Kinetic Modeling Tools Using Cell-Free Experiments to Predict Metabolic Network Behavior in Non-Model Systems

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Project Goals: We aim to develop a predictive model of metabolism in bacterial cell-free systems for the purpose of rapidly prototyping heterologous metabolic pathways. This model may be used to both optimize the production of metabolites in cell-free systems, as well as to understand how results in these systems should inform design in living organisms.

Metabolic engineering efforts with non-model organisms present many opportunities in small molecule production due to the wide variety of metabolic capabilities, including the anaerobic fermentation of waste gas streams or plant-based byproducts to valuable chemicals. However, these non-model microbes are slow growing, less well-characterized, and more difficult to genetically modify than model organisms. Additionally, the same complexity that allows these microbes to produce so many interesting compounds makes the task of cloning and testing all possible factors within the cell experimentally infeasible. While the maturation of cell-free technologies has allowed the rapid testing of many experimental conditions for a heterologous pathway outside the cell, there remains a need for methods to analyze these data and understand the connection between these cell-free results and how these experimental conditions will perform once translated to *in vivo* production strains. To accomplish this goal, we are using kinetic models, based on the metabolic ensemble modeling (MEM) framework, to elucidate the underlying kinetic parameters of the pathways of interest in these cell-free systems. Currently, we are working towards a model of cell-free metabolism which will both predict conditions to optimize production in cell-free, as well as recommend future experiments which will most efficiently train our model. Ultimately, we aim to construct a model framework which will utilize these high-throughput cell-free experiments to predict, for a given heterologous pathway of interest, which enzyme homologs and expression levels are most likely to maximize target production rate when applied *in vivo.* We have developed several novel features to the MEM framework which will allow us to uniquely utilize this cell-free data to accomplish these goals.

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