Phosphoproteomics- Reanalysis of mass spectrometry data

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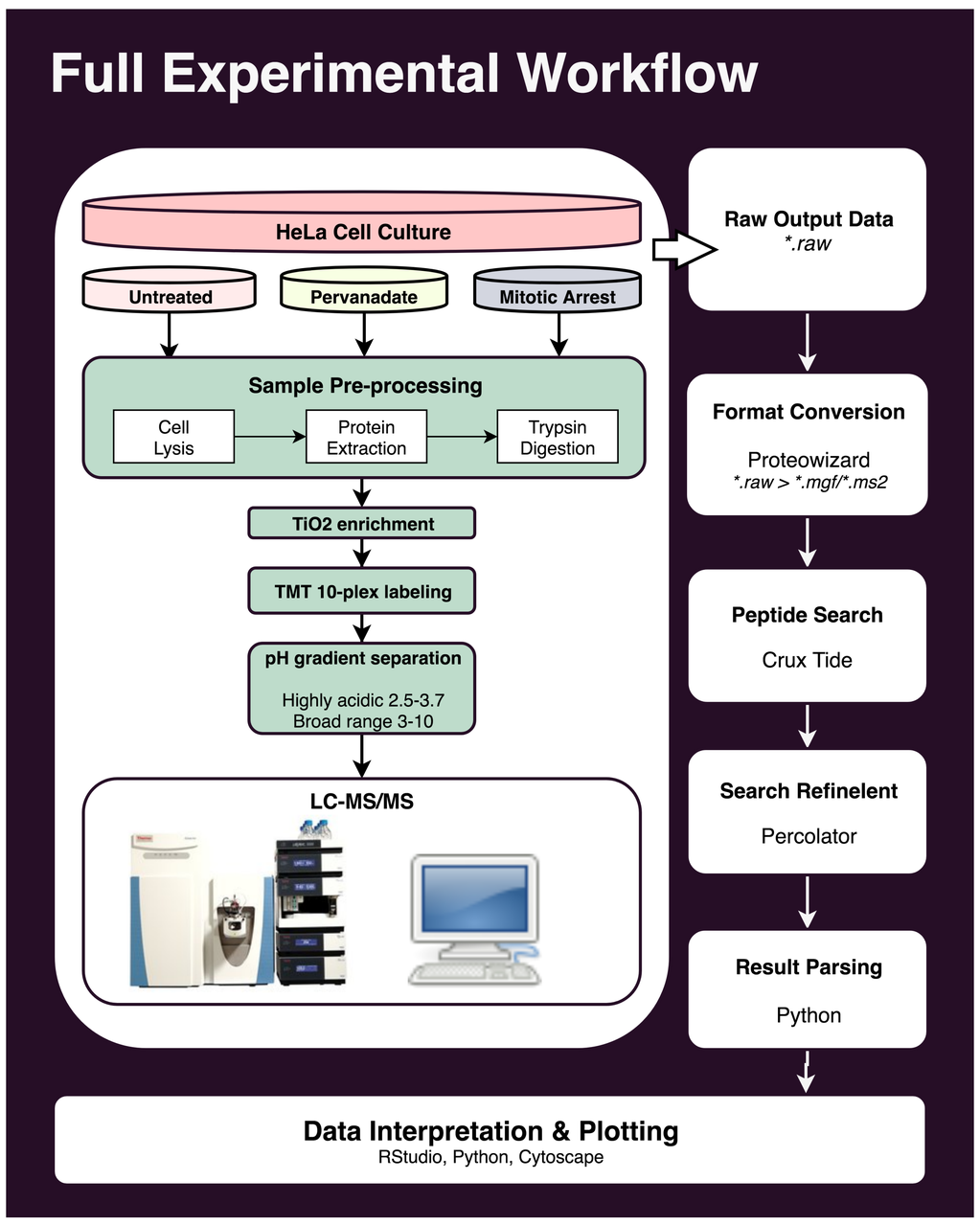
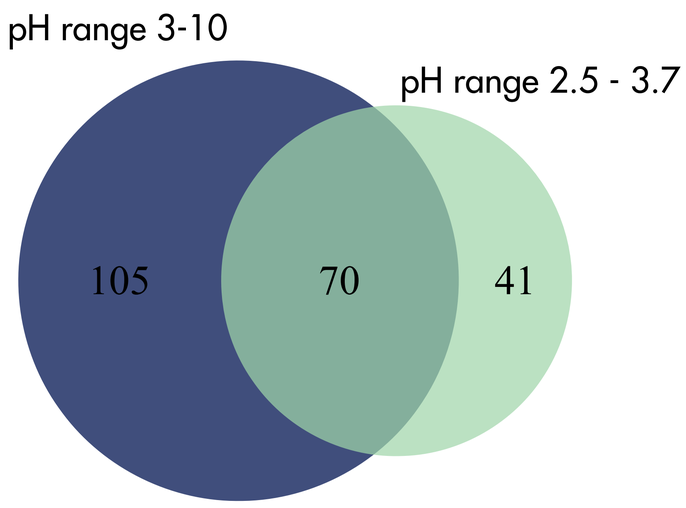
Introduction

