AUTOMATING DATA PROCESSING, PROTEIN ANNOTATION, AND INTER-SAMPLE COMPARISONS OF INTACT PROTEIN LC/MS ANALYSES USING NOVEL SOFTWARE TOOLS

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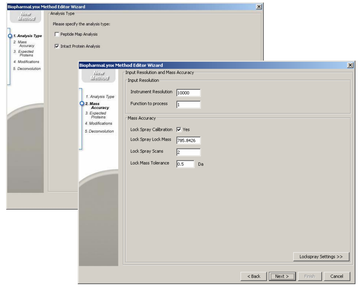
OVERVIEW

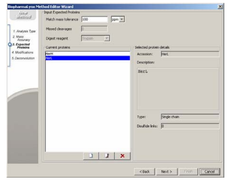
- Intact protein LC/MS analysis can provide a wealth of information about a biopharmaceutical, and the process by which it was created.
- Processing of the resulting data is repetitive and tedious, and constitutes the productivity-limiting step for many laboratories.
- Automated MaxEnt1 protein mass spectral deconvolution has been supported using the open access program OpenLynx, but this application has not permitted annotation of deconvoluted masses to their likely structures, nor facilitated easy comparisons between results.
- This poster describes the integration of all such capabilities in a new MassLynx application manager (BiopharmaLynx) for automated processing of protein and peptide TOF MS data.
- In this poster, we highlight the workflows used to process intact protein LC/MS data, assign the deconvoluted masses to proteins and their variants, and illustrate the various display tools for inter-sample comparisons.

DEFINING ACQUISITION PARAMETERS

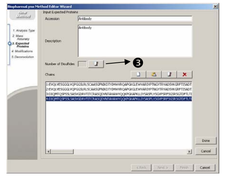


Selecting the intact protein processing workflow enables the entry of basic acquisitions parameters (instrument resolution, lockspray information) for protein deconvolution. These values facilitate automated spectral deconvolution of smaller proteins and improved mass accuracy for LC/MS data sets acquired with a lockmass function.

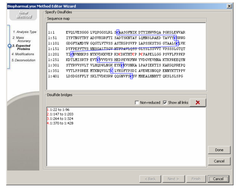
PROTEIN ASSIGNMENT PARAMETERS



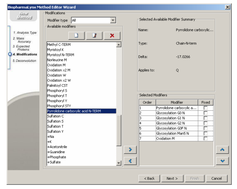
❶ Setting search parameters: What protein(s) will be searched, and at what initial mass search tolerance.



❷ Defining a protein: The basic unit of any protein is the protein chain. Multiple chains can associate noncovalently, or covalently through one or more disulfide linkages.

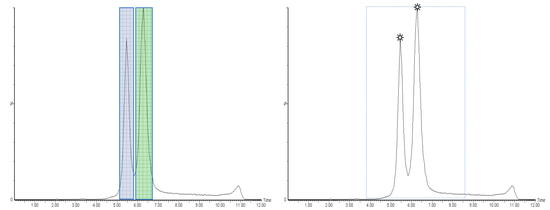


❸ Disulfide linkages within a chain or between chains can be specified.



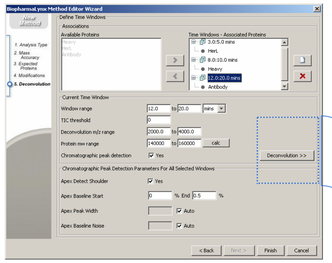
❹ Proteins searches can include both fixed and variable modifications. These can be restricted to a given amino acid (e.g. methionine oxidation) or to a specific location (e.g. N-terminal modifications).

RATIONALE FOR PROCESSING INTACT PROTEIN LC/MS DATA

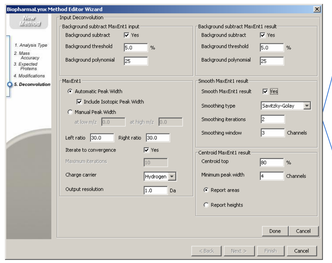


LC/MS runs can be divided into time-based processing segments where unique sets of deconvolution parameters and protein search targets are defined. Within each time segment, all spectra can be summed (LEFT), or individual peaks (RIGHT) recognized for processing and inter-sample comparisons.

