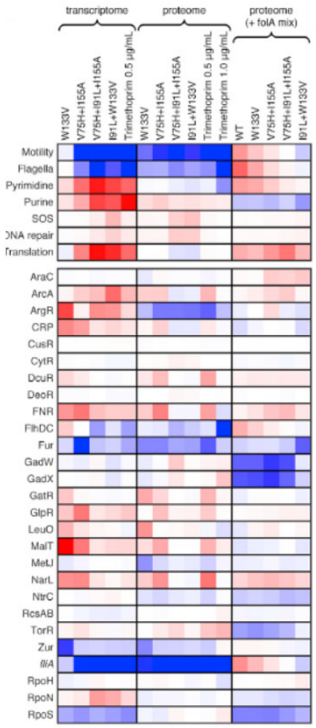
FAS Division of Science
Mass Spectrometry Proteomics and Research Laboratory (MSPRL)

proteomics@fas.harvard.edu http://proteomics.fas.harvard.edu/

Whole Proteome Quantitative Proteomics

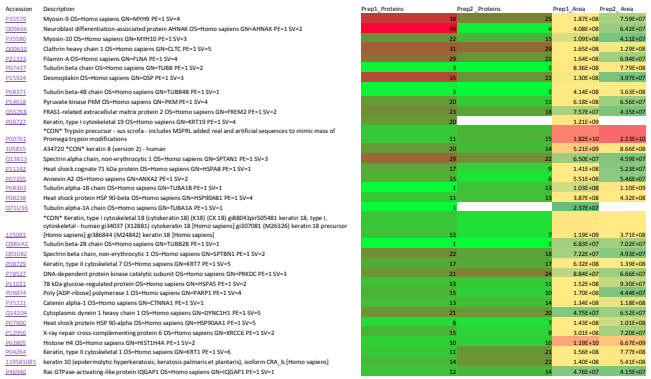
Quantitative whole proteome analysis via high throughput quantitative profiling of complex protein mixtures is essential for an understanding of function and control of biological systems. For this type of human analysis we generally use a focused-ultrasonicator to lyse cells followed by Chloroform/Methanol precipitation and FASP (Filter Aided Sample Prep) digestion. We label from 6 to 10 samples with TMT (Targeted mass tags) and pool the samples followed by fractionation. With fractionation into 20 fractions we may see 5,000 – 6,000 proteins, with 40 fractions from 7,000 – 8,000 and with 60 fractions we may see more than 10,000 proteins.

Here we show that a global proteome and transcriptome response to destabilizing mutations in a core metabolic, enzyme dihydrofolate reductase (DHFR), quantitatively links the molecular effects of mutations to bacterial fitness. [1]



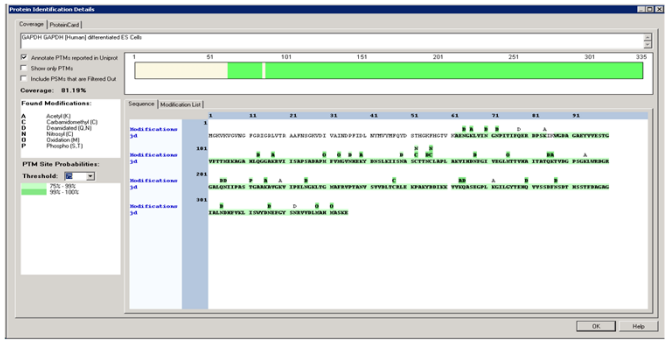
Pull Down Comparisons

Scientists studying cellular pathways do so by looking at complex protein-protein interactions in an effort to determine the function of protein products which may then illuminate the gene function. We can compare different states of interest or compare a control pulldown to a compromised or modified state via a pull down comparison analysis.



PTM Characterization

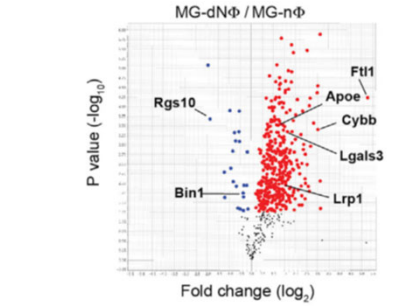
Determining sites of Post Translational Modifications (PTM's) is essential to disease inhibition, treatments and prevention as well as general studies of cell biology. Modifications can include phosphorylation, glycosylation, ubiquitination, nitrosylation, methylation, acetylation, lipidation and proteolysis as well as addition of user bonded modifications and sites of cleavage or degradation.
We are able to provide a comprehensive picture of most sites of modification.

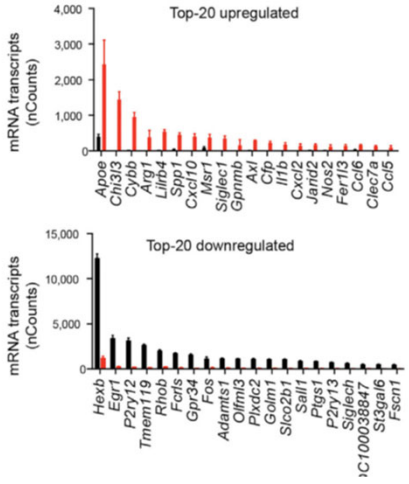


Sensitive Proteomics

Quantitative Mass Spectrometry was used to demonstrate upregulation of several significant proteins in young vs. old mice microglia from both a disease prone cell line and wild type with very low amounts of cells to work from. In this case we had only 5,000 microglia cells to work with post FACS sorting. This type of approach led to our current work flow for single cell analysis.

We found significant differentiation between low level proteins: homeostatic non-phagocytic phenotype (MG-No) and amoeboid-phagocytic phenotype (MG-dNo) [2]





Mass Spectrometry and Proteomics Resource Laboratory (MSPRL)
52 Oxford Street Room B251
Cambridge MA 02138
ph: 617-495-4043
proteomics@fas.harvard.edu
http://proteomics.fas.harvard.edu/

Lab Members:
Dr. Bogdan Budnik - Director
John Neveu
Renee Robinson

References:

1. Bershtein et al., 2015, Cell Reports 11, 645–656, April 28, 2015
2. Krasemann et al., 2017, Immunity 47, 566–581, September 19,