A New Software Tool for Interpretation of Accurate Mass LC/MS Peptide Maps

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INTRODUCTION

Protein characterization requires efficient tools to establish a complete chemical structure. Ideally, the analysis would be sensitive to minor changes in the structure. Peptide mapping by liquid chromatography linked to high mass accuracy mass spectrometry (LC/MS) has the benefit of chemical structure identification. During protein characterization comparisons are made between batches and a control or reference standard.

A software tool which makes visual and quantitative comparisons readily reported and exported can provide efficient data interpretation. We have developed prototype software to address the large data sets found in protein characterization laboratories. The peaks are detected by the Apex3D/Peptide3D algorithms to deconvolute multiply-charged ions and combine isotopes. Apex3D and Peptide3D are specifically designed to interpret the resolved isotope clusters produced by oa-Tof mass spectrometers. The results can be viewed as a table, as a combined spectrum, or as a processed chromatogram.

GOALS

- To provide comparative views of raw and processed data
- To report qualitative and quantitative differences
- To Analyze LC/MS data from oa-Tof mass spectrometers
- To Match observed peptides with the sequence of the protein based on molecular weight.
- To identify site-specific modifications

DATA REDUCTION

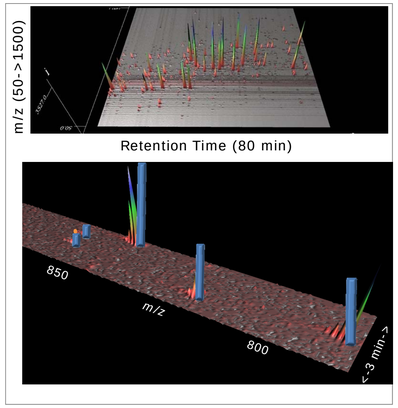

Figure 1. 3D view of LCMS data from an oa-Tof instrument.
Panel A. Full 3D view of one LC/MS analysis of a tryptic digest.
Panel B. Individual isotope clusters are assigned accurate retention times, accurate neutral molecular weight, and summed intensity as indicated by the blue bars.

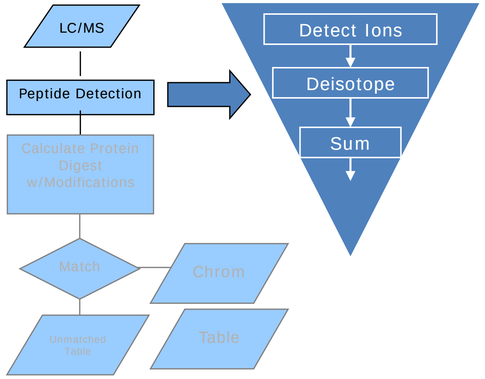

Figure 2. Workflow of Biopharmalynx Data Reduction. First the LC/MS data has been peak detected, deisotoped, charge state reduced, and intensity summed. Matching occurs to calculated

USER CONTROLS

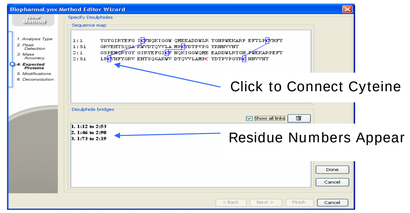

Figure 3. Protein Sequences can be linked by disulfide bonds.

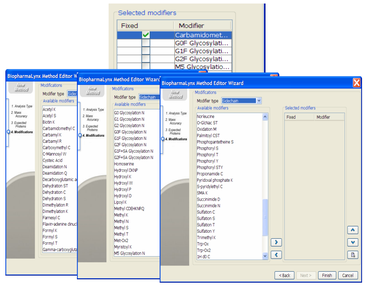

Figure 4. Extensive Modification List includes common antibody glycans.

PROCESSING

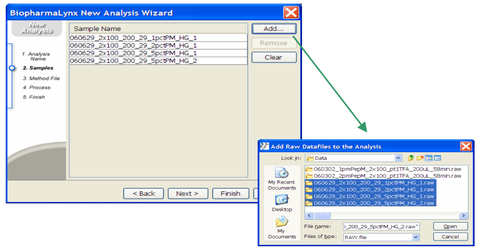

Figure 5. Data files from LC/MS oa-Tof runs can be processed from any storage device which can be browsed from the Biopharmalynx computer.

BROWSER VIEWS

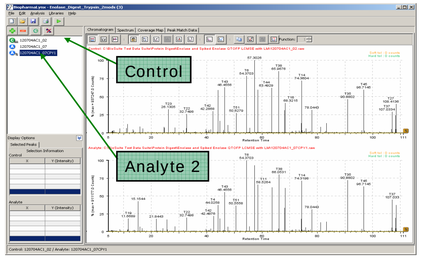

Figure 6. Compared chromatogram view of processed LC/MS data files.

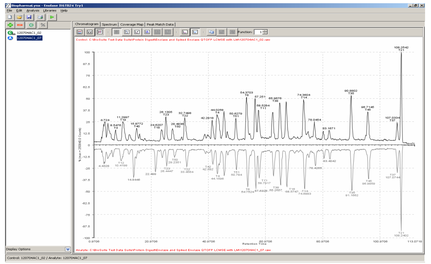

Figure 7. Compared chromatogram view of raw LC/MS data files.

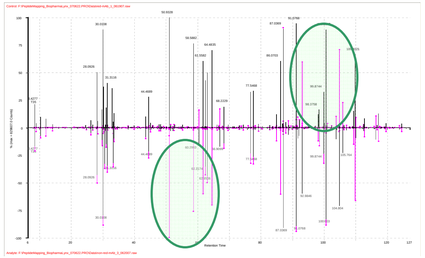

Figure 8. Processed data in a mirror plot with differences displayed in magenta. A digest was analyzed with reduction (top) and without reduction (bottom). Significant differences between the data files are highlighted. Detailed annotation is available in the browser by pointing the cursor at individual peaks.

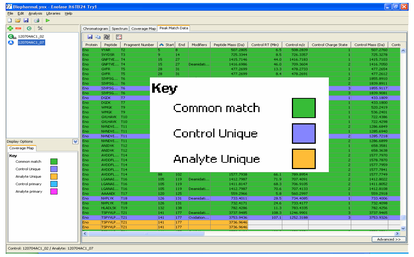

Figure 9. Data table showing matched peptide peaks. Color coding makes differences between samples readily visible.

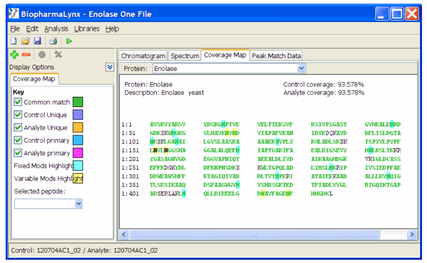

Figure 10. Coverage Map showing results from two files, a control and an analyte. Color coding makes modifications readily visible.

WHAT BIOPHARMALYNX DOES

- Automates time consuming and tedious manual data analysis
- Produces annotated chromatograms, coverage maps, and tabular data
- Facilitates comparison between a reference standard and any number of batches or variant samples
- Outputs include formal reports, figure cut/paste, and tabular data export
- Frees users to concentrate on important questions

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