Antibody Sequence Validation by Peptide and Disulfide Bond Mapping

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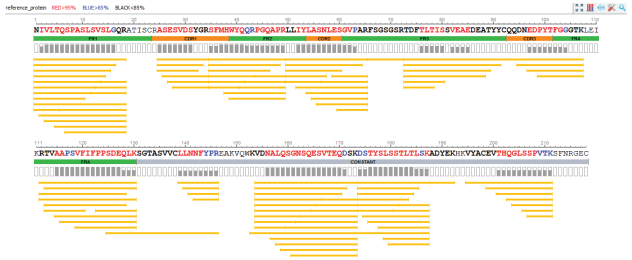
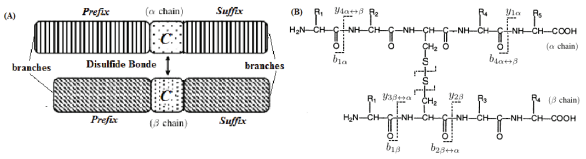
Summary
The purpose of antibody sequence validation is to unambiguously confirm reference sequences and verify the integrity of the analytical data from orthogonal enzyme digestions. Typically, the protease digestion of a sample protein is achieved under reduction conditions, which deliberately erases the native disulfide bond connections. In this work, we introduced a new algorithm for interpreting disulfide linkage patterns from tandem mass spectra. Furthermore, we present an new workflow for antibody sequence validation by combining both peptide mapping and disulfide bond mapping to increase sequence coverage.

Sample Preparation and Peptide Mapping
The mAB sample is digested under non-reduction conditions to avoid the cleaving of native disulfide bonds within and between light and heavy chains. The LC-MS/MS datasets generated from the non-reduced conditions are refined and search against the reference sequences by PEAKS AB® to identify confident peptides. Peptides reported with a score above a specific threshold are kept and mapped to the reference sequences.

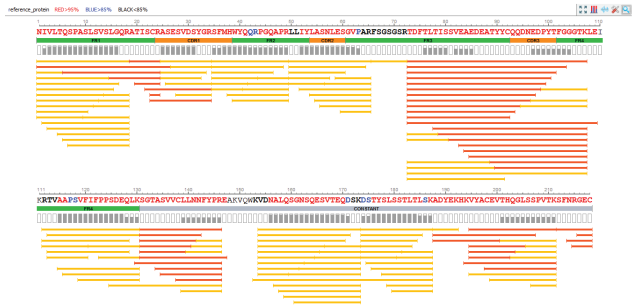


Coverage 89%

Matching Spectrum with Disulfide Linkage Structure
All unmapped MS/MS spectra from the non-reduced dataset, including the unidentified spectra and identified spectra with a score lower than the specific threshold are collected. They are then analyzed by a special module integrated into PEAKS AB® that can identify disulfide linked peptides from the input tandem mass spectra. PEAKS AB® is capable to consider each disulfide linkage structure as a tree in which the bonded location between two cysteines is treated as root of the tree.

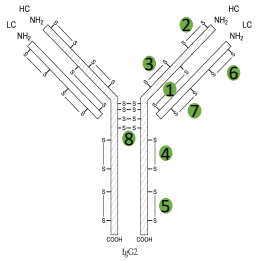


Disulfide Bond Mapping
The purpose is to map the identified disulfide structures to related locations on the reference sequences. A typical disulfide linkage structure containing two individual peptide chains will normally be mapped to two different locations on the reference sequences.

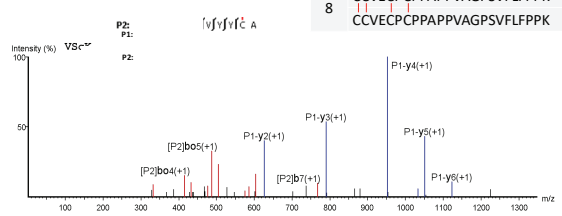


Coverage 96.8%

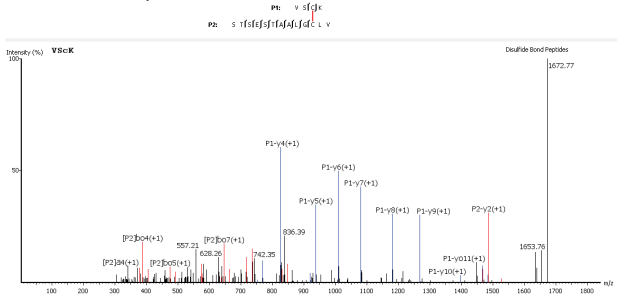
Native Disulfide Bond Mapping
The PEAKS AB® sequence validation module, equipped with disulfide bond analysis, is used to validate the given reference antibody sequences using LC-MS/MS datasets, which are collected from the digestion of an IgG2 antibody sample prepared under non-reduction conditions.



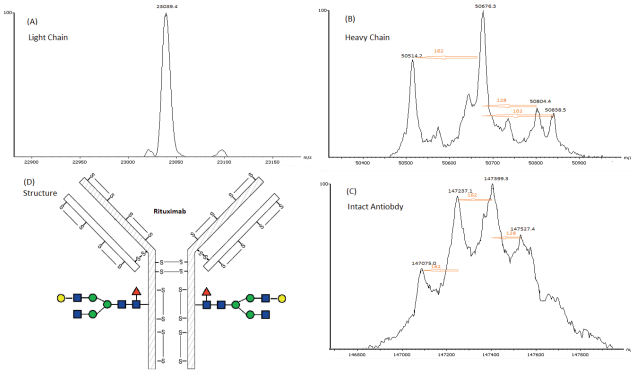
	Peptide
1	SFNRGEC GPSVFPLAPCSR
2	VSCK SEDTAVYYCAR
3	STSESTAALGCLVK HC[174]-C[201]-HC[206]
4	HC[253]-C[258]-HC[285] CK
5	NQVSLTCLVK WQQGNVFSCSVMHEALHNHYTQK
6	ATISCR TDFTLTISSVEAEDEATYYCQQDNEDP YTFGGGTK
7	SGTASVVCLLNNFYPR VYACEVTHQGLSSPVTK
8	CCVECPCPPAPPVAGPSVFLFPPK CCVECPCPPAPPVAGPSVFLFPPK



Non-Native Disulfide Bond Findings
Besides the mapping of native disulfide bonds in an antibody, the integrated module has the capability to find some non-native disulfide bonds, as demonstrated below.



Intact Mass Verification
PEAKS AB® has the ability to characterize an antibody sequence from different perspectives. A new integrated module can be used to determined the intact mass of the heavy and light chains of the antibody. Comparative studies regarding intact mass determination for the intact antibody, as well as the reduced heavy/light chains are shown below.



The mass of the major peak for the intact antibody is 147399.3, the major mass values for heavy chain and light chain are 50676.3 and 23039.4 respectively. We can observe that there exists a mass difference, calculated as 147399.3-(50676.3+23039.4)*2≈32, corresponding to the mass reduction of 16 native disulfide bonds in the antibody Rituximab. Besides the major peaks, we are also able to observe some minor peaks that correspond to different glycoforms in the heavy chain.

References
[1] Tran, N.H. et al., Complete De Novo Assembly of Monoclonal Antibody Sequences. Scientific Reports. 01/09/2016.
[2] He, L. & Shan, B., PEAKS AB: A Software Platform for LC-MS/MS based Therapeutic Protein Characterization, 20/3/2017, US HUPO.

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