Thermo Scientific
PepFinder Software

A New Paradigm for Peptide Mapping

For Conclusive Characterization of Biologics

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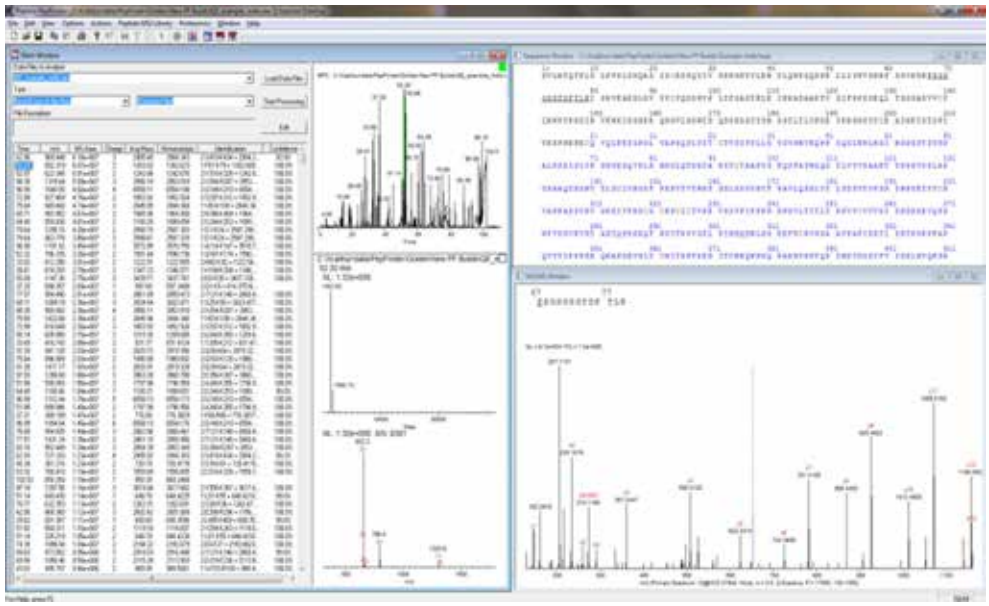
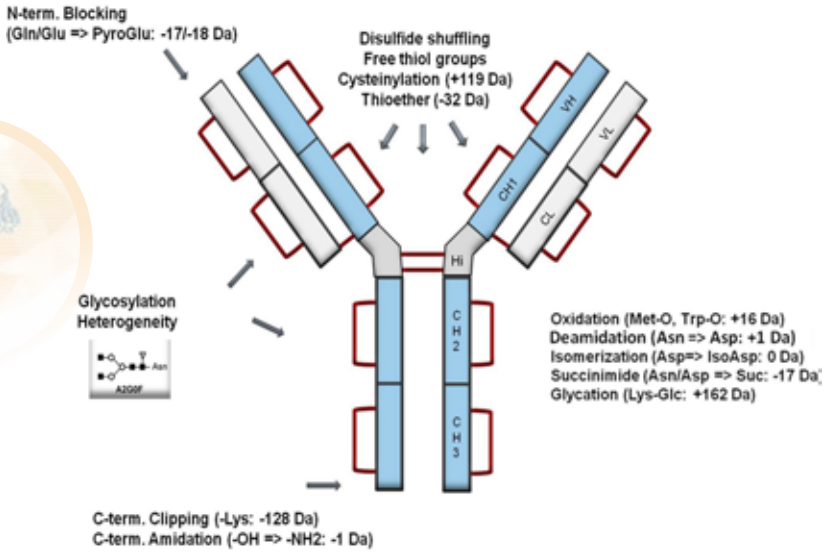
Deep Protein Characterization Is Crucial

Pharmaceuticals have historically been small molecules. Today however, many newly approved drugs are derived from proteins. For protein therapeutics to be effective, they must be produced in biologically active forms, which require proper folding, and post-translation modifications (PTMs). Detailed characterization of these modifications is crucial. A comprehensive verification of the protein's (amino acid) sequence, assessment and identification of impurities in a recombinant protein drug product along with a detailed characterization of the existing PTMs, is a regulatory requirement prior to its approval for clinical use.



Thermo Scientific™ PepFinder™ software provides accurate characterization of biologics and uses multidimensional dynamic search capabilities to automatically reduce complex data into a concise and informative report. With this new paradigm for peptide mapping, confidence in the results is increased while significantly reducing the data analysis time.

