



# FAS Division of Science

## Mass Spectrometry Proteomics and Research Laboratory (MSPRL)

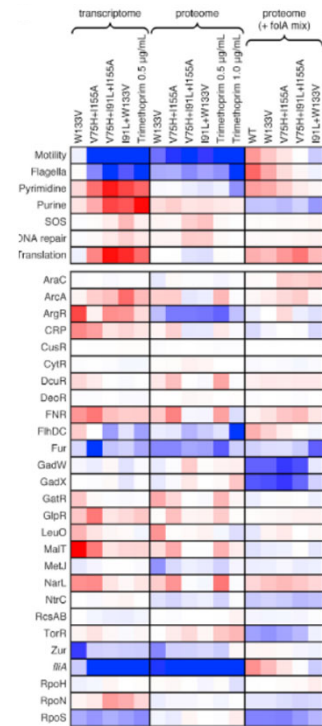
proteomics@fas.harvard.edu http://proteomics.fas.harvard.edu/



### 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. <sup>1</sup>



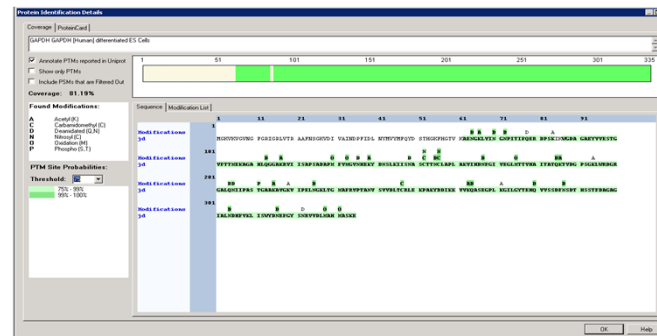
### Pull Down Comparisons

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 pull-down to a compromised or modified state via a pull down comparison analysis.

