comprehensive peptide mapping module, furnished with advanced peptide feature detection, peptide-feature association, comparative analysis/view, and other bundled functions. The module offers a detailed, user-friendly interface to easily interpret the extracted chromatogram (XIC), and peptide mapping to analyze the MS spectra. By highlighting multiple features from the features results table, direct comparisons can be made between multiple samples in the (XIC) view as seen in Figure 3.

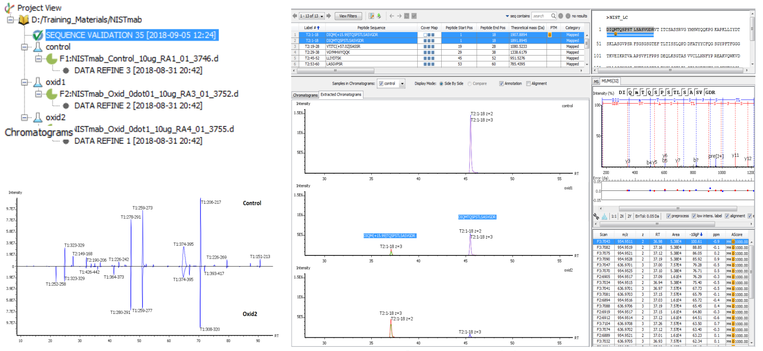


Figure 3. Comparative view of PEAKS BioValidate

Complete Protein *De Novo* Sequencing & Validation by PEAKS AB

To facilitate the mAb discovery stage, PEAKS AB (1) an efficient and accurate protein *de novo* sequencing module within PEAKS MAPS was used. Furthermore, PEAKS AB provided (2) a comprehensive sequence validation function for the amino acid level verification between the given antibody protein sequences and the mAb sample.

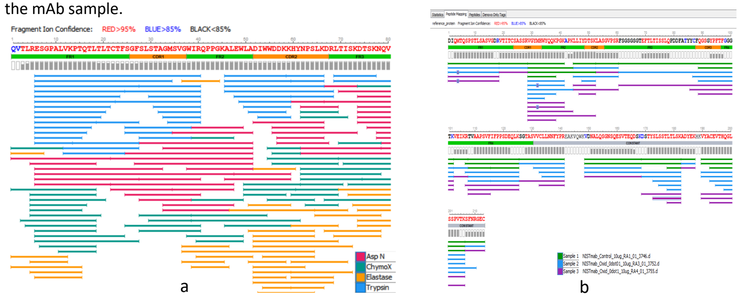


Figure 4. Protein view of PEAKS AB

(a) Complete protein *de novo* sequencing using four-enzyme digests of the NIST mAb. (b) Sequence validation using tryptic digests from three differently treated NIST mAb samples.

Beyond Traditional LC/MS-Based Peptide Mapping

The design of the PEAKS MAPS platform was intended to help further analyze each sample by detecting post-translational modifications (PTMs) and sequence variants, identifying disulfide bond linked peptides, detecting N-linked glycans, and exporting abundant un-mapped peptide features. An example of how the software suite extracts this information is shown below in Figure 5.

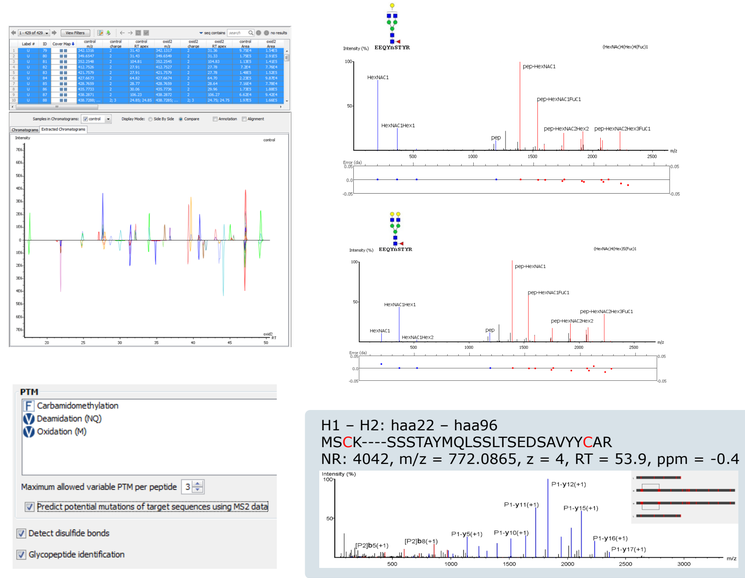


Figure 5. Beyond peptide mapping in PEAKS BioValidate

Conclusion

With three stand-alone modules for intact biotherapeutic mass analysis, complete protein *de novo* sequencing & amino acid-level sequence validation, and automated comparative peptide mapping, PEAKS MAPS can accelerate the biopharmaceutical analysis with LC-MS Multi-Attribute Method.

References

[1] N. Tran, et. al, Complete De Novo Assembly of Monoclonal Antibody Sequences. Scientific Reports, 6, 2016.

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

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