Automated Identification and Relative Quantitation of Proteins and Variants



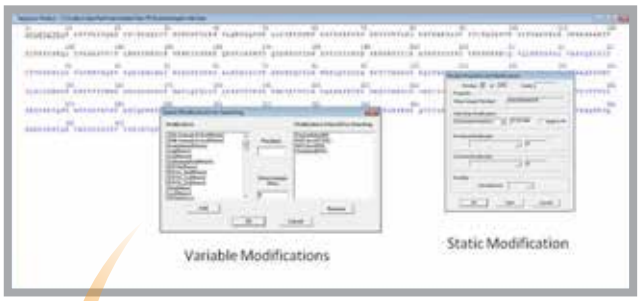
Analytical Challenge in Characterization of Biologics

Thorough characterization of heterogeneities is necessary for the reproducible and safe production of biologics such as antibodies. High resolution accurate mass MS provides accurate information on various protein properties, such as intact molecular mass, glycosylation forms, amino acid sequence, post-translational modifications, disulfide linkages as well as higher-order structural information.

PepFinder software is a powerful new tool for in-depth characterization of biotherapeutic proteins. It automates the analysis of LC-MS and MS/MS data to provide identity and relative quantitation of proteins, variants, and low level post translational modifications (PTMs). Using the industry-leading, high-resolution, accurate-mass Thermo Scientific™ Orbitrap™ technology with PepFinder software, the most accurate and confident PTM profiles and identifications can be achieved more quickly than ever before. This novel software allows for:

- Fast automated component detection- peptide mass, retention time and abundance, with background subtraction to improve overall signal to noise.
- Peptide identification is performed using full MS data or MS/MS.
- Extra confidence in peptide identification is achieved by using a novel ms/ms predictive algorithm.
- Quantification of modifications, including glycoforms, deamindation, oxidation, custom modification (e.g. drug conjugates) and many more.
- Error tolerant search allows for identification of unspecified modifications and amino acid substitutions.
- Characterization of disulfide linkages including non predictive bonds and disulfide scrambling.
- Automated modification summary report for rapid monitoring of modification site in a single sample or across multiple samples in a study.
- Comprehensive sequence coverage map for target proteins

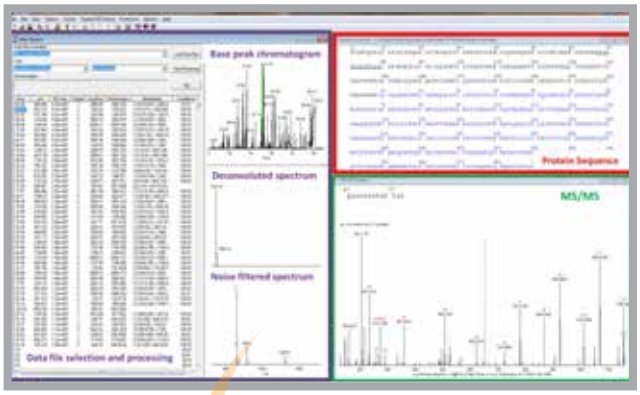
Step 1 Sequence window



Step 1: Experimental Setup - Target proteins and data file selection:

- Define target protein sequence, and PTM's to search including mass window for unspecified modifications.
- Select raw data file
  - Multiple files can be processed at the same time allowing for comparison across studies.

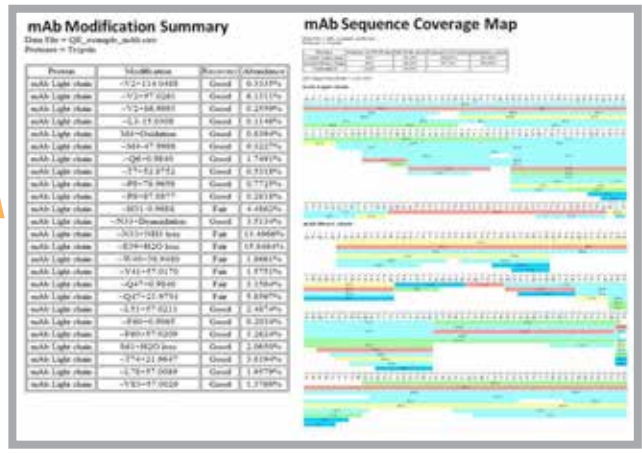
Step 2 Main, Sequence, and MS/MS windows



Step 2: Data Processing

- Component detection:
  - Fast intelligent peak detection and integration.
- Peptide identification
  - A novel identification algorithm predicts MS/MS fragmentation which provides more confidence in peptide identification for all modes of fragmentation types such as CID, ETD and HCD.

