BIOPHARMALYNX: A NEW BIOINFORMATICS TOOL FOR AUTOMATED LC/MS PEPTIDE MAPPING ASSIGNMENT

Joomi Ahn, Beth Gillece-Castro, and Scott Berger
Waters Corporation, Milford, MA, U.S.

OVERVIEW

The productivity of many biopharmaceutical characterization groups is limited by the manual and repetitive process of data analysis. LC/MS peptide mapping analysis is an archetype of this problem, as a LC/MS peptide map can take days to weeks to fully characterize for the first time. As peptide mapping remains a common technique to confirm the primary structure and modified forms of a recombinant protein, a solution for data analysis, which is often the bottleneck, is needed. This work features a software tool capable of handling the combinatorial complexity of peptides and their potential modifications.

BiopharmaLynx™ Application Manager, a new informatics package for MassLynx™ Software, significantly reduces data analysis times for LC/TOF-MS peptide mapping studies. The automatically annotates peptides detected from LC/TOF-MS data. This software package also automates batch data processing of multiple peptide maps. Faster data processing and a user-friendly interface are some of the attractive features designed to reduce the burden of data analysis.

In this application note, the practical application of this innovative software is described for automated processing and map interpretation of a bovine hemoglobin digest analyzed by LC/MS.

INTRODUCTION

Several key features of the BiopharmaLynx Application Manager for qualitative peptide mapping are:

- Automated data processing enables annotation of peptide sequences to LC/MS peaks using accurate mass assignments.
- These peak assignments are displayed in the form of chromatograms, spectra, and coverage maps.
- Automated peak annotations include recognition of modified peptides present within the sample.
- Graphical tools for easy visualization of raw and processed chromatographic and mass spectral features.
- Tabulated results permit interactive data sorting and editing.

Figure 1 illustrates the overall workflow of BiopharmaLynx Application Manager and the effectiveness of key features proceeding automated peptide map data analysis. The software is also capable of processing and comparing a batch of LC/MS data to identify the differences among peptide samples.

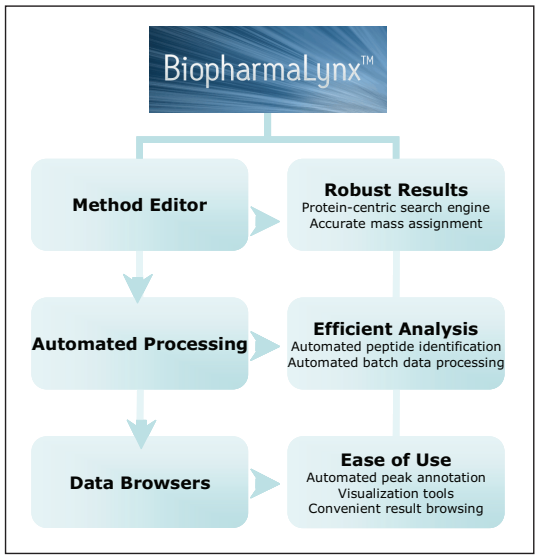

Figure 1. Key capabilities of BiopharmaLynx Application Manager for peptide mapping analysis.

Method editor

A streamlined wizard interface creates methods that can be used to batch process LC/TOF-MS peptide map data. The method editor includes the ability to:

- Define a list of protein sequences to be searched.
- Set search criteria for producing peptide map assignments (mass tolerance, proteolytic enzymes).
- Select fixed and variable modifications from a comprehensive list, with support for customized modifications.
- Specify lockmass parameters for increased mass accuracy.

An example hemoglobin map processing method (Figure 2A) defines a targeted search against the individual alpha and beta chain sequences. The products of cysteine alkylation by iodoacetamide, asparagine deamidation, and methionine oxidation were searched as variable modifications against these sequences.

Automated processing

Raw data processing is facilitated by linking a set of MassLynx data files with a processing method created in the editor. Data files from multiple MassLynx projects (on local and networked drives) can be processed by batch analysis (Figure 2B). LC/MS maps process in minutes on typical desktop workstations.

