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OVERVIEW

- Protein-level MS analysis produces data that are complementary to peptide-level analysis (e.g. revealing PTM's, processing, etc.)
- We have developed an automated processing approach (Automated Maximum Entropy, or AutoME) for analysis of complex intact protein LC/MS data sets.
- The maximum entropy deconvolution algorithm (MaxEnt1) used in this approach can transpose both the qualitative and quantitative features of raw mass spectra to neutral mass spectra.
- In this poster, the reproducibility and quantitative capacity of AutoME processing are demonstrated from the LC-ESI-TOFMS analysis of a standard protein spiked soluble *E. coli* protein fraction.

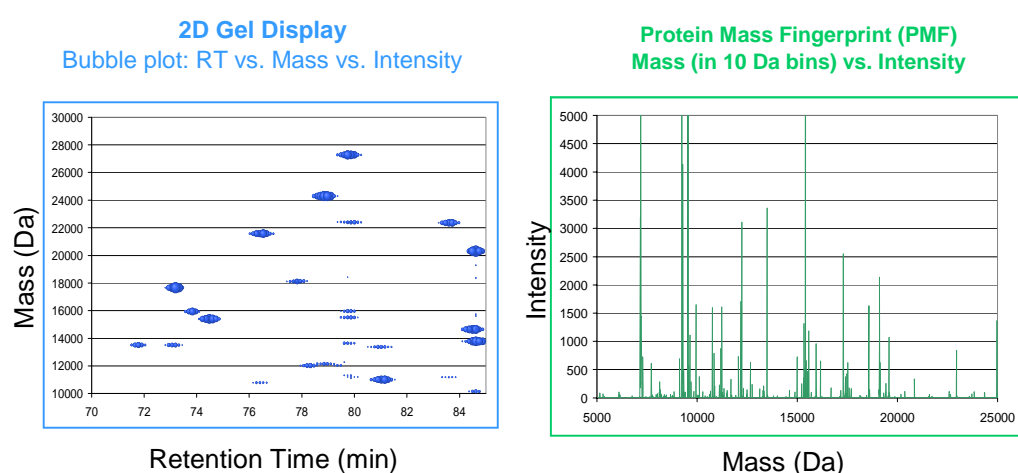
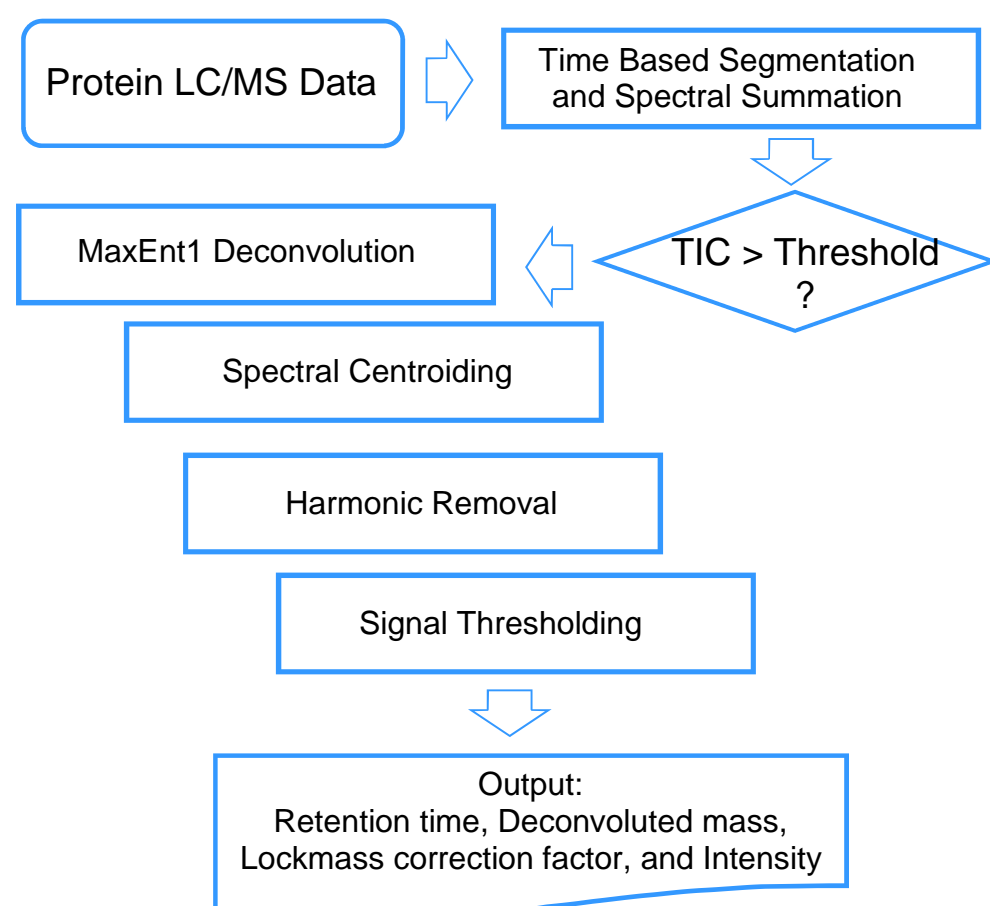
METHODS