Step 3 Modification summary and sequence coverage map



Step3: Report Generation and Manual Review

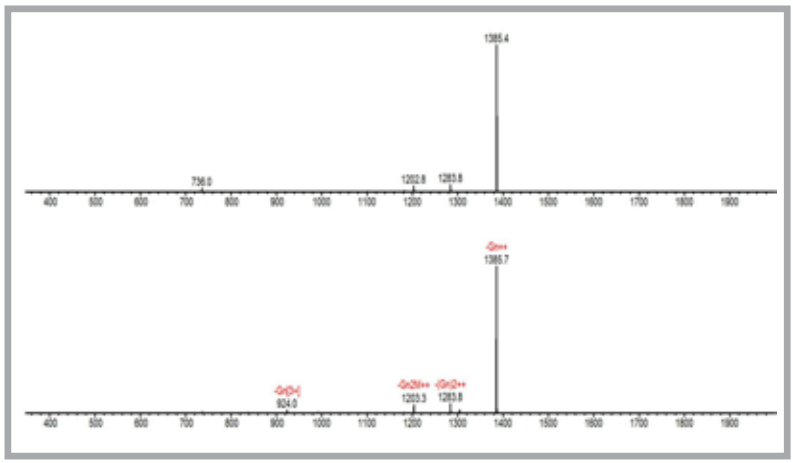
- Reports:
  - Automated sequence coverage map and modification site summary report with recovery information and abundance of specific modifications in one sample or comparison across samples.
- Data review:
  - Interactive visual display of software predicted and experimental MS/MS spectrum.

Increased Confidence Using a Kinetic Model for Peptide MS/MS Fragmentation Prediction

PepFinder software uses a novel predicted MS/MS algorithm to identify peptide sequences. Other algorithms are available which calculate the *m/z* of the fragment ions, the empirical predicted model. But unlike others, PepFinder software also determines the fragment's relative abundance, using the unique kinetic model which is vital when fragmentation is limited.

Glycoeptide identification for a Glycopeptide CSRFPNATDKE (N34+A4Go)

The identification of this glycopeptide was assigned by comparing the measured spectrum (B) to the kinetic model predicted spectrum (A). The fragmentation comes almost exclusively from the sugar. With the lack of peptide sequence information, other search engines would be unable to identify the peptide. PepFinder software is able to predict the spectra and make a confident assignment based on the prediction even though the MS/MS spectra has very few fragment ions and no peptide backbone ions. The lack of information in the MS/MS spectra proves the match.

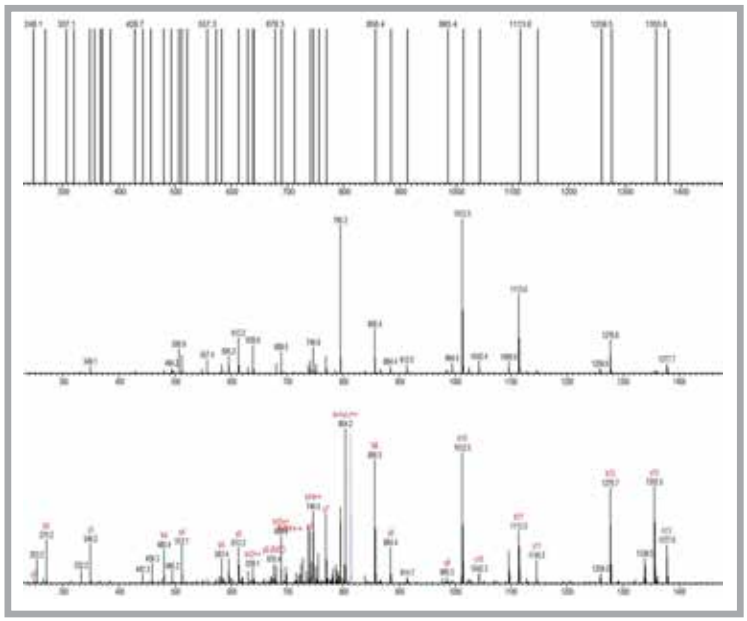


A. Kinetic Model Predicted

B. Measured Spectrum

Peptide identification for a medium size peptide LRPICGTDGVTYTND

The identification was assigned by comparing the measured spectrum (C) to the kinetic model predicted spectrum (B) unique to Pepfinder software. Other software programs can provide an empirical model (A), but these lack relative abundance information of the fragment ions.