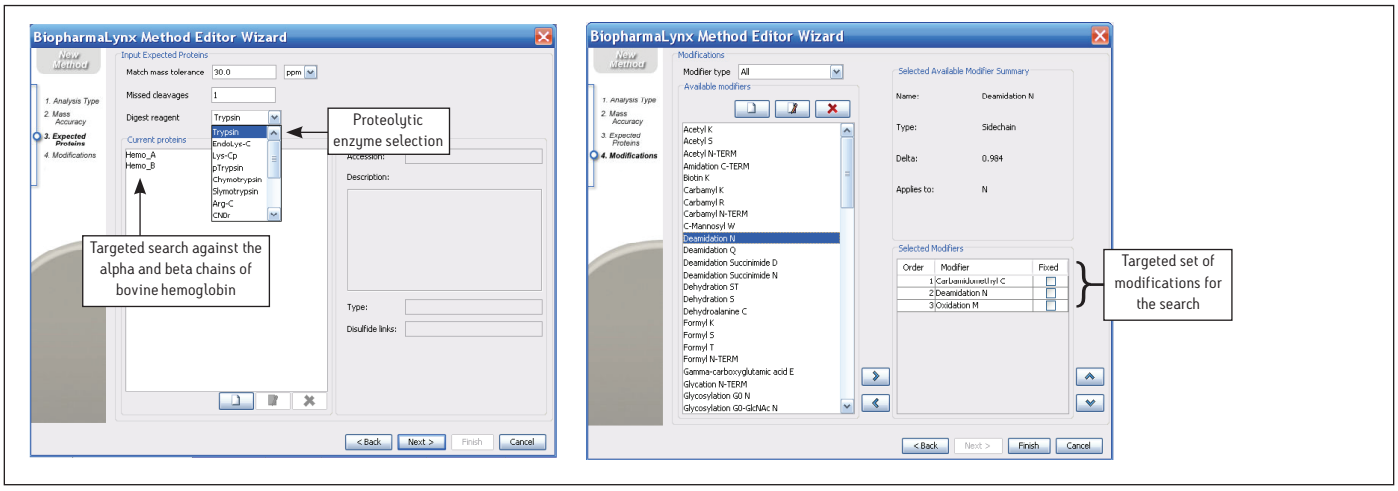

Figure 2A. The method editor created for a bovine hemoglobin LC/MS peptide map.

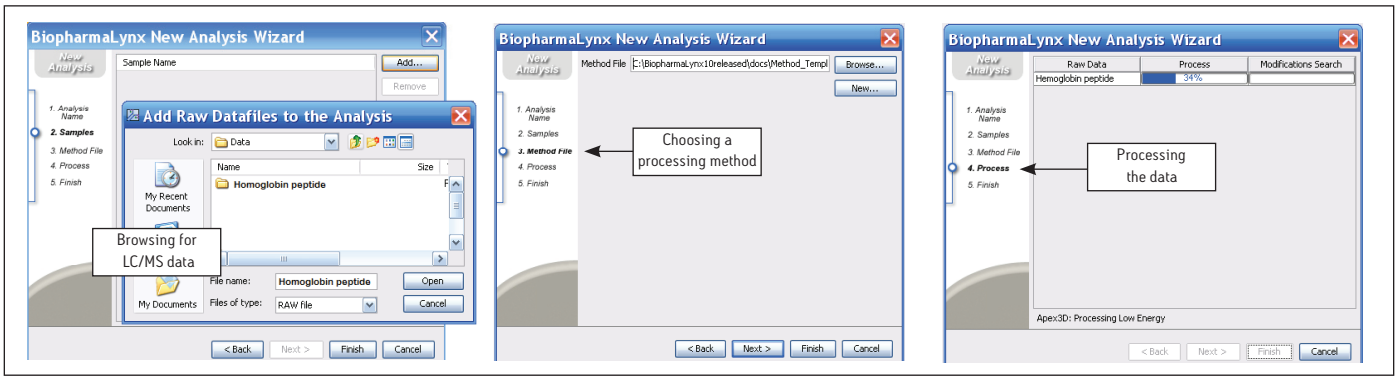

Figure 2B. Automated data processing.

Data browsers

Peptide mapping results can be browsed in several graphical and tabular presentation formats:

- Annotated displays of raw and processed chromatograms.
- Annotated raw and processed mass spectral views.
- Tabular result views that can be customized.
- Protein-centric views of the processed data can be superimposed on coverage maps or a list of digested peptides.

The sequence coverage shown in Figure 3 for the hemoglobin peptide mapping indicates high protein coverage for both alpha and beta chains (95.0% and 94.4%, respectively). The color-coded coverage view provides additional information, such as peptides that have been matched, modified, and not detected. The unmatched peptides highlighted in gray were usually in low intensity signals or short peptides, which were not well retained on reversed-phase columns.

