

## **Quantification of Multiple Post-Translational Modifications by ‘One-Pot’ Affinity Enrichment - Applications in *E. coli***

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### **Project Goals:**

**The goal of this project is to gain deeper insights into posttranslational remodeling of engineered microorganisms, and specifically their protein acylomes under different growth conditions. Lysine acetylation, for example is a common post-translational modification (PTM) that eukaryotes, archaea, and bacteria employ to regulate protein activity. In fact, most metabolic enzymes are subject to lysine acetylation. Due to the dynamic nature of protein acetylation and deacetylation mechanisms in the cell, lysine acetylation can likely be considered a global mechanism to regulate metabolism in response to their energy and redox status. Here, we are presenting a novel workflow that will enrich multiple posttranslational modifications, such as acetylation and succinylation in a ‘one-pot’ affinity enrichment procedure, which will greatly improve throughput and enable PTM crosstalk. The significance of this work is that it will provide new tools to address a fundamental gap in our understanding of bacterial metabolism and identify new approaches for overcoming the problems associated with the production of advanced biofuels.**

### **Abstract:**

Quantitative proteomic studies of post-translational modifications (PTMs) are increasingly of widespread interest in biomedical research; however, rarely do these studies consider multiple modifications in parallel. One barrier to multi-PTM studies is the time costs for both sample preparation and instrument acquisition, which scale linearly with the number of modifications studied. Perhaps the most prohibitive requirement of PTM studies is the need for large amounts of sample material, which typically must be increased proportionally with the number of PTM enrichments. Here, we describe an innovative, streamlined, quantitative label-free proteomic workflow (“one-pot” enrichment), which allows for comprehensive identification and quantification of acetylated and succinylated peptides from a single sample containing 1 mg of mitochondrial protein. We show that simultaneous immunoaffinity enrichment of acetylated and succinylated peptides by ‘one-pot’ enrichment is the most efficient enrichment method, requiring the least sample preparation and instrument time without compromising data quality. We further show that coupled with a label-free, data-independent acquisition (DIA, e.g. SWATH), ‘one-pot’ enrichments from frozen mouse liver samples could identify and quantify 2235 acetylated and 2173 succinylated peptides from small amounts of input protein. We also demonstrate that the peak areas

of sites identified following one-pot enrichment are highly correlated with single-antibody pull downs. Finally, we show that this method makes it possible to detect both acetylation and succinylation modifications that occur on the same peptide. One-pot enrichment is a novel quantitative PTM workflow for enriching post-translational modifications as reproducibly as single-antibody enrichments, and enables the direct assessment of PTM crosstalk from biological samples with limited tissue material. This improvement in technology and workflow will be highly relevant for our acylation studies in *E. coli*, and will allow us to achieve higher throughput and better understanding of multiple PTM regulation during bacterial growth under diverse environmental and nutritional conditions.

#### References:

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