Using Orthogonal Techniques for Protein-Peptide Separation to Generate Comprehensive HDMSe Mass Spectral Libraries from an E. coli Model System

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Introduction
Spectral libraries are increasingly used for peptide identification as they can take full advantage of all spectral features, including relative ion intensities as well as precursor and fragment masses. Such libraries have been constructed by synthesizing individual peptides or by capturing spectra from genetically-encoded proteome data sets. Libraries generated from experimental data tend to emphasize peptides that are most likely to be observed in typical in vivo experiments and may even preserve characteristics of the underlying biological system. We report a third library generation strategy that builds upon the benefits of implementing orthogonal, multi-dimensional protein and peptide separation techniques to acquire a comprehensive HDMSe spectral library data set for E. coli.

Methods
Escherichia coli (K-12, MG1655) was grown under approximately 60 different growth conditions including variation of growth phases and carbon sources, limitations of nutrients and stress response induction. Individual culture conditions and a composite cell mixture were used to produce corresponding protein lysates. The resulting proteins were fractionated by denaturing electrophoresis using a prototype of the IOMix platform from Sage Science to produce 12 in-solution protein fractions, spanning 5000-15,000Da. Fractionated protein samples were reduced, alkylated, digested with trypsin, and desalted prior to LC-MS analysis. Peptides were analyzed by multidimensional 2D-RP/HPLC-HDMSe on a Waters nanoacquity UPLC and a SYNAPT-G2S HDMS system using data-independent acquisition methods. Assignment of HDMSe spectra to peptide sequences and label-free quantitation were performed using TransOmics software.

Escherichia coli Growth Conditions

Figure 1. Common Media and Growth Phases (black), Carbohydrate Metabolism (gray) and Energy Shunt (red). Growth Conditions (green) generated for the pooled Escherichia coli reference sample. The growth conditions are red shaded if they are required for global protein measurement and bold conditions are red shaded if they are required for all individual protein measurement.

Spectral Library Work Flow & Results

Figure 2. Analysis Workflow. Protein fractionation, reduction, alkylination, digestion and desalting (step 1, 2d), LC-HDMSe acquisition (step 2, 5d), TransOmics Data Processing and Analysis (step 3, 3d).

Spectral Library Results

Figure 3. Sage Science ELF Apparatus for protein fractionation (A) and NLC analysis of fractionated samples using SDS and UREA solutions (B).

Figure 4. HDMSe Acquisition. (A) Illustration of time-resolved precursor and product ion acquisition (LCQAr) with ion mobility separation (IMS) for HDMSe data acquisition, (B) Separation of +1 (blue), +2 (blue) and +3 (red) precursors with IMS (C) IMS separation of co-eluting peptides from replicate analysis of ELF-FractoF1 of E. coli reference sample.

Figure 5. (A) Heat map of proteins identified from replicate analyses of the 11 protein fractions and (B) the sum total intensity of characterized proteins identified in each protein fraction using TransOmics analysis.

Quantitative and Qualitative Results

Figure 8. Distribution of peptide abundance ratio (FPFR; mass 1.0-4.56 Da).

Figure 9. Binary comparisons of the TransOmics features from the four growth conditions (log2(ratio) versus control intensity (denominator)).

Quantitative and Qualitative Results

Figure 11. Protein abundance observed among the four E. coli growth conditions (CST1, CST2, CST3 and CST4), including replicates, for select protein groups or pathways (Glyoxylate Shunt, Pyruvate Metabolism and Ribosomes). Relative protein abundance is estimated using the Top1 peptides calibrated against internal standard (green ADH1).

Summary
Generation of spectral libraries by coupling the orthogonal techniques of intact protein fractionation with LC-HDMSe shows great promise. Future work will include refinement of the intact protein fractionation step, and utilisation of 2D-RP/HPLC-HDMSe to increase the percent sequence coverage of each protein, and increase the percent of coverage of the E. coli proteome from a single multi-dimensional analysis study.

References


Figure 12. Self-Organizing map of the scaled relative intensity for all peptide features from the four E. coli growth conditions (see Figure 8).

Figure 10. Self Organizing Map of the scaled relative intensity for all peptide features from the four E. coli growth conditions (see Figure 8).

Figure 6. (A) Fraction of identified proteins from E. coli proteome; (B) Distribution of protein coverage (C) Ion Map of AceA (mass, RT, drift time)

Figure 7. HDMSe drift resolved fragment ion spectra for Acce and AceA for E. coli growth conditions (see Figure 8 for sample descriptions).