Coverage map

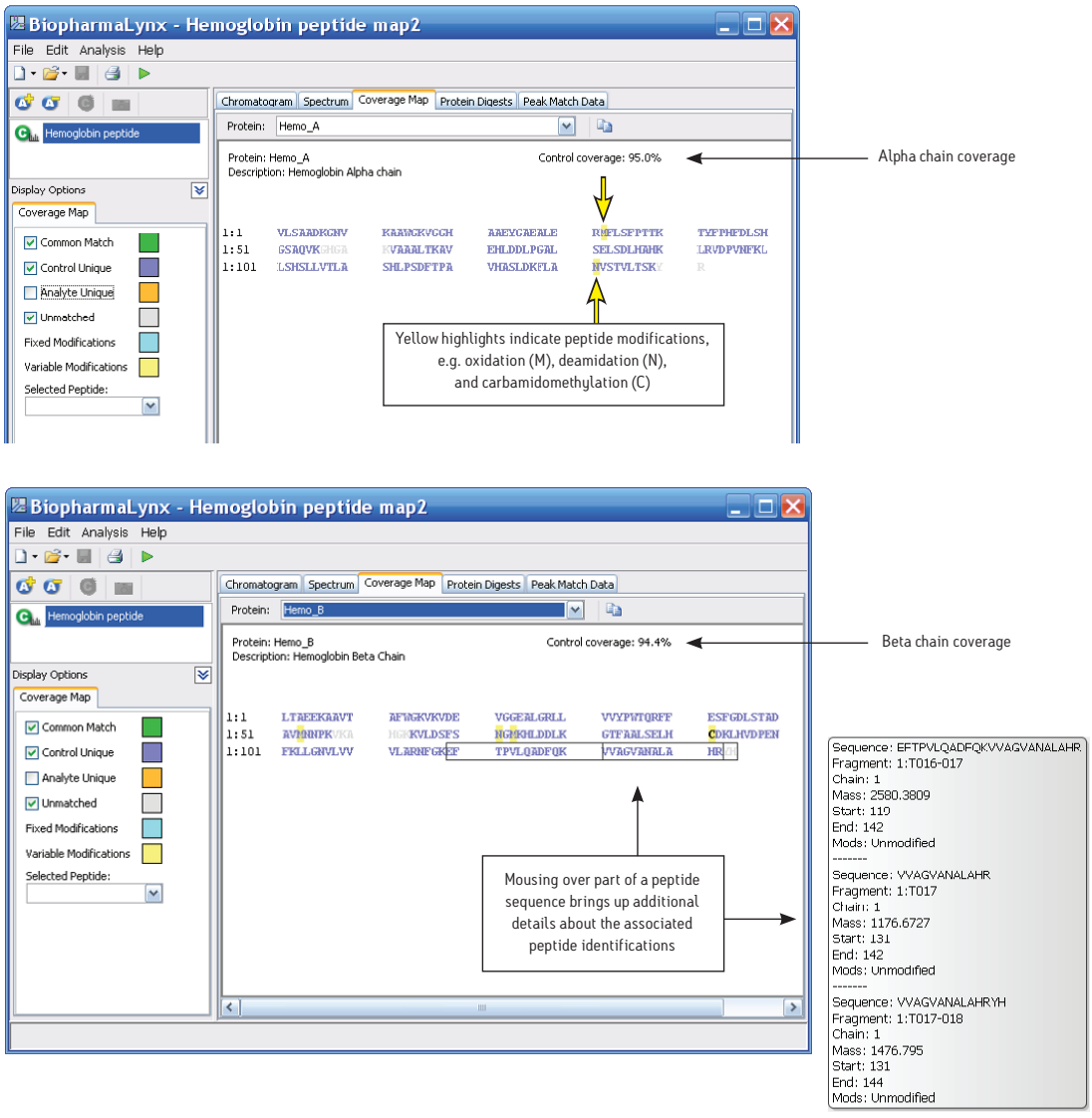

Figure 3. The coverage map overlays LC/MS mapping results on the searched protein sequences.

Displays of the raw and processed chromatogram views in Figure 4 depict annotation of the peptide identifications on the LC/MS data. Tryptic peptides in both chromatograms are labeled as protein digest fragment numbers (T_n) along with their retention times. Additional peptide information is accessible by mousing over any given peak.

In the processed chromatogram in Figure 4B, the peptides are shown as centroided "sticks" at the peak apex retention time. Each centroid represents the summed intensity (ion counts) of all isotopic peaks for all detected charge states of that peptide over the full chromatographic elution profile.