Accession	Description	Prep1 Proteins	Prep2 Proteins	Prep1 Area	Prep2 Area
P12312	Myosin II OS-Homo sapiens GN-Myo9 PE-1 S0+4	18	15	1.81E+08	9.76E+07
P12313	Neurofilament-associated protein ANNA6 OS-Homo sapiens GN-ANNA6 PE-1 S0+2	18	15	4.08E+08	4.42E+07
P12314	Myosin-20 OS-Homo sapiens GN-Myo20 PE-1 S0+4	22	15	5.09E+08	2.83E+08
P12315	Cadherin-10 OS-Homo sapiens GN-CDH10 PE-1 S0+5	11	10	1.05E+08	1.29E+08
P12316	Filamin-A OS-Homo sapiens GN-FIL1A PE-1 S0+4	18	15	1.64E+08	6.94E+07
P12317	Tubulin beta chain OS-Homo sapiens GN-TUBB1 PE-1 S0+2	1	1	8.30E+08	1.79E+08
P12318	Desmoplakin OS-Homo sapiens GN-DSP PE-1 S0+1	15	15	1.30E+08	5.97E+07
P12319	Tubulin beta 4B chain OS-Homo sapiens GN-TUBB4B PE-1 S0+1	1	1	4.14E+08	1.63E+08
P12320	Pyruvate kinase PKM OS-Homo sapiens GN-PFKFB3 PE-1 S0+4	19	18	1.18E+08	1.55E+07
P12321	FRAG-related extracellular matrix protein 2 OS-Homo sapiens GN-FREM2 PE-1 S0+2	11	11	7.93E+07	4.93E+07
P12322	Keratin, type I cytoskeletal 39 OS-Homo sapiens GN-KRT39 PE-1 S0+4	1	1	1.21E+09	
P12323	"CDN" Topoisomerase, nuclear, includes KAPPA, added real and artificial sequences to mimic case of Promega Topoisomerase	11	15	1.82E+10	2.28E+10
P12324	ADAMTS-1/CDN keratin 1 (keratin 21, human)	14	14	1.22E+09	8.65E+08
P12325	Spectrin alpha chain, non-erythrocytic 1 OS-Homo sapiens GN-SPTAN1 PE-1 S0+1	20	22	6.52E+07	4.95E+07
P12326	Heat shock cognate 70 kDa protein OS-Homo sapiens GN-HSP70 PE-1 S0+1	17	19	1.41E+08	5.29E+07
P12327	Annexin A2 OS-Homo sapiens GN-ANXA2 PE-1 S0+2	11	11	1.51E+08	3.46E+07
P12328	Tubulin alpha-1B chain OS-Homo sapiens GN-TUBA1B PE-1 S0+1	1	1	1.09E+08	1.10E+08
P12329	Heat shock protein HSP 90-alpha OS-Homo sapiens GN-HSP90A PE-1 S0+4	11	11	1.87E+08	4.33E+08
P12330	Tubulin alpha-1A chain OS-Homo sapiens GN-TUBA1A PE-1 S0+1	1	1	2.97E+08	
P12331	"CDN" Keratin, type I cytoskeletal 18 (cyokeratin 18) (K18) (CK 18) gBBD4bort504E1 keratin 18, type I, cytoskeletal, human (K18) (CK18) (cyokeratin 18) (Homo sapiens) (Keratin 18) (Homo sapiens)	11	11	1.19E+09	1.71E+08
P12332	Tubulin beta 3B chain OS-Homo sapiens GN-TUBB3B PE-1 S0+1	1	1	8.02E+07	7.02E+07
P12333	Spectrin beta chain, non-erythrocytic 1 OS-Homo sapiens GN-SPTBN1 PE-1 S0+2	12	17	7.22E+07	4.93E+07
P12334	Keratin, type II cytoskeletal 7 OS-Homo sapiens GN-KRT7 PE-1 S0+5	17	17	6.32E+08	1.39E+08
P12335	CDN-dependent protein kinase-related substrate OS-Homo sapiens GN-PRKDC PE-1 S0+1	21	20	8.40E+07	6.66E+07
P12336	78 kDa glucose-regulated protein OS-Homo sapiens GN-HSPA1 PE-1 S0+2	13	13	1.52E+08	8.30E+07
P12337	Prolyl 4-hydroxylase 1 OS-Homo sapiens GN-PH4 PE-1 S0+4	15	15	1.70E+08	3.68E+07
P12338	Galactose alpha-1 OS-Homo sapiens GN-C1NA1 PE-1 S0+1	11	11	1.34E+08	1.18E+08
P12339	Cytoplasmic dyxins 1 heavy chain 1 OS-Homo sapiens GN-DYX1H1 PE-1 S0+5	11	11	4.79E+07	6.52E+07
P12340	Heat shock protein HSP 90-alpha OS-Homo sapiens GN-HSP90A PE-1 S0+5	9	9	1.41E+08	1.01E+08
P12341	X-ray repair cross-complementing protein 6 OS-Homo sapiens GN-RCC6 PE-1 S0+2	11	11	1.01E+08	7.20E+07
P12342	Malware M4 OS-Homo sapiens GN-MST4A PE-1 S0+2	11	11	1.99E+10	6.97E+08
P12343	Keratin, type I cytoskeletal 2 OS-Homo sapiens GN-KRT1 PE-1 S0+6	11	11	1.56E+08	1.77E+08
P12344	Keratin 20 (epidermal) (hyperkeratotic keratosis palmorum et plantarum), isoform CRA_b (Homo sapiens)	14	14	1.40E+08	1.41E+08
P12345	Hex GTPase-activating-like protein (G24P) OS-Homo sapiens GN-G24P1 PE-1 S0+1	11	11	1.76E+07	1.73E+07

### PTM Characterization

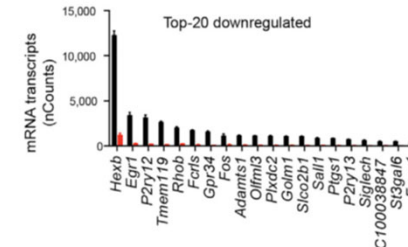
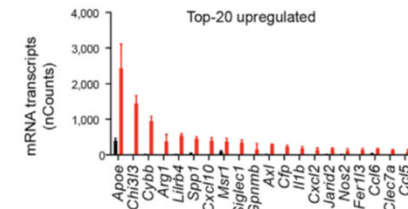
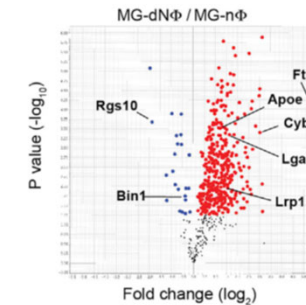
Determining sites of Post Translational Modifications (PTM's) is essential to disease inhibition, treatments and prevention as well as general studies of cell biology. Modifications can include phosphorylation, glycosylation, ubiquitination, nitrosylation, methylation, acetylation, lipidation and proteolysis 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) <sup>2</sup>



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:

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