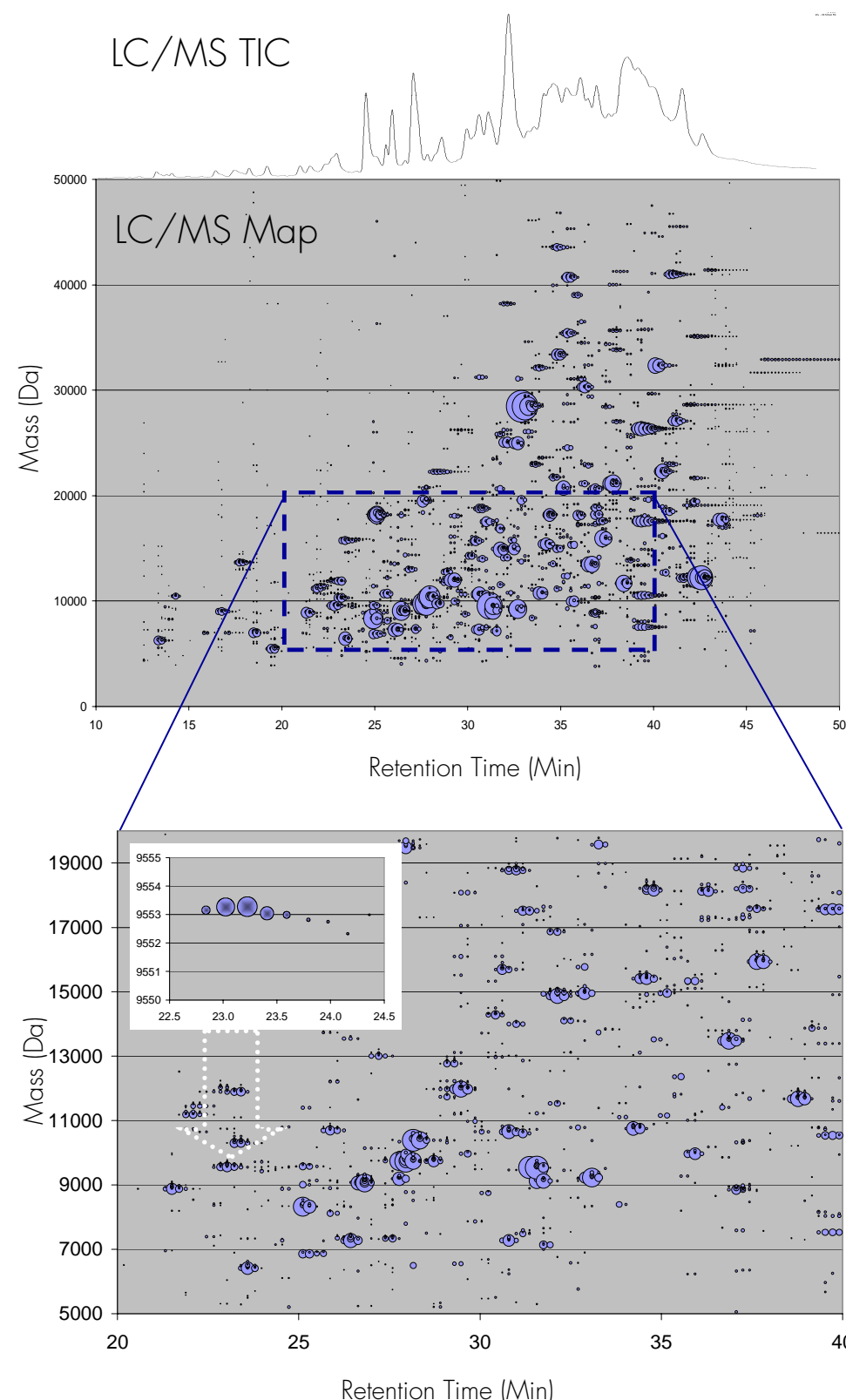
LC System: Waters 2796 Alliance™
 Bioseparations Module
 MS System: Waters LCT Premier™
 ESI-TOF MS
 Chemistry: Waters Symmetry® 300
 2.1 x 100 mm C4 3μ