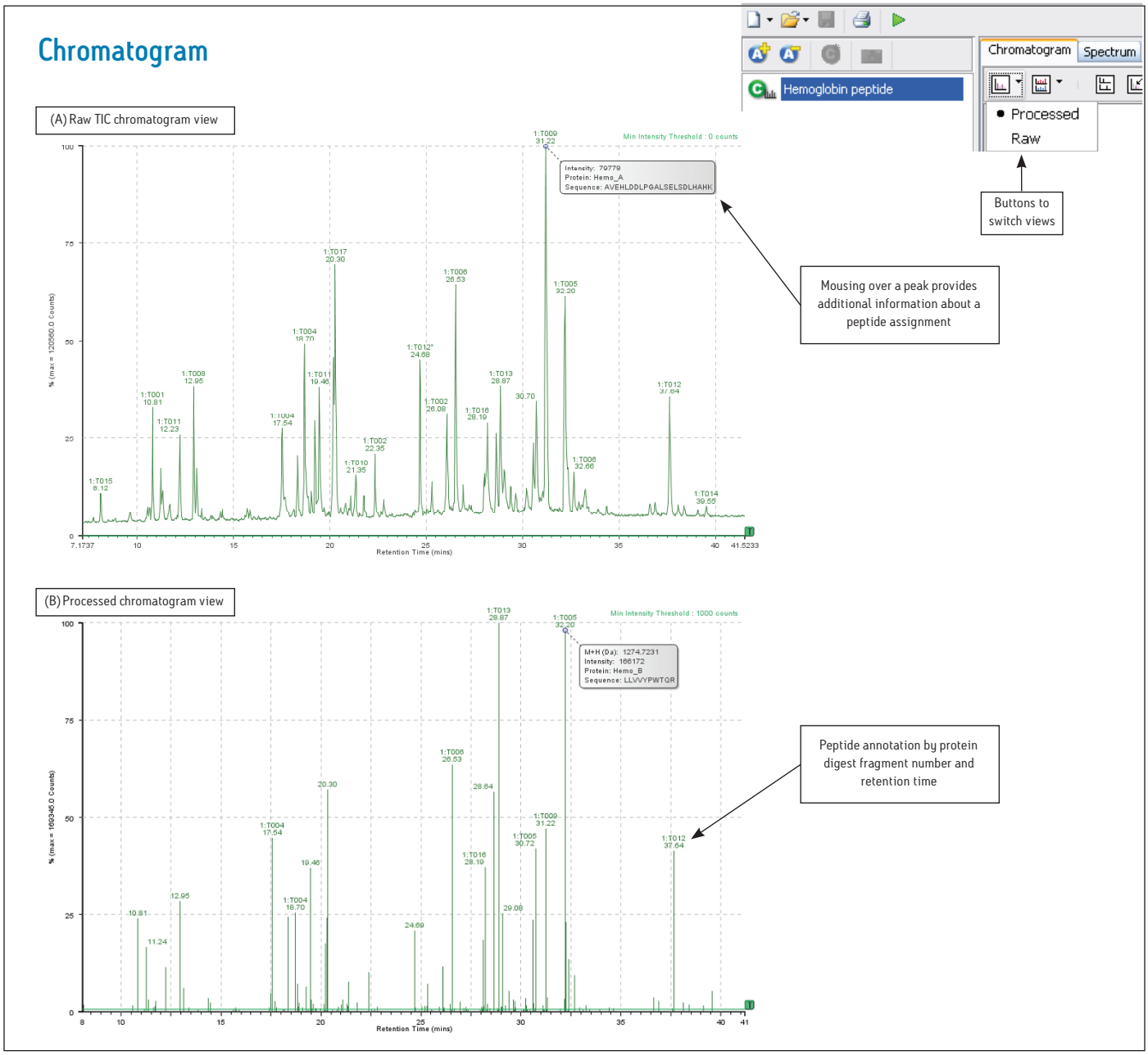

Figure 4. The raw (A) and processed (B) chromatogram views of the bovine hemoglobin peptide map.

