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MODELING ENZYMATIC REGULATION IN METABOLIC NETWORKS

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ABSTRACT

Metabolic network modeling is a fundamental aspect of systems biology for metabolic engineering applications. Constraint-based modeling has become the most popular framework for modeling metabolic networks at the genome scale, due to its simple formulation. However, the price to pay for this simplicity is the neglect of the enzymatic mechanisms and their kinetic behavior. In order to overcome these limitations, a new generation of kinetic models built from constraint-based models and approximative kinetic formats is emerging. We identify the lack of representation for enzymatic regulation mechanisms as a major limitation in this new approach and propose the utilization of Extended Petri nets as a better scaffold for the creation of kinetic reconstructions that account for this kind of regulation. We evaluate the impact of accounting for enzymatic regulation in the central carbon metabolism of *E. coli* for the simulation of the steady-state phenotype of mutant strains. The work shows evidence that enzymatic regulation influences the mutant phenotype and its representation can improve rational strain design methods.

INTRODUCTION

Systems biology aims to understand cellular behavior through the creation of mathematical and computational models of the cell. One of its applications is in metabolic engineering, where metabolic models are used in methods to guide rational strain design.

Constraint-based modeling is one approach to build metabolic models that accounts for the reaction's stoichiometry and reversibility (Price et al. 2003). This simplicity as allowed its application in the construction

of genome-scale metabolic models. However this approach imposes several limitations, it defines a space of solutions rather than a single solution and it does not account for kinetic behavior.

In order to overcome the current limitations in metabolic network modeling new approaches are emerging (Smallbone et al. 2010, Jamshidi and Palsson 2010). These involve the automatic generation of large-scale kinetic models by adding kinetic rate laws based on approximative kinetic formats to constraint-based models. Kinetic models express temporal profiles and determine unique steady-states for the metabolic phenotype. However, since constraint-based models do not account for enzymatic regulation, the generated kinetic models also lack this important aspect of metabolism.

Petri nets are a graphical and mathematical formalisms that have been applied in the modeling of several biological pathways, including signaling, gene regulatory and metabolic (Reddy et al. 1993). Extended Petri nets are an extension to the original formalisms that includes special types of arcs.

This work proposes the utilization of Extended Petri nets as a better scaffold for generation of large-scale kinetic models that account for enzymatic regulation. The central carbon metabolism of *E. coli* is used as a case-study.

MODELS AND METHODS

Central carbon metabolism of *E. coli*

The model of the central carbon metabolism of *E. coli* (Chassagnole et al. 2002) was used to generate pseudo-experimental data for parameter estimation and validation of the simulation results.

Overall methodology

The overall methodology is exemplified in Fig. 1.



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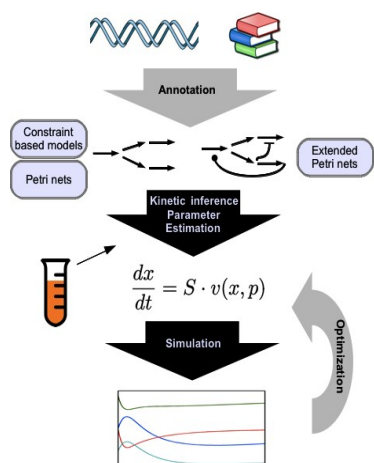


Figure 1: Overview of the Methodology

The topology of metabolic networks is built from genome annotation and literature data. This information can be modeled with constraint-based models or simple Petri nets. Extended Petri nets are more expressive as they can also represent regulatory interactions within the network topology. The next step is to use the previous models to generate kinetic models. Experimental data is used for parameter estimation. The resulting models can be used for simulation and optimization applications.

RESULTS

In order to evaluate the impact of enzymatic regulation in kinetic reconstructions of metabolism we have generated kinetic models of the central carbon metabolism based on their Petri net representation with and without enzymatic regulation. Generalized mass action kinetics (Horn and Jackson 1972) were used to define the rate laws, and the kinetic parameters were estimated using the steady-state flux distribution and metabolite concentrations from the original model.

Both models were used to simulate the steady-state phenotype of mutant strains upon gene knockout and changes in enzyme expression levels. Fig. 2 compares the results of both models against the experimental data generated with the original model for a 5-fold underexpression of *pepCxlase*. It is possible to observe a small shift in the flux from the glycolytic pathway to the pentose-phosphate pathway. This shift was correctly predicted by the model with regulation but not by the model without regulation.

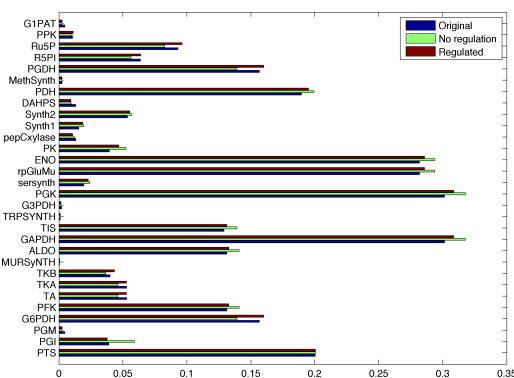


Figure 2: Underexpression of *pepCxlase*

CONCLUSIONS

The results show that enzymatic regulation influences the steady-state metabolic phenotype. Our framework, based on Extended Petri nets, is able to model these interactions and can be used to generate kinetic models that account for this kind of regulation. Such models can be used to uniquely determine the steady-state flux distributions of the metabolism under different conditions. Therefore they are able to guide the rational design of mutant strains that will account for new regulatory targets.

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