Dynamic Metabolic Model Building Based On The Ensemble Modeling Approach

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Project Goals: The goal of this project is to develop a novel modeling approach to describe the dynamic behavior of metabolic systems (in particular, flux changes upon enzyme tuning) by integrating multiple data platforms including flux, metabolite, transcriptome, and enzyme tuning data. Although the utility of such models is undeniable, their development has been impaired by inadequate modeling approaches, the sheer size of the problem, and difficulties in accessing the intracellular environment. As a result, little progress has been made in realizing such dynamic models despite the continuously increasing number of intracellular measurements that are becoming available by high throughput methods. The resulting models from the proposed research will account for pathway enzyme kinetics and aim to predict the effects of genetic manipulations designed to bring about changes in metabolic flux and overproduction of metabolites, such as tuning various enzyme levels or the Michaelis-Menten constants (K_m) of key enzymes. In this context, such models will be instrumental for constructing microbial strains to produce various biofuels such as ethanol, 1-butanol, and isobutanol from renewable resources. We will use production of these fuels in *Escherichia coli* as a model system, because of *E. coli*'scentral role as a test bed in systems biology, the wealth of kinetic and regulatory information available and its successful usage for the production of biofuels. While the *E. coli* focus will facilitate model development, the approach developed will be general and applicable to other microorganisms and eventually plants. The project is based on the Ensemble Modeling (EM) approach, robust flux and metabolite measurements, and an efficient optimization scheme developed in the PIs' laboratories.

Presently, there are no satisfactory dynamic models of cellular function. This unique deficiency persists despite recent advances in the areas of high throughput measurement of cell-wide intracellular biomolecules and molecular level simulations of various systems. Current approaches for creating dynamic models of cellular function attempt to do so by fitting transient metabolite concentration data to various kinetic rate expressions. These data are difficult to obtain and often have large experimental errors, making it impossible to scale up to the levels required by our current understanding of cellular metabolism. The Ensemble Modeling (EM) framework was proposed to address this issue by relying only on steady-state measurements (although transient measurements can also be used) to create accurate kinetic models of cellular metabolism. Here we introduce the EM framework and show how it can be integrated with robust flux measurement techniques and efficient optimization schemes in order to arrive at such models.