

## **Collaborative Proteomics- Description of the 1 Hour Yeast Proteome and its use in GLBRC**

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### **Project Goals**

This project was initiated to exploit contemporary proteomic methodologies to achieve two goals of the GLBRC. The first is to investigate the mechanism of toxicity of specific fermentation inhibitors (e.g.  $\gamma$ -valerolactone (GVL), ionic liquids, isobutanol). The second is to improve our understanding how engineered and evolved strains of *Saccharomyces cerevisiae* metabolize xylose into biofuels.

### **Abstract**

The ability to measure differences in protein expression has become key to understanding biological phenomena. Owing to cost, speed, and accessibility, transcriptomic analysis is often used as a proteomic proxy. However, mRNA is a genetic intermediary and cannot inform on the myriad of post-translational regulation processes. For the past decade considerable effort has been invested in maturing proteomic technology to deliver information at a rate and cost commensurate to transcriptomic technologies. Recently, a new Orbitrap hybrid mass spectrometer comprised of a mass filter, a collision cell, a high-field Orbitrap analyzer, and a dual cell linear ion trap analyzer was introduced (Q-OT-qIT, Orbitrap Fusion, Thermo). This system offers a high MS<sup>2</sup> acquisition speed of 20 Hz, and with this fast scan rate, is capable of providing whole proteome analysis in record time. To maximize instrument performance we developed an optimized cellular lysis approach, employed trypsin digestion, and used dimethyl sulfoxide (DMSO, 5%) as an LC additive to increase abundance of acidic peptides and unify charge state. Using this novel system we report the comprehensive analysis of the yeast proteome (4,002 @ 1% FDR) following 1.3 hours of nLC-MS<sup>2</sup> analysis (70 minute gradient). Over the chromatographic method, the Q-OT-qIT hybrid collected an average of 13,447 MS<sup>1</sup> and 80,460 MS<sup>2</sup> scans per run to produce 43,400 peptide spectral matches and 34,255 peptides with unique amino acid sequences. These experiments delivered an extraordinary 67 proteins per minute and demonstrate that complete analysis of the yeast proteome can be routinely performed in approximately one hour.

We have applied our single shot yeast proteomic analysis to validate chemical genomic analyses studies that revealed a number of mitochondrial gene mutants had increased sensitivity to the ionic liquid, and that EMIM-Cl and BMIM-Cl alter mitochondrial membrane polarization similar to the ionophore valinomycin. High-throughput quantitative proteomics validated this prediction, as there was significant enrichment for proteins involved in mitochondrial processes among proteins with depleted abundance in the presence of EMIM-Cl. From this study we also find 36 genes that have both significantly altered protein expression and affect viability when knocked out, in

the presence of EMIM-Cl. These genes represent proteins that are both important to the *in vivo* response to EMIM-Cl treatment and have a measurable effect on susceptibility to EMIM-Cl. Additionally, we found the expression of *hsp30*, a negative regulator of Pma1p, is decreased by ~10 fold after EMIM-Cl treatment. From chemical genomics experiments we know deletion of *ptk2*, an activator of Pma1p, imparts resistance to EMIM-Cl toxicity, yet its expression is not affected by treatment. Thus over expression of *hsp30* may impart EMIM-Cl resilience similar to *ptk2* removal and may also be a pivotal gene in explaining EMIM-Cl toxicity or a new point of rational engineering.

In addition to the above, we have also analyzed proteomic profiles of engineered and evolved *S. cerevisiae* in biological triplicate under aerobic and anaerobic conditions to elucidate how genetic changes combinatorially promote *S. cerevisiae* growth and metabolism of xylose under aerobic and anaerobic conditions. Interestingly, we found that enzymes mediating the metabolism of non-fermentable carbon sources were significantly downregulated in xylose metabolizing strains. This suggested that the engineered and evolved mutations cause *S. cerevisiae* to recognize xylose as a fermentable carbon source. This analysis demonstrates the power of combining complimentary metabolomics and proteomics approaches to tease out molecular details.

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