181. Dynamic 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 networks (in particular, flux changes upon enzyme tuning) by integrating multiple data platforms, including flux, metabolites, 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 biofuels in *Escherichia coli* as a model system, both because of its central 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 the extraordinary advances that have taken place during the past decade in the areas of high throughput measurement of cell-wide intracellular biomolecules and molecular level simulations of various systems. Present models of microbial metabolism suffer from serious drawbacks that limit their applicability as a robust and versatile tool for re-engineering metabolic networks. Such limitations include: (a) reliance on coarsely lumped kinetic and regulatory information, (b) sparse and/or unreliable kinetic parameters derived under mostly in vitro conditions that poorly approximate the cellular environment, (c) small scale models describing only fractions of the cellular metabolism, (d) difficulty in scaling up to levels required by our current understanding of cellular function and, also, potentially allowed by available genomic and cell-wide measurements. This difficulty stems from the inherent problems in accurately estimating the large number of parameters required for kinetic cell-wide models. Consequently, current cell-wide models are mainly stoichiometric in nature and capitalize on genomic sequence information to define cell-wide bioreaction networks whose rates are determined such as to optimize a cell objective (such as maximizing growth rate) subject to constraints derived from metabolite balances (the FBA approach).

Ensemble Modeling (EM) was recently introduced to address the many shortcomings of conventional theory-based models. The basic approach of EM is to construct an ensemble of dynamic models that span the entire kinetic parameter space allowable by thermodynamics. All such models attain the same steady state in terms of flux distribution and metabolite concentrations. However, these steady states are altered upon introduction of specific genetic perturbations. Ultimately, we expect to develop models that will encompass all reactions involved in current metabolic reconstructions of the bacterium *Escherichia coli,* a sum total of approximately 150-200 reactions that carry significant flux. (Other reconstructed networks carry zero or negligible fluxes under most conditions.) Besides being able to predict the outcomes of manipulation of any enzyme within the model, it will have the flexibility to be adapted to incorporate the production of novel compounds. This flexibility will allow experimentalist to quickly pinpoint targets for genetic manipulation regardless of the novel pathway they have introduced.

In this work, we use parameter continuation methods for analyzing the robustness of metabolic models. Parameter continuation allows us to quickly observe the metabolic effects of perturbations, such as enzyme activity changes, as function of perturbation magnitude without the need to run computationally expensive time domain integrations. This gives us a more complete view of a perturbation's effect on metabolism and allows us to examine a model's robustness. Parameter continuation will play a big role in the development of cell-wide models, because robustness is a key element of living organisms and should be a property of its models and numerical integration of the time-domain system becomes computationally infeasible as models increase in scope.