Post-translational modifications of cellular proteins can have a strong impact on protein interactions or whole pathways. To detect and interpret phosphorylation modifications, a novel workflow was developed by Panizza et al. [1], integrating titanium dioxide phospho-enrichment HiRIEF fractionation, isobaric labeling and LC-MS/MS.

Our goal was to uncover a robust strategy for analysis of such high-throughput experimental data, by evaluating performance of various existing interpretation tools



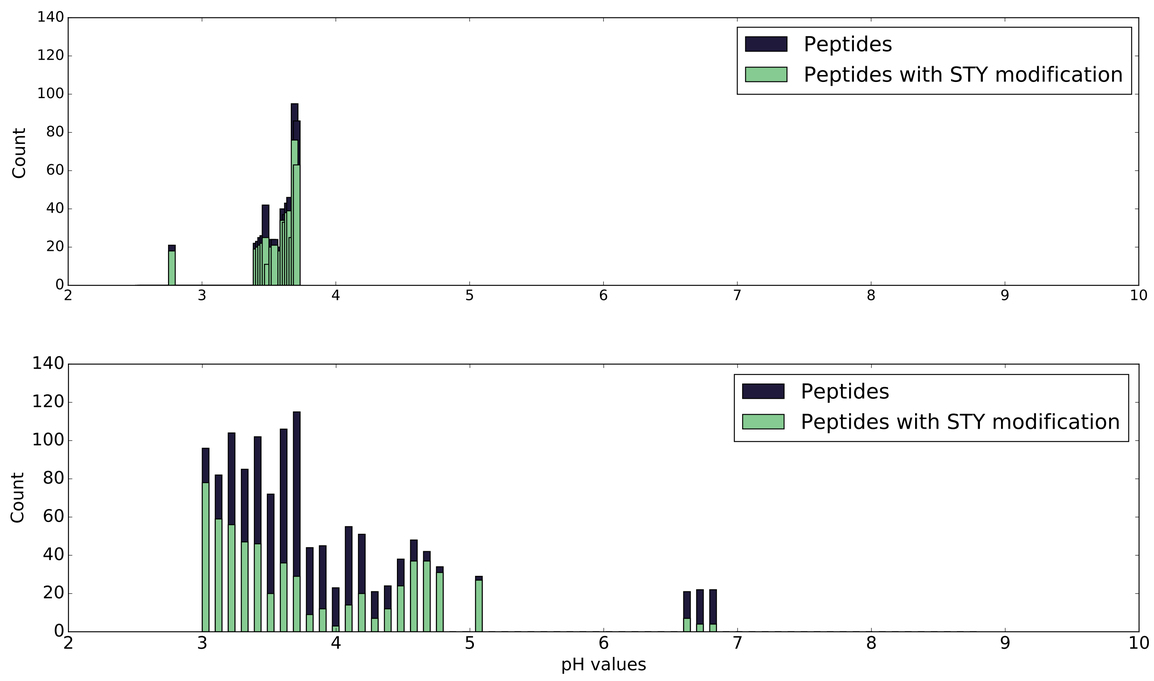


Overlap of phopshorylated proteins identified using two different pH ranges with isoelectric point-based fractionation by HiRIEF.

