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Control analysis of the impact of allosteric regulation mechanism in a *Escherichia coli* kinetic model: Application to serine production

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ABSTRACT

Kinetic modeling is a key aspect of systems biology with biotechnological applications. However, a limitation of building kinetic models of metabolism (particularly from stoichiometric reconstructions of metabolic networks) is that they often ignore the allosteric regulators. This can cause discrepancies in the model predictions. In this paper, we derived an approximated lin-log ODE model of the *Escherichia coli* central carbon metabolism, with and without metabolite-enzyme regulators. Next, we analyzed the influence of incorporating this level of metabolite-enzyme interactions in the metabolic network by performing several *in silico* single-gene knockouts and enzyme under-/over-expression changes. Through comparing these model predictions with those generated with a reference mechanistic kinetic model for *E. coli*, it is shown that including of allosteric regulation affects the flux control patterns over serine production and reveals more details of the model behavior in a general sense. The present work demonstrates that the regulatory (allosteric) structure in metabolic networks plays an essential role to further improve kinetic model prediction capabilities. The incorporation of allosteric regulation interactions in building a kinetic model can lead to different hypotheses in order to suggest enzyme targets for strain design through metabolic engineering.

1. Introduction

The ultimate goal of Systems Biology is to explain and quantitatively predict the dynamic behavior of complex systems, by combining theory, simulation and laboratory experiments [1]. Metabolic Engineering is one of the fields where this perspective has proven useful through the optimization of relevant industrial strains under different perturbations for the optimization of the production of compounds of industrial interest (see for example Park and Lee, [2]). To achieve this goal, mathematical models of cellular metabolism have become an important tool for performing *in silico* experiments.

The mathematical models that are usually used in metabolic engineering can be grouped into stoichiometric and kinetic [3–5]. The stoichiometric metabolic models are based on network topology, thermodynamic and enzyme capacity constraints and can be based on reconstructions from genome-scale data [6]. The simulations with these models are performed under a steady-state assumption using constraint-based methods like Flux Balance Analysis (FBA) [7]. Another approach, called metabolic flux analysis (MFA), also assumes a steady-state condition and provides a flux distribution without the need of kinetic information [8]. However, the dynamic behavior of the system cannot be captured and in general the effects of regulatory mechanisms are not considered.

The traditional detailed kinetic models, on the other hand, allow one to numerically simulate the dynamic behavior of the system over time in response to changes in specific cellular components or environmental parameters. They also allow one to perform other analyses such as Metabolic Control Analysis (MCA) [9] and optimal re-design of biological systems [10]. For decades, non-linear ordinary differential equations (ODE's) have been the most predominant dynamic modeling techniques for metabolic systems [11]. They require *a priori* knowledge on the network structure and a large amount of experimental information, such as initial concentration of metabolites, several kinetic parameters and reaction rate laws.

Kinetic models have been used for a large variety of applications including the estimation of optimal drug concentrations [12] and rational strain design of metabolic systems [56]. Recently, kinetic models with a significantly large number of reactions have been developed for processes like the central metabolism in *Escherichia coli* [13], glycolysis in lactic

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Nomenclature

Metabolites

Gluc.Ext	Glucose extracellular
g1p	Glucose-1-phosphate
g6p	Glucose-6-phosphate
pep	Phosphoenolpyruvate
pyr	Pyruvate
6pg	6-Phosphogluconate
f6p	Fructose-6-phosphate
xyl5p	Xylulose-5-phosphate
sed7p	Sedoheptulose-7-phosphate
pgp	1,3-Diphosphoenolpyruvate
e4p	erythrose-4-phosphate
gap	Glyceraldehyde-3-phosphate
dhap	Dihydroxyacetonephosphate
3 pg	3-Phosphoglycerate;
2 pg	2-Phosphoglycerate
ribu5p	Ribulose-5-phosphate
rib5p	Ribose-5-phosphate
fdp	Fructose-1,6-bisphosphate
atp	Adenosintriphosphate
adp	Adenosindiphosphate
amp	Adenosinmonophosphate
nadp	Diphosphopyridindinucleotide-phosphate oxidized
nadph	Diphosphopyridindinucleotide-phosphate reduced
nad	Diphosphopyridindinucleotide oxidized
nadh	Diphosphopyridindinucleotide reduced
oxa	Oxalate
akg	Alpha-ketoglutarate
cit	Citrate
succi-CoA	Succinyl-CoA
asp	Aspartate
mal	Malate
fum	Fumarate
cit	Citrate
succ	Succinate
pi	Phosphate
oaa	Oxaloacetate
glycp	Glycerol 3-phosphate
f1p	Fructose-1-phosphate

