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 curating spectra from pre-existing proteomic 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 existing empirical strategies by 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, limitation 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 fractioned by denaturing electrophoresis using a prototype of the ELF platform from Sage Science to produce 12 in-solution protein fractions, spanning 5000 -150000 Da. Fractionated protein samples were reduced, alkylated, digested with trypsin, and desalted prior to LC-MS analysis. Peptides were analyzed by multidimensional 2D-RP/RP-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

	Growth Condition		Growth Condition
	LB (lag phase)		M9 + 0.4% thymidine
CST1	LB (log phase)		M9 + 0.4% galactose
	LB (stationary phase)		M9 + 0.4% α-ketoglutarate
	SOC (lag phase)		M9 + 0.4% tryptone
CST2	SOC (log phase)		LB
	SOC (stationary phase)		M9 + 0.25% glucose, 0.25% glycerol
	BHI (lag phase)		M9 + 1% glycerol, 100mM sodium fumarate
	BHI (log phase)		M9 + 0.5% glycerol, 100mM potassium nitrate, 1uM ammonium molybdate
	BHI (stationary phase)		reduced nitrogen - M9 + 0.4% glycerol, 10mM arginine
	M9 + 0.4% glucose (lag phase)		reduced sulfur - M9 + 0.4% glycerol, 0.25mM glutathione
CST3	M9 + 0.4% glucose (log phase)		reduced phosphate - M9 + 0.4% glucose, 2mM monopotassium phosphate
	M9 + 0.4% glucose (stationary phase)		reduced iron - LB + 200uM 2,2'-dipyridyl
	BHI in log --> M9 + 0.4% glucose (lag)		osmotic stress - M9 + 0.4% glucose, 0.8M NaCl
	BHI in stationary --> M9 + 0.4% glucose (lag)		acidic - LBK, pH 5.5 (MES)
	M9 agar		acute response to pH change - LBK, pH 5.5 (by adding 6M HCl to culture)
	M9 + 0.4% gluconate		basic - LBK, pH 9.0 (AMPSO)
	M9 + 0.4% lactose		superoxide - LB + 0.5mM methyl viologen (2hrs)
	M9 + 0.4% acetate		hydrogen peroxide - LB + 200ug/ml (40 minutes)
	M9 + 0.4% lactate		stringent response - M9 + 0.05% glucose, 0.15% lactose (30 min after OD = 0.6
	M9 + 0.4% glycerol		biofilm - LB + 0.1% mannitol, 100uM nickel chloride (adherent & planktonic cc
CST4	M9 + 0.4% L-alanine		metal toxicity - LB + 0.2mM Cd, 0.6mM Zn, 1.2mM Co, 0.05mM Hg (all chlorid
	M9 + 0.4% L-proline		SOS response - UV induced 1', 60' growth recovery (lamp)
	M9 + 0.4% malate		SOS response - UV induced 1', 60' growth recovery (gel doc)
	M9 + 0.4% mannitol		DNA structure change induction - LB + 0.075ug/ml norfloxacin (3hrs)
	M9 + 0.4% L-arabinose		cold shock (16°C, 40 minutes)
	M9 + 0.4% succinate		heat shock (46°C, 45 minutes)
	M9 + 0.4% maltose		M9 + 0.05% glucose (30 min after OD = 0.6)

Figure 1. Common Media and Growth Phases (**black**), Carbohydrate Sources (blue), Stress Conditions (red) and Anaerobic Conditions (green) generated for the pooled *Escherichia coli* reference sample. The growth conditions that were evaluated for global protein measurement are noted as CST1, CST2, CST3 and CST4.

Spectral Library Work Flow & Results

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

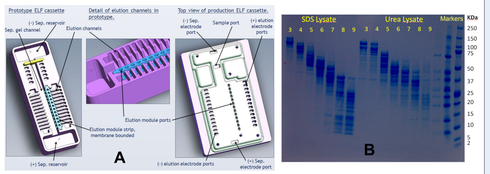


Figure 3. Sage Science ELF Apparatus for protein fractionation (**A**) and PAGE analysis of fractionated samples using SDS and UREA buffers (**B**).

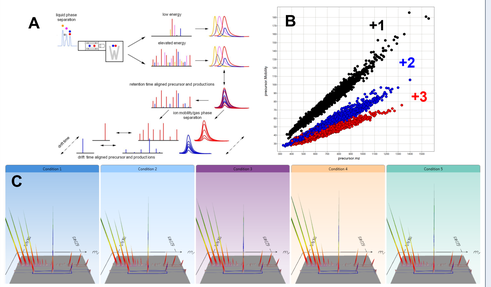


Figure 4. HDMSe Acquisition. (**A**) Illustration of time-resolved precursor and product ion acquisition (LCMSe) with ion mobility separation (IMS) for HDMSe data acquisition, (**B**) Separation of +1 (**black**), +2 (blue) and +3 (red) precursors with IMS (**C**) IMS separation of co-eluting peptides from replicate analysis of ELF-Fraction07 of *E. coli* reference sample.

