Global Phosphoproteomic Analysis from Low Sample Amounts Enabled by Effective Phosphopeptide Enrichment

Fang Liu[1], Momei Zhou[2], Kratika Singhal[1], Rowan Matney[1], Stefan L. Oliver[2], Ann M. Arvin[2], Ryan D. Leib[1], Allis S. Chien[1]

[1] Stanford University Mass Spectrometry, Stanford University, Stanford, CA;

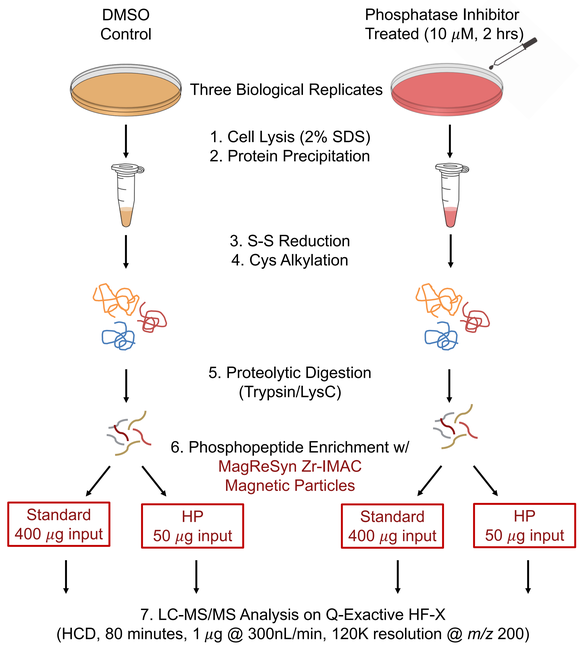
[2] Departments of Pediatrics and Microbiology & Immunology, Stanford University School of Medicine, Stanford, CA.

Introduction

Protein phosphorylation is an important regulatory mechanism of protein function and cellular signal transduction. Comprehensive analysis of a phosphoproteome by mass spectrometry can provide valuable information on the molecular level to better understand cellular biology and disease mechanisms. However, due to the low stoichiometry and transient nature of most phosphorylation events, enrichment for phosphoproteins and phosphopeptides is often necessary for their identification.

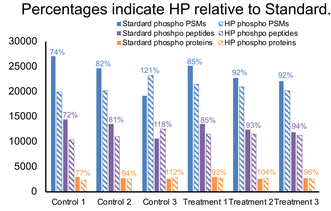
This study examines phosphoproteomic changes in the MeWo human melanoma cell line upon treatment with a phosphatase inhibitor. Discussed here will be both technical evaluation of the phosphopeptide enrichment method using Zr-IMAC magnetic beads with low sample amounts, and in-depth data analysis of the differential phosphoproteomic profiles with and without phosphatase inhibitor.

Experimental Workflow

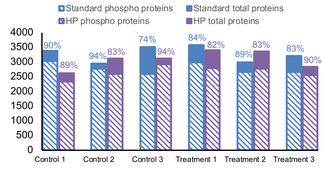


Results and Discussion

A. Overall phosphoproteomic coverage. Percentages indicate HP relative to Standard.

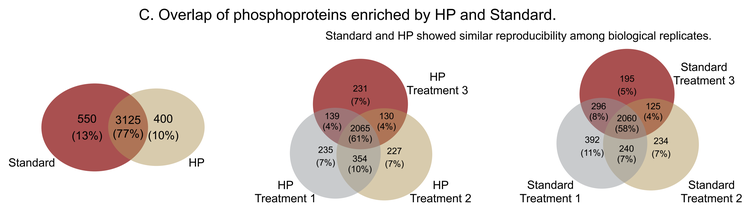


B. Similar enrichment efficiency between HP and Standard. Percentages indicate phosphoproteins relative to total proteins.

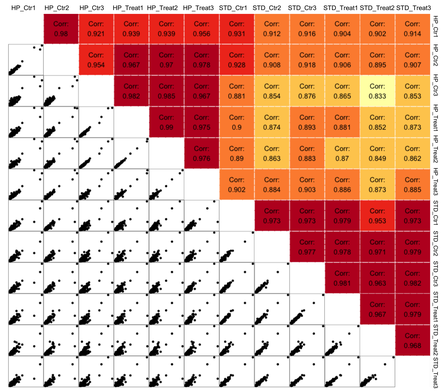


C. Overlap of phosphoproteins enriched by HP and Standard.

Standard and HP showed similar reproducibility among biological replicates.