Reactions

PTS	Phoshotransferase system
PGI	Glucose-6-phosphate isomerase
PFK	Phosphofrutokinase
ALDO	Aldolase
TIS	Triosephosphate isomerise
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
PGK	Phsphoglycerate kinase
PGluMu	Phosphoglycerate mutate
ENO	Enolase
PK	Pyruvate dehydrogenase
PDH	Pyruvate dehydrogenase
PEPcarbox	PEP carboxylase
PGM	Phosphoglucomuatse
G1PAT	Glucose-1-phosphate adenyltransferase
PPK	Ribose phosphate pyrophoskinase
G3PDH	Glycerol-3-phosphate dehydrogenase
SerSynth	Serine synthesis
MurSynth	Mureine synthesis
DAHPS	DHAP synthase
TrpSynth	Tryptophan synthesis
MetSynth	Methionine synthesis
G6PDH	Glucose-6-phosphate dehydrogenase
PGDH	6-Phosphogluconate dehydrogenase
Ru5p	Ribulose phosphate epimerase
R5PI	Ribose phosphate isomerise
TKa	Transketolase A
TKb	Transketolase B
TA	Transaldolase
Synth1	Synthesis 1
Synth2	Synthesis 2
PPS	Phosphoenolpyruvate synthetase
IDH	Isocitrate dehydrogenase
LDH	D-lactate dehydrogenase
PGCD	Phosphoglycerate dehydrogenase

bacteria [14,15] or the pentose phosphate pathway in *Saccharomyces cerevisiae* [16]. A major challenge of such mechanistic kinetic models, however, is that they possess many mechanistic rate equations and free kinetic parameters [17]. Usually, for a large number of enzymes, the *in vivo* kinetic parameters are unknown or are available in the literature and databases only as general values obtained by *in vitro* measurements by enzymologists because it is very complex to measure the exact values [18]. These parameters should be used with care by modelers, since enzymologists in general work under optimal conditions for the enzyme and do not perform the enzyme characterization under physiological conditions [19], restricting their *in silico* applicability [20]. Therefore, an alternative approach to address this issue has been the use of a variety of *in vivo* data that usually includes time-course measurements of metabolite concentrations in response to a stimulus experiment [16,21,22]. These set of experimental data are then used to fit the model output by minimizing an objective function using a variety of nonlinear optimization algorithms [23]. Although these high-throughput data are becoming more and more available, kinetic parameter estimation, reaction expression and allosteric regulators of enzymes identification remain very challenging tasks [24].

In order to overcome the gaps in stoichiometric and mechanistic kinetic models of metabolism various new "top-down" approaches to move large-scale modeling from stoichiometric models to the kinetic domain have started to emerge. For example, in [25] the authors proposed an approximate modeling approach composed of mass-action kinetics by integration of genomic, proteomic, metabolomic and fluxomic measurements. The authors also evaluate the impact of regulation by including the regulatory interactions. However, one disadvantage of this approach is the need of concentrations of a large number of reaction intermediates. Another approach was developed by Smallbone et al. [26,27] combining two modeling approaches (approximated lin-log kinetics and constraint-based modeling), in which the parameters (elasticities) are given by the negative stoichiometric coefficient for the respective metabolites and/or are derived from available kinetic models within the BioModels database [28]. The reference steady-state fluxes are estimated by the FBA approach. However, the parameters estimated with these methodologies are rough approximations and may result in false predictions. In Miskovic and Hatzimanikatis. [29,30] the authors propose an approach, known as ORACLE (Optimization and Risk Analysis of Complex Living Entities). ORACLE is a framework based on MCA, that consists of a set of successive computational procedures where biological data from several sources (*i.e.*, metabolomics, transcriptomics, fluxomics and thermodynamics) are integrated. It generates a population of large-scale kinetic models of cellular metabolism using a Monte Carlo approach that satisfy thermodynamic and physico-chemical