A. Empirical Model Predicted

B. Kinetic Model Predicted

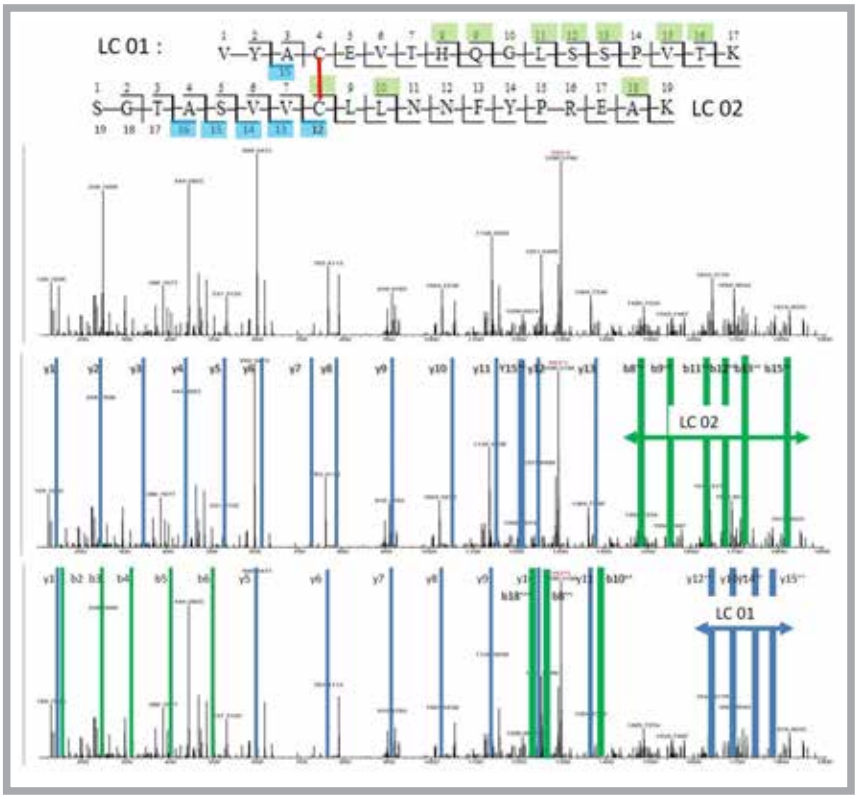
C. Measured Spectrum

(1) Large-Scale Identification and Quantification of Covalent Modifications in Therapeutic Proteins Zhongqi Zhang Anal. Chem. 2009, 81, 8354–8364

Determination of Disulfide Linkage

Using PepFinder software, disulfide mapping is easily achieved by processing a non-reduced peptide map data file. No previous knowledge of linkage sites is required, therefore novel linkages and potential disulfide bond scrambling can be identified and confirmed by MS/MS. Non-reduced and reduced data sets also can be compared at the same time for additional confidence.

Below is an example of a disulfide bond identified by PepFinder software. The bond was present and confirmed in a peptide map produced with the enzyme Lys C. As shown, PepFinder software is able to display the coverage of each peptide individually from within the mixed MS/MS spectrum.



A. MS/MS disulfide bonded peptides

B. Y and b series ions of LC01

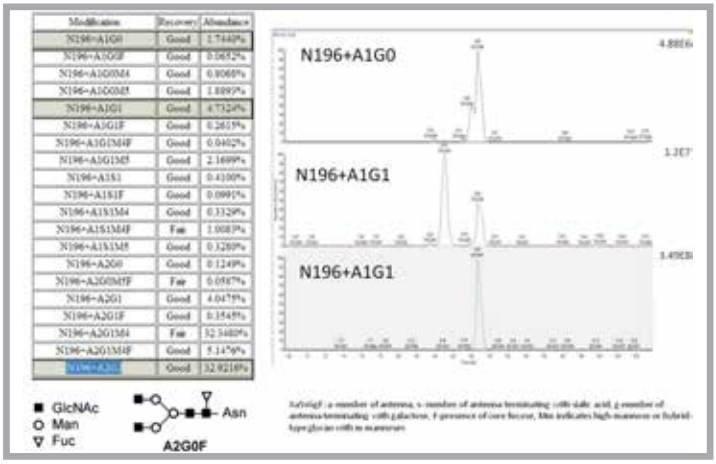
C. Y and b series ions of LC02

Fragment ion spectrum for the disulfide bonded dipeptide
The HCD MS/MS spectrum of the +3 parent ion of the disulfide linked dipeptide is shown in (A). The MS/MS evidence supporting the light chain peptide 1 (LC01) is illustrated in (B) and the evidence for LC02 shown in (C).

For the MS/MS spectra (B) and (C), the heavy green lines show the multiply charged fragment ions which identify and confirm the location of the disulfide bond.