E. coli cytosol was resolved by reversed phase chromatography using a 60 min gradient of acetonitrile (0-60%) in 0.5% formic acid. A post-column split directed 20% of the effluent (~50 μl/min) to the lockspray ESI source of the mass spectrometer. Acquired spectra (ESI+, V-mode (6,000 resolution), 1Hz, 650-2990 m/z) were processed using an internally developed Masslynx processing module (AutoME, see below), using the following conditions: 10 scans/ processing segment, input segment TIC threshold (10,000 counts), MaxEnt1 (0.75 Da width, 3,500-50,000 output mass range, 1 Da resolution, 15 iterations), Data reduction (Harmonic removal with 1 Da tolerance, 100 count minimum component intensity, Top30 peaks recorded/processing segment).

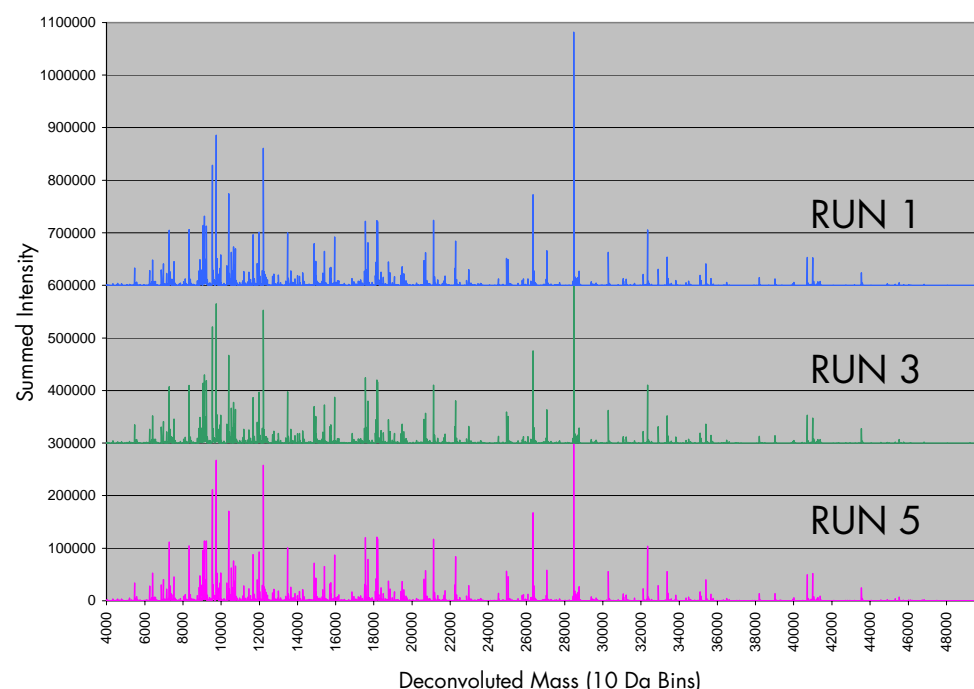
AutoME Processing Workflow



The LC/MS Map of *E. coli* Cytosol: *E. coli* cytosol (250 μg) was analyzed by a 1 hour LC/MS analysis.