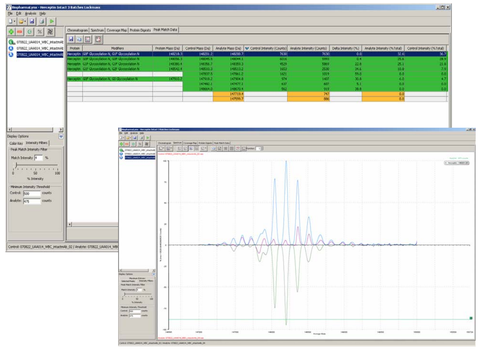
Even simple samples (e.g. reduced antibodies) can generate LC/MS data sets where the individual proteins are optimally detected using distinct deconvolution parameters. In this window, the user assigns proteins and deconvolution parameter sets to the defined time segments of an LC/MS run.



While the default deconvolution parameters should suffice for automated processing of smaller proteins (<50kD), a newly optimized MaxEnt1 TOF spectral deconvolution algorithm permitted manual assignment of these parameters for improved characterization of large proteins and complexes.

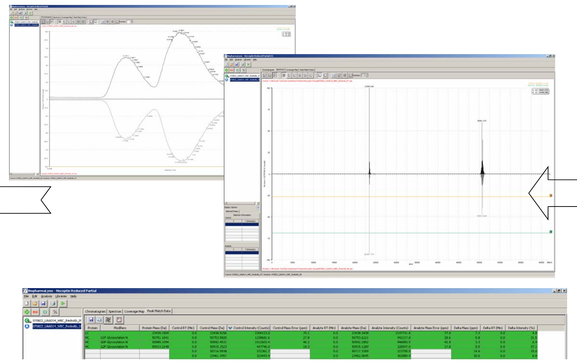


COMPARISON OF TWO PRODUCTION LOTS OF A COMMERCIAL ANTIBODY



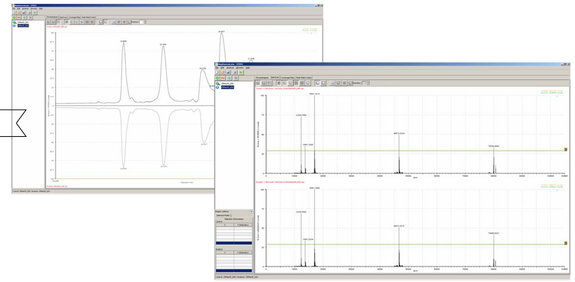
Intact antibody analysis of two commercial production lots of monoclonal antibody showed both qualitative and quantitative batch-to-batch differences. Results are shown in a tabular format (TOP), and as a differential mirror plot of the two MaxEnt1 deconvoluted spectra (BOTTOM).

COMPARISON OF REPLICATE REDUCED ANTIBODY LC/MS RUNS



Two reduced antibody preparations were analyzed by LC/MS. The mirror-plot TIC (TOP), and a mirror plot of the processed deconvoluted spectra (LOWER) show consistency between the two preparations. The most abundant components in the antibody run corresponded to the light chain and a common series of biantennary heavy chain glycovariants (TABLE AT BOTTOM).

COMPARISON OF STANDARD PROTEIN LC/MS RUNS



Five standard proteins (Cytochrome C MW 12,230, Myoglobin 13,680, RNase A 16,950, Enolase 46,672, and apoTransferrin ~80 kD for the average of 60 major glycoforms) were analyzed by LC/MS. Two repeat injections produced comparable results in a mirror TIC plot (TOP) and a stacked deconvoluted spectra plot (BOTTOM). Individual processing segments were defined for optimal deconvolution of each protein peak.

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