D. Heatmap of Pearson correlation of phosphoproteins enriched with different amounts of starting material shows high quantitative reproducibility of over 80%.



E. Fold change analysis with Log2 values of spectral count ratios (Standard/HP) to further examine outlier proteins in the Pearson correlation analysis (see Fig 1D).

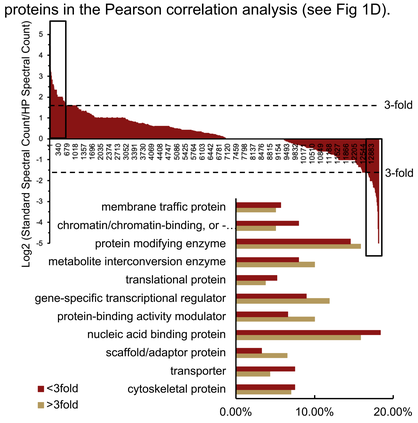
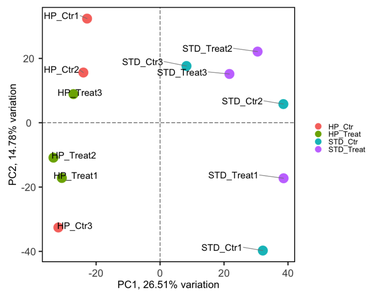
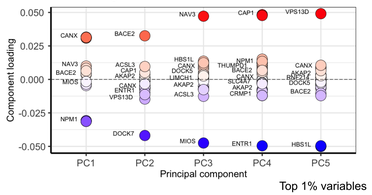


Fig 1. Global evaluation of the phosphopeptide enrichment performance using MagReSyn Zr-IMAC magnetic beads with different amounts of starting material (Standard: 400 μg; HP: 50 μg).

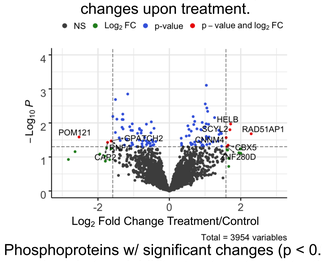
A. Principal component analysis (PCA) comparing all samples.



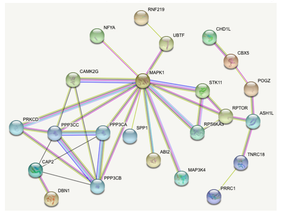
PCA Loadings



B. Volcano plot comparing phosphoproteome changes upon treatment.



C. Phosphoproteins w/ significant changes ($p < 0.05$) upon phosphatase inhibitor treatment as potential substrates of the phosphatase under study.



D. Unsupervised hierarchical clustering of phosphoproteins w/ significant changes ($p < 0.05$) upon phosphatase inhibitor treatment.

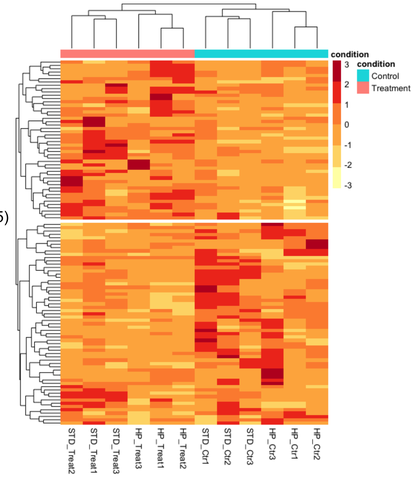


Fig 2. Global analysis of phosphoproteome changes upon treatment with phosphatase inhibitor.

Conclusions

Technical:

1. The overall phosphoproteomic coverage obtained w/ 50 μg starting material was ~90% of that obtained w/ 400 μg starting material.
2. Similar enrichment efficiency and reproducibility was observed w/ either 400 μg or 50 μg of starting material.
3. Different amounts of starting material did not introduce bias in terms of enriched protein class.
4. Therefore, the results have demonstrated the feasibility of phosphopeptide enrichment with relatively low sample amounts.

Biological:

1. PCA analysis showed greater variance among Control samples compared to Treatment samples.
2. Top PCA loading proteins and differentially regulated proteins (p-value < 0.05) included many that have known interactions w/ the phosphatase under study.
3. Unsupervised hierarchical clustering showed that phosphatase inhibitor-treated samples had enriched MAPK signaling pathway, while control samples had enriched mTOR, HIF-1, neurotrophin, and PI3K-Akt signaling pathways.

ACKNOWLEDGEMENTS

This work is supported by the Stanford Dean of Research, the Vincent and Stella Coates Foundation, and SUMS Affiliate Program Seed Funding Grant.