Modeling Tools to Predict Metabolic Network Behavior in Non-Model Systems

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Project Goals: We aim to develop computational tools to aid in the design and understanding of metabolic pathways for the production of novel compounds. Our work utilizes promiscuous reaction rules to enumerate candidate pathways and filters these pathways based on network topology, thermodynamics, and theoretical yield. Once candidate pathways are prototyped in cell-free systems, we utilize kinetic modeling to determine rate-limiting steps in the pathway along with system-level interactions which would not be detected through steady-state models alone.

Non-model organisms present many opportunities for the production of small molecules due to a 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, making it difficult to quickly test potential pathways. Cell-free systems provide a platform to overcome these challenges by allowing rapid prototyping of novel pathways in the context of a cell's native metabolism. Despite this ability to rapidly test pathways, there is still a need to efficiently design, filter, and interpret the immense number of potential pathways. To accomplish this task, we are focused on two areas: novel pathway design, and kinetic modeling of cell-free metabolism. The successful development of these tools will aid in the utilization of cell-free prototyping to efficiently design and implement pathways into non-model organisms.

To identify promising novel pathways, we are developing a computational workflow that generates, analyzes, and filters pathways from specified starting compounds to a set of desired target compounds. Metabolic networks are generated by using Pickaxe, a reaction network generation software that utilizes promiscuous reaction rules to transform compounds, allowing for the generation of pathways consisting of both known and novel reactions. These pathways are then analyzed to score important features such as their yield determined by flux balance analysis, number of known enzymes, pathway length, and thermodynamics. Thermodynamic feasibility is calculated by determining the max-min driving force using eQuilibrator, which has been modified to accept arbitrary compounds for inputs. These pathway features are then used to determine overall pathway feasibility and potential performance to rank the pathways into a set of the most promising candidates for experimental consideration.

Once a pathway has been designed and is prototyped in cell-free systems, there remains a need for methods to uncover the kinetics of this system. To this end, we are developing a framework to construct dynamic kinetic models to understand how the pathway of interest interacts with native metabolism. While past kinetic models have been parameterized from literature kinetic

values or extensive fluxomics datasets, the modeling of these cell-free systems do not have these data due to limited characterization of non-model organisms and the lack of detailed multi-omics from rapid prototyping. Therefore, this method first uses a limited set of metabolomics, proteomics, and thermodynamics to constrain a system of linear equations, which guide and constrain the sampling of underlying fluxes and metabolic states. We subsequently apply the metabolic ensemble modeling (MEM) framework to sample and prune best-fit parameters. By modeling this system with a dynamic metabolic model, we capture complex time-dependent interactions between the novel pathway and native metabolism, providing detailed understanding of these connections and allowing for better control and optimization of the pathway of interest. This work will ultimately allow for both deeper understanding of cell-free metabolism as well as improved engineered pathway titers in both *in vitro* systems and translated to *in vivo* production strains.

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