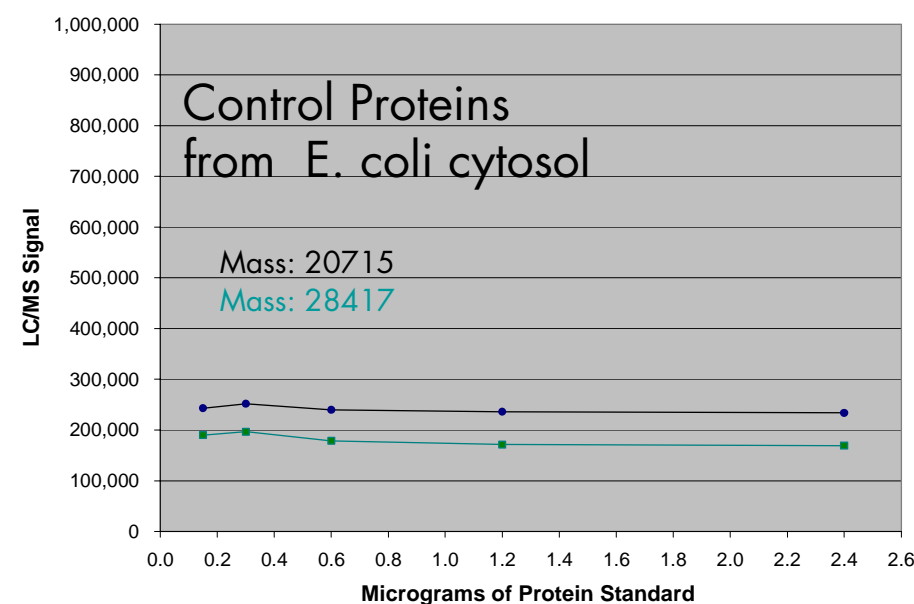
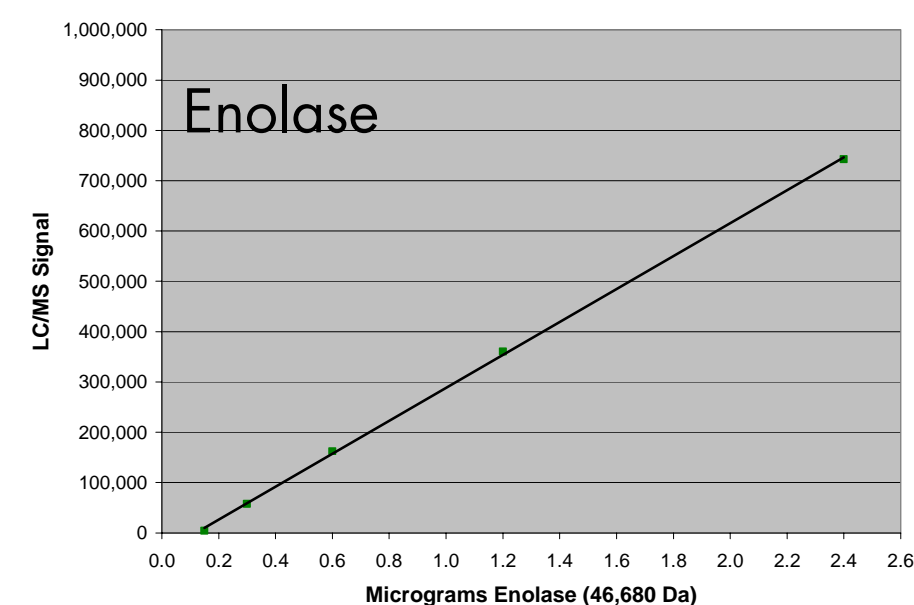
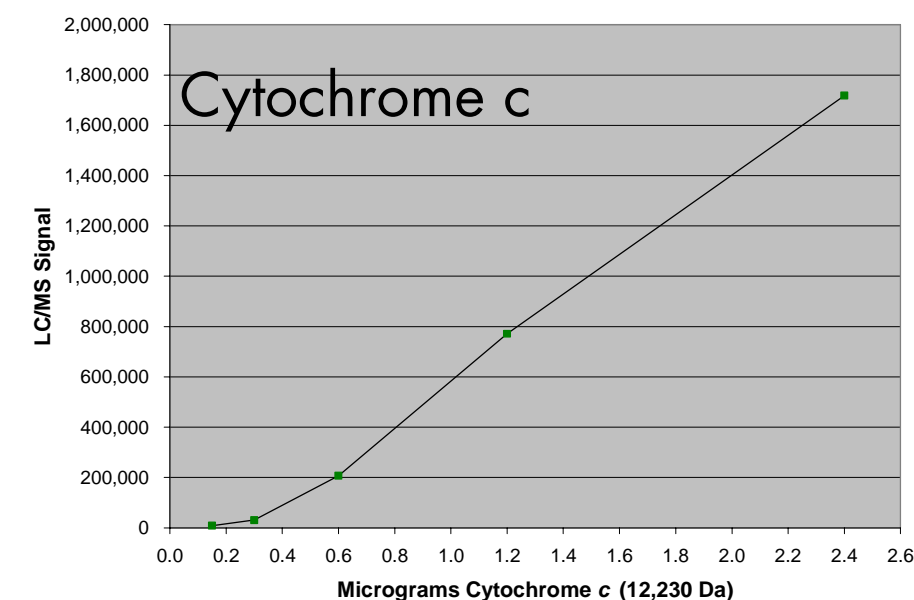
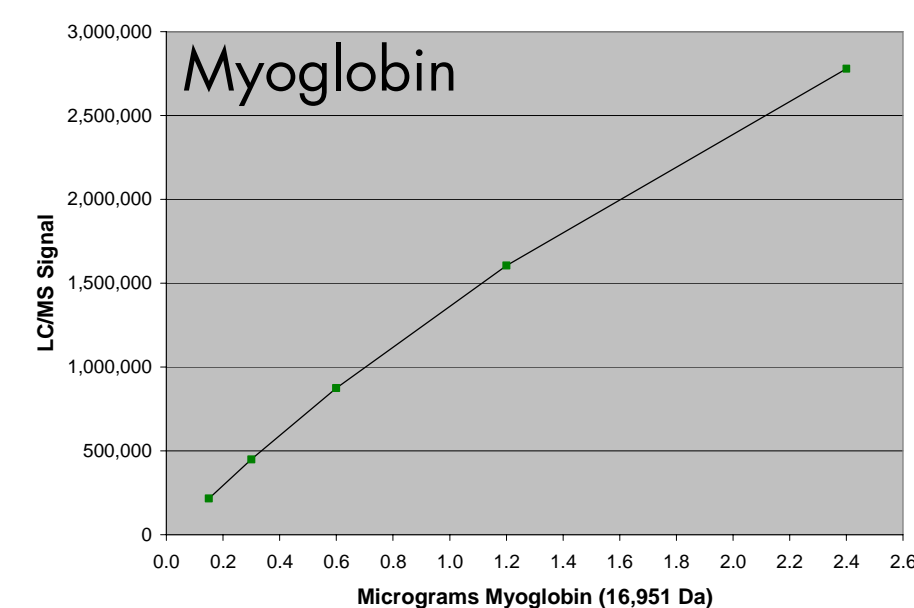


Reproducibility of *E. coli* cytosol LC/MS Analysis: *E. coli* cytosol (250 μg) was subjected to replicate LC/MS analysis, and Protein Mass Fingerprints were generated.



RESULTS

Spiking of standard proteins into *E. coli* cytosol: Several purified proteins (Cytochrome c, Myoglobin, Enolase) were spiked into 250 ug *E. coli* cytosol at levels of 0.15 to 2.4 ug per protein.



CONCLUSIONS

- Intact protein LC/MS data sets are amenable to automated data processing approaches.
- Proper selection of data processing parameters permits intact protein masses and intensities to be measured with high precision and accuracy.
- Processing parameters can be selected such that the underlying chromatographic profiles of individual components are retained.
- Protein Concentration-Response curves can be produced for individual proteins within a complex sample.