Peak match data table

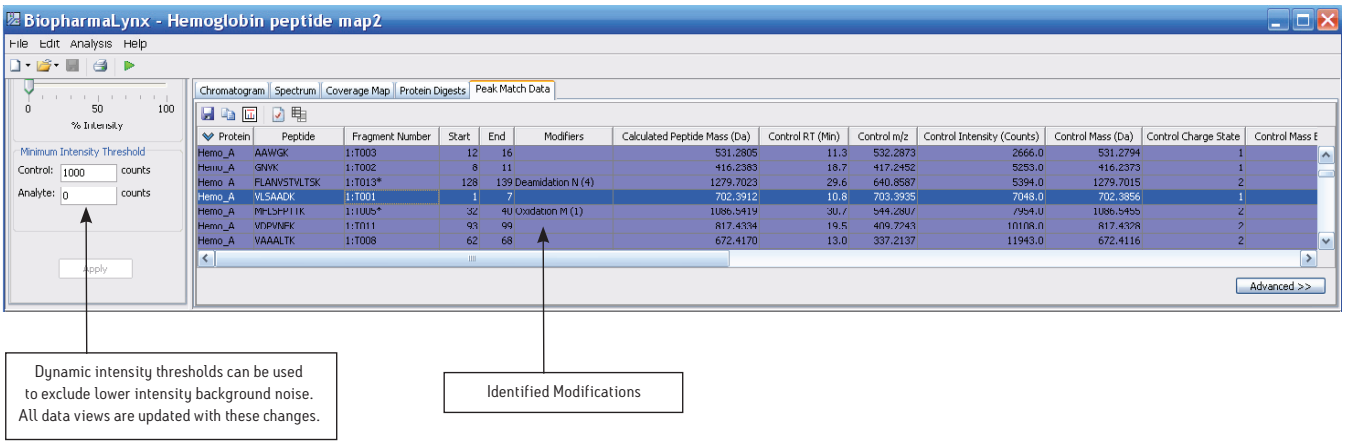

Figure 5. The peak match data table.

The peak match data table display (Figure 5) contains processed results for all detected LC/MS components, including protein and peptide annotations produced by the targeted search. This tabular display of peptides can be customized, sorted by any category, and thresholded to suppress false identifications from low intensity background ions. The results in this table are exportable to Microsoft Excel or other spreadsheet applications. Scientists can also use this view to select alternative assignments for peptides in question or manually annotate an entry.

EXPERIMENTAL

MassPREP™ Hemoglobin (alpha and beta chains) Digestion Standard (P/N 186002327) was prepared (2 pmol/µL in 0.1 % formic acid). This standard is a purified tryptic digest of bovine hemoglobin (Swiss Protein database files HBA P01966 and HBB P02070) that was reduced with DTT and alkylated by iodoacetamide. 20 pmol of the standard digest was injected for LC/MS peptide mapping analysis.

LC conditions

LC system:	Waters® ACQUITY UPLC®
Column:	ACQUITY UPLC BEH 300 C_{18} 2.1 x 150 mm, 1.7 µm, 300 Å
Flow:	200 µL/min
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in acetonitrile
Gradient:	0 to 50% B over 60 min.
Column temp.:	40 °C
Weak wash:	95% buffer A/5% buffer B
Strong wash:	20% buffer A/80% buffer B

MS conditions

MS system:	Waters SYNAPT™ MS
Ionization mode:	ESI Positive
Capillary voltage:	3.0 kV
Cone voltage:	35 V
Desolvation temp.:	250 °C
Desolvation gas:	350 L/Hr
Source temp.:	120 °C
Acquisition:	50 to 1700 m/z
Lockmass:	100 fmol/µL Glu-Fibrinopeptide B $(M+2H)^{2+}$ =785.8426

CONCLUSION

The BiopharmaLynx Application Manager is a powerful bioinformatics tool that automates data processing and peptide annotation for LC/TOF-MS peptide mapping data sets. Data browsing with graphical and tabular tools quickly provides the information needed to make decisions about protein quality or effectiveness of an analytical method or process. Obtaining better information faster can decrease development costs and streamline development timelines.

Waters provides a total system solution for peptide map analysis that includes ACQUITY UPLC-based separations to enable faster more resolving peptide maps, multiple UPLC® chemistries to exploit map selectivity differences, and sensitive TOF mass spectrometers for accurate mass and modified peptide identifications. BiopharmaLynx complements Waters peptide mapping solutions, allowing for integrated peptide mapping data analysis with greater speed and confidence. Automated processing and annotation of peptide maps can be achieved in minutes, removing the most tedious and productivity-limiting element of a peptide mapping analysis.

In conclusion, BiopharmaLynx decreases the expense and time-to-market of protein therapeutics by reducing data analysis time for peptide mapping studies. This software offers fast data processing and an easy-to-use interface that saves time and allows scientists to engage in other high-value tasks.

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Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com