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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

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# **USER CONTROLS**

#### YGYGIRYEFG IGFNOKIGGW OMEEADDWLR YGNPWEKARP EFTLPCVHFY GRVEHTSGGA KWVDTOVVLA MECHTPVPG YRNNVWT GSPEMGFYGY GIRYEFGICF NOKIGGWOME EADDWLRYGM POEKARPEFT LPCVHFYGRV EHTSQGAKWV DTQVVLAMPC YDTPVPGYRC NNVVNT 2. Peak Detection 3. Mass Accuracy 4. Expected Proteins Modifications Deconvolutio Click to Connect Cyteine ✓ Show all links 1. 1:12 to 2:53 2. 1:46 to 2:90 3. 1:73 to 2:19 **Residue Numbers Appear** Done Cancel Cancel

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

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Additional Model Callon Control Callon	Image: Second	BiopharmaLynx Method Editor Wizard           Modifications         Modifications           1 - Analysis Type         Subschaft           2 Mass Accuracy         Profescine           3 Expended Proteins         Or-GicNac ST           9 - Modifications         Or-GicNac ST           9 - Modifications         Painticity (CST           9 - Amodifications         Phosphory (ST           9 - Phosphory (ST)         Phosphory (ST)           9 - Springle Vertications         C           9 - Widowal phosphate K         S-pyrologicity (C           5 - Mark (Station C         Stuffation S           Stuffation S         Sulfation T           Sulfation T         Sulfation T           Sulfation T         Sulfation T           Sulfation Y         Trip-Ox           Trp-Ox         Trp-Ox           Trp-Ox         Trp-Ox	Sek	ed Modifier

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.





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



Figure 8. Processed data in a mirror plot with differences displayed in magenta. the browser by pointing the cursor at individual peaks.



# **BROWSER VIEWS**

A digest was analyzed with reduction (top) and without reduction (bottom). Significant differences between the data files are highlighted. Detailed annotation is available in



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

🎾 BiopharmaLynx - Enolase One File											
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Key Common match	Description: Enolase	yeast	Analyte coverage: 93.578%								
Control Unique Control Unique Control primary Analyte primary Fixed Mods Highlight Variable Mods Highlight Selected peptide:	1:1 AVSKVYARSV 1:51 GDKSKWMGKG 1:101 IKKSKLGANAT 1:151 LIVLINGGSHA 1:201 YGASAGNVGD 1:251 EFFKDGKVDL 1:301 DDWEAWSHFF 1:351 TLSESIKAAO 1:401 ARSERLAKLM	YDSRGIPTVE VLHAVKIVID LGVSLAASRA GGALALQEFM EGGVAPNIQT DFKNIMSDKS KTAGIQIVAD DSFARGWGVM QLLRIEEELG	VELTTEKGVF VIAPAFVKAN RAREKNYPLY IAPTGAKIFA REEALDLIVD KMLTGPQLAD DLIVTMPKRI VSHRSGETED DMAVFAGENF	RSIVPSGAST IDVKDQKAVD KHLADLSKSK EALRIGSEVY AIKAAGHDGK LYHSLINKRYP ATAIEKKAAD TFIADLVVGL HHGDKL	GVHEALEMRD DFLISLDGTA TSPYVLPVPF HULKSLTKKR VKIGLDCASS IVSIEDPFAE ALLLKVNQIG RIGQIKTGAP						
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Control: 120704AC1_02 / Analyte: 120704AC1_02											

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