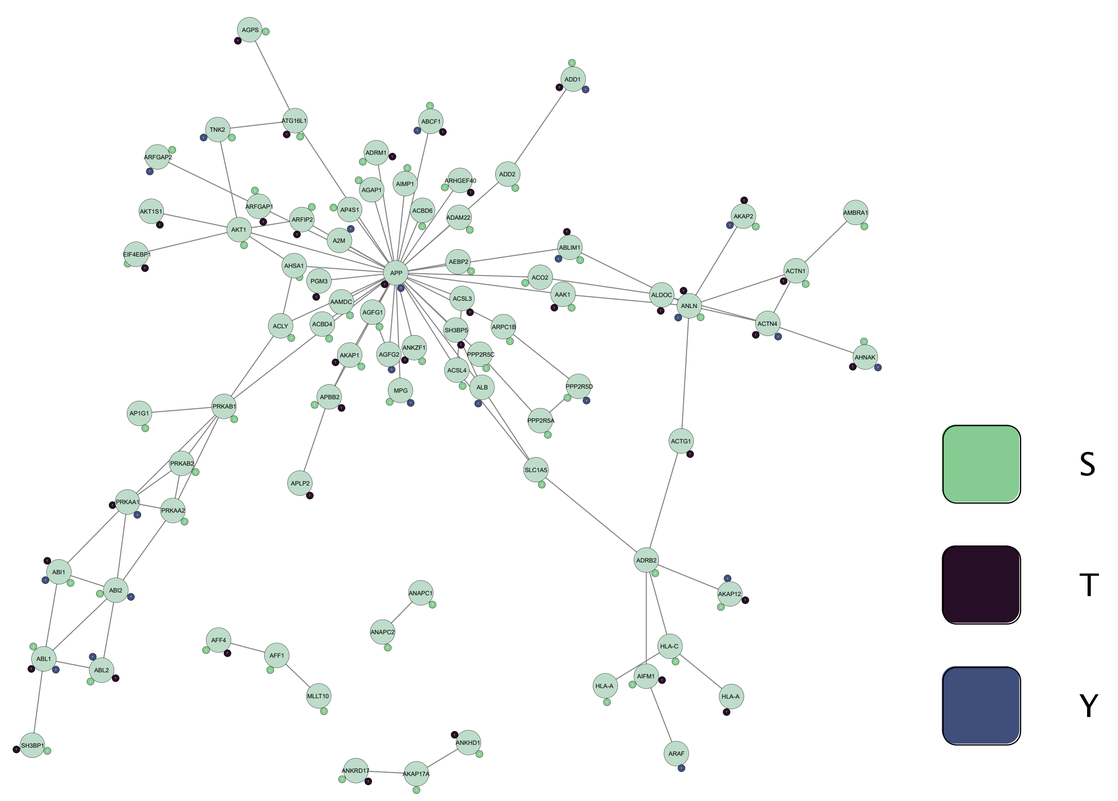
The authors used two different immobilized pH gradient gel strips with the pH ranges "ultra - acidic" (pH 2.5 - 3.7) and "wide" (pH 3-10) [1]. Each strip is divided into 72 fractions. The authors did not identify any phosphopeptides in the last 11 fractions of wide range samples and discarded these fractions from the study.

The figures below shows the total number of peptides we identified in different pH ranges (with q-value<0.05). In the ultra-acidic and wide pH ranges, we found peptides in 17/72 and 26/60 fractions. The authors reported 7,338 and 16,153 unique phospho-peptites at pH ranges whereas we only found 279 and 458.

Number of peptides and peptides with STY modifications based on pH - ultra acidic range (top) and wide range (lower)



Network of phosphorylated proteins identified using isoelectric point-based fractionation by HiRIEF followed by LC-MS



Network visualisation of the phosphorylated proteins was performed using the Cytoscape software platform with the PhosphoPath plugin. Using the 179 proteins (3 - 10 pH range) with their 828 phosphorylation sites, we obtained an interaction network that was more complex than the one we obtained with 110 proteins (2.5 - 3.5 pH range) with their 484 phosphorylation sites. Visualized in the network are 83 out of 179 proteins that interact with one or more other phosphorylated proteins and conclude that using the approach of a wider pH range enabels the charactarisation of more proteins but also more phosporylations. We identified phosphorylated proteins and could show that they in interact with other phosphorylated proteins. Hence we assume that **the identified phosphorylation belong to functional aspects of the proteins.**

Conclusions

We were unable to fully replicate the results. We identified fewer phosphorylated peptides and proteins. However most of the identified phosphoproteins were found to interact with each other, hence leading to the conclusion that the modifications are functional.

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References

1 Panizza, Elena, et al. "Isoelectric point-based fractionation by HiRIEF coupled to LC-MS allows for in-depth quantitative analysis of the phosphoproteome." Scientific reports 7.1 (2017): 4513.
2 Raaijmakers LM, et al. PhosphoPath: Visualization of Phosphosite-centric Dynamics in Temporal Molecular Networks. J. Proteome Res. 2015;14:4332–4341. doi: 10.1021/acs.jproteome.5b00529.
3 Christopher Y. Park, Aaron A. Klammer, Lukas Käll, Michael J. MacCoss and William Stafford Noble. "Rapid and accurate peptide identification from tandem mass spectra." Journal of Proteome Research. 7(7):3022-3027, 2008.