Identification of Glycoforms

PepFinder software automatically identifies N-linked glycosylated peptides and provides a report of the glycoforms that are present and their relative abundances. Glycoforms anticipated by PepFinder software include N-glycans with 1-4 antenna, each antenna terminating with sialic acid, galactose, or N-acetylgucosamine, with and without core fructose, plus hybrid-type and high-mannose type; a total of 147 possible N-glycans commonly observed in IgG monoclonal antibodies.



PepFinder software modification site summary for different glycoforms is compared to three specific glycoforms in Qual Browser software The relative abundance order of different glycoforms reported by PepFinder software (automatic) match the raw results from Qual Broswer software(manual). The modification site summary from PepFinder software can significantly increase data processing by eliminating the need for manual calculations.

Lot-to-Lot comparison

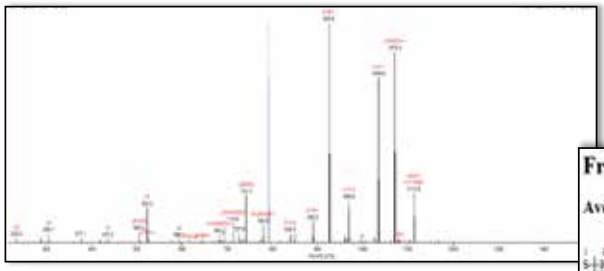
PepFinder software automatically performs a comparison when more than one data file is uploaded into the software.This workflow includes retention alignment for shifts in chromatography along with gap filling, so that a peptide identified in one file will be automatically detected and integrated in other files, even if it was below the designated threshold. Relative abundance of each site-specific modification is reported across each sample, along with the component peak area values, for easy export into other software packages for further statistical and trend analysis.

Shown to the left is the modification site summary report for a four sample comparison. Sample 2 was untreated, sample 1 was oxidized, and samples 3 and 4 were treated with different amounts of glycine. The relative abundance for several different modifications are shown in the columns. Highlighted in red are lysine glycation which is elevated in samples 3 and 4 as expected. Highlighted in green are two different oxidations which are increased in sample 1.

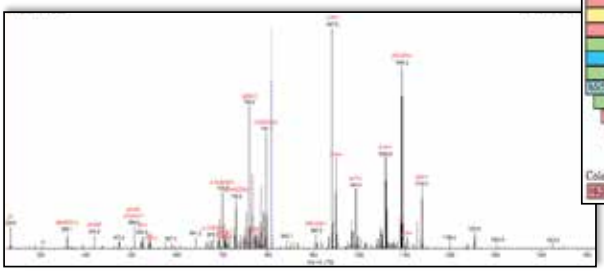
Error Tolerant/Amino Acid Substitution

PepFinder software automatically performs error tolerant searches and will identify unspecified modifications and amino acid substitutions with confidence. Shown in this example is the CID spectra of a peptide with a substitution of V->F. The fragmentation coverage map is easily generated for additional confirmation.

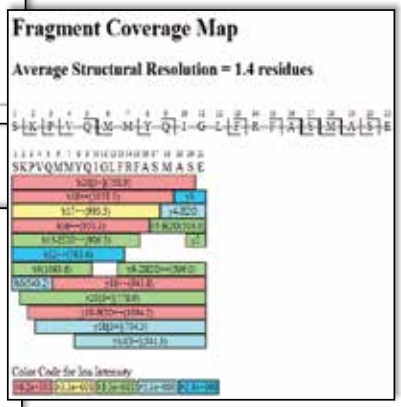
A. CID spectra for SKPVQMMYQI GLFRVASMAS E



B. CID spectra for SKPVQMMYQI GLFRFASMAS E



C. Fragmentation map for confirmation



Amino acid substitution of V->F is confirmed by the CID spectra (B) and the peptide fragmentation map (C) shows both y9 and b13 (site of substitution) were identified for extra confidence in the assignment. The unmodified peptide spectra is displayed in (A).

With its fast, comprehensive peptide characterization and automated modification summary reports, PepFinder software can dramatically reduce the time and effort required for the detailed characterization of biologics. To learn more information about PepFinder software contact your Thermo Fisher Scientific representative today or visit us online at www.thermoscientific.com/pepfinder.

Minimum System Requirements:

Hardware

- 2.0 GHz processor with 1 GB RAM
- 30 GB hard drive
- DVD/CD-ROM drive
- Resolution display 1680×1050

Software

- Microsoft™ Windows™ 7 SP1 (64-bit and 32-bit) operating system
- Adobe™ Reader™ 10
- Microsoft .NET 4.5
- Microsoft Office 2010 (optional)
- MSFileReader 3.0

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