Spectral Library Results

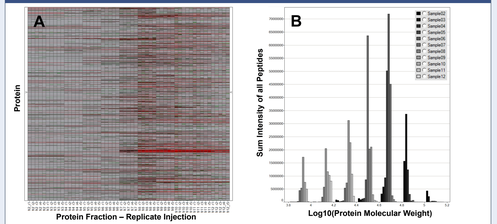


Figure 5. (**A**) Heat map of proteins identified from replicate analyses of the 11 protein fractions and (**B**) the sum total intensity of characterized proteins identified in each protein fraction using TransOmics analysis.

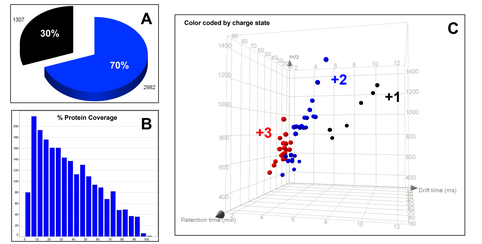


Figure 6. (**A**) Fraction of identified proteins from *E. coli* proteome (**B**) Distribution of protein coverage (**C**) Ion Map of AceA (mass, RT, drift time).

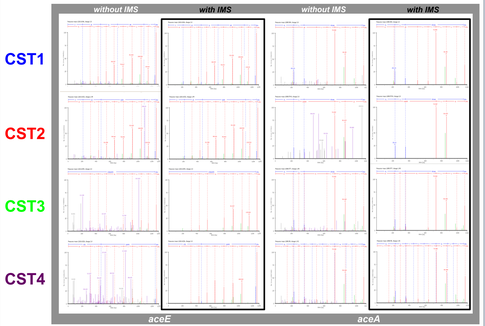


Figure 7. HDMSe drift resolved fragment ion spectra for aceE and aceA for *E. coli* growth conditions (see Figure 8 for sample descriptions).

Quantitative and Qualitative Results

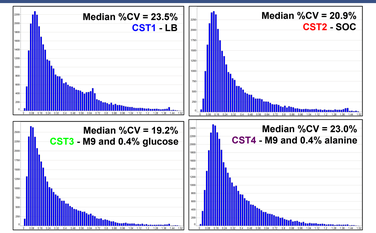


Figure 8. Distribution of variation among replicate injections of four *E. coli* growth conditions (LB, SOC, M9/0.4% glucose and M9/0.4% alanine).

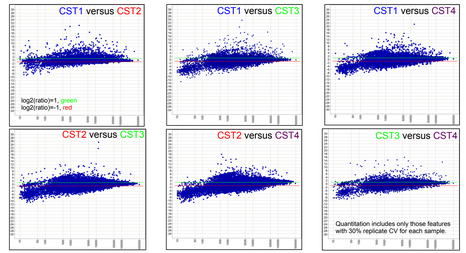


Figure 9. Binary comparisons of the TransOmics features from the four growth conditions (log2(ratio) versus control intensity (denominator)).

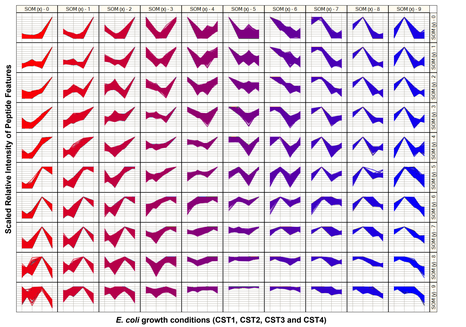
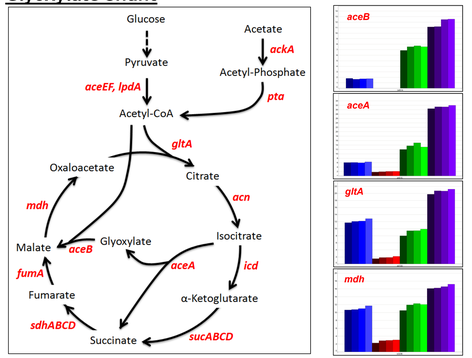


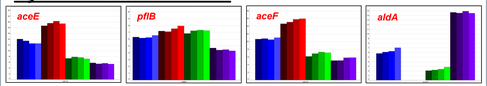
Figure 10. Self Organizing Map of the scaled relative intensity for all peptide features from the four *E. coli* growth conditions (see Figure 8).

Quantitative and Qualitative Results

Glyoxylate Shunt



Pyruvate Metabolism



Ribosomes

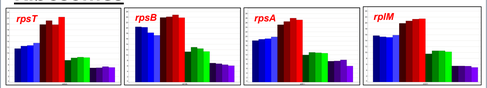


Figure 11. Protein abundance observed among the four *E. coli* growth conditions (CST1, CST2, CST3 and CST4), including replicates, for select protein groups or pathways (Glyoxylate Shunt, Pyruvate Metabolism and Ribosomes). Relative protein abundance is estimated using the Top3 peptides calibrated against internal standard (yeast 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 utilization of 2D-RP/RP (pH 10/pH 3) LC-HDMSe to increase the percent sequence coverage of each protein, and increase the percent coverage of the *E. coli* proteome from a single multi-dimensional analysis study.

References

S.J. Geromanos etal (2009) Proteomics, 9: 1683-1695.

J.C. Silva etal (2006) Molecular & Cellular Proteomics, 5: 589-607.

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