Computational Tools to Predict Novel Chemical Reactions and Metabolic Network Behavior

Jake Martin, Joseph Ni, Linda Broadbelt, Keith E.J. Tyo* (k-tyo@northwestern.edu), and **Michael Jewett**

Northwestern University, Evanston, IL

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Project Goals: The *Clostridium* Foundry for Biosynthetic Design (cBioFab) goal is to provide tools and engineering strategies 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. The project combines *in vitro* (cell-free) and *in vivo* work to interweave and advance state-of-the-art pathway design, computational modeling, genome editing, and systems-biology analyses. The cBioFAB goal is to engineer complex biological systems by linking pathway design, prospecting, validation, and production in an integrated framework with system-level omics data.

Advances in high throughput biology have enabled the routine collection of large, comprehensive datasets, which cannot be easily interpreted manually. In the design-build-test-learn cycle, there is an urgent need to extract actionable conclusions from these datasets (i.e. learn) to enable more sophisticated and successful designs. The *Clostridium* Foundry for Biosystems Design (cBioFab) is reconceptualizing the way we engineer complex biological systems by linking pathway design, prospecting, validation, and production in an integrated framework that relies on computational modeling, cell-free technologies, and system-level omics data. Our computational efforts are focused on two important questions: (1) how to utilize high throughput cell-free metabolic engineering data to inform the design of metabolic pathways in microorganisms that are challenging to engineer? and (2) how to utilize enzyme activity and promiscuity data to predict novel biochemical reactions that have utility in biosynthesis of chemicals and fuels?

To inform microorganism engineering from cell-free measurements, we are exploiting metabolic ensemble modeling (MEM) as a multiparameter sampling approach toward estimating enzymatic parameters. As the cell-free environment has several distinct differences from the cytoplasmic milieu, strong metabolic performance in a cell-free reaction may not translate to strong performance in the cell. By estimating enzyme parameters that are independent of environment, we can replace cell-free environmental parameters with appropriate *in vivo* parameters. We have developed a number of unique features in the MEM framework that will enable us to model cell-free systems, a first step toward our larger goal.

Toward the discovery of useful, novel biochemical reactions, we are using the Biochemical Network Integrated Computational Explorer (BNICE) to predict possible products from known metabolites and retrosynthetically predict precursors that lead toward target compounds. We are currently improving prediction accuracy by developing algorithms to better 'learn' from many available metabolic databases. The pathways and candidate enzymes predicted by BNICE can be rapidly tested in cell-free systems to identify lead enzymes for implementing in microbes. Altogether, these computational tools should enable faster and more sophisticated engineering of metabolic pathways to produce fuels and chemicals.

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