**Integrating Proteomic and Metabolomic Analyses to Optimize Cellular Extract Preparation for Enhanced Cell-Free Protein Synthesis**

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**Project Goals: The interdisciplinary clostridia Foundry for Biosystems Design (cBioFAB) project addresses the complex challenge of designing, building, and optimizing biosynthetic pathways in biological systems. The goal of the project is to accelerate engineering efforts in non-model organisms through *in vitro* and *in vivo* metabolic pathway prototyping, computational modeling, and integrated omics analysis. Through these diverse approaches, the project seeks to provide the tools to enable high-level synthesis of next-generation biofuels and bioproducts from lignocellulosic biomass and expand the breadth of platform organisms that meet DOE bioenergy goals.**

Although biocatalysts offer economic and efficient solutions for many biomanufacturing processes, there are limitations with using live cells for industrial production of potentially toxic compounds. Cell-free expression (CFE) systems provide an alternative solution for high-throughput enzyme prototyping and subsequent biomanufacturing of valuable chemicals that may or may not be compatible with living cells. A key component of CFE systems are cellular extracts, which provide the molecular machinery (i.e. transcription, translation) and metabolic intermediates required for biosynthesis. The overall activity, productivity and/or capability of these systems is thus intrinsically dependent on the extract preparation methodology itself. Routine procedures employed during extract preparation include sonication, ribosomal runoff, dialysis, and application of exogenous energetic molecules – slight modifications to any of these can affect CFE system output. Although the sonication energy applied to lyse cells has been optimized for generating highly productive extracts, the effect of runoff and dialysis has not been studied in detail. Proteins and metabolites form key components of any extract and improper processing can result in the loss of important factors required for optimal biosynthesis. To further investigate the impact of these processing steps on extract quality and to better inform future optimization efforts, an integrated omics analysis was performed. Cell-free extracts from *E. coli* BL21 utilizing different processing steps – no processing, after runoff, after dialysis, and after dual runoff and dialysis – for two commonly used salt conditions (acetate and glutamate) were prepared for proteomic and metabolomic analysis. Initial results indicate 87 proteins to be differentially abundant in the acetate extracts after both runoff and dialysis were applied; the condition in which the most dramatic decrease in activity was observed. Three of these proteins were related to transcription, a critical step for CFE, and were also altered after application of runoff alone. The metabolite profile was

also altered after dual processing, with the greatest effect driven by dialysis. The largest reduction was observed in nitrogen-containing metabolites, including amino acids, polyamines, purines, and pyrimidines, all of which are likely important for lysate productivity. Overall, this integrated analysis will help optimize cell-free extract preparation and provide information about the molecules required for cell-free activity in CFE systems.

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