Journal of Theoretical Biology 365 (2015) 469–485

Contents lists available at ScienceDirect

Journal of Theoretical Biology

journal homepage: www.elsevier.com/locate/yjtbi

Dynamic optimization of metabolic networks coupled with gene expression

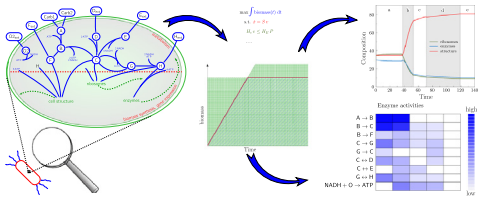
Steffen Waldherr [a,*], Diego A. Oyarzún [b], Alexander Bockmayr [c]

[a] Institute for Automation Engineering, Otto von Guericke University Magdeburg, Universitätsplatz 2, 39106 Magdeburg, Germany
[b] Department of Mathematics, Imperial College London, SW7 2AZ London, United Kingdom
[c] DFG Research Center Matheon, Freie Universität Berlin, Arnimallee 6, 14195 Berlin, Germany

HIGHLIGHTS

- A dynamic optimization framework integrating a metabolic network with the dynamics of biomass production and composition.
- Predicting the temporal regulation of gene expression from an optimization principle.
- No knowledge of regulatory interactions required

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 12 March 2014
Received in revised form
22 October 2014
Accepted 27 October 2014
Available online 6 November 2014

Keywords:
Flux optimization
Constraint-based methods
Metabolic–genetic networks
Bacterial growth

ABSTRACT

The regulation of metabolic activity by tuning enzyme expression levels is crucial to sustain cellular growth in changing environments. Metabolic networks are often studied at steady state using constraint-based models and optimization techniques. However, metabolic adaptations driven by changes in gene expression cannot be analyzed by steady state models, as these do not account for temporal changes in biomass composition.

Here we present a dynamic optimization framework that integrates the metabolic network with the dynamics of biomass production and composition. An approximation by a timescale separation leads to a coupled model of quasi-steady state constraints on the metabolic reactions, and differential equations for the substrate concentrations and biomass composition. We propose a dynamic optimization approach to determine reaction fluxes for this model, explicitly taking into account enzyme production costs and enzymatic capacity. In contrast to the established dynamic flux balance analysis, our approach allows predicting dynamic changes in both the metabolic fluxes and the biomass composition during metabolic adaptations. Discretization of the optimization problems leads to a linear program that can be efficiently solved.

We applied our algorithm in two case studies: a minimal nutrient uptake network, and an abstraction of core metabolic processes in bacteria. In the minimal model, we show that the optimized uptake rates reproduce the empirical Monod growth for bacterial cultures. For the network of core metabolic processes, the dynamic optimization algorithm predicted commonly observed metabolic adaptations, such as a diauxic switch with a preference ranking for different nutrients, re-utilization of waste products after depletion of the original substrate, and metabolic adaptation to an impending nutrient depletion. These examples illustrate how dynamic adaptations of enzyme expression can be predicted solely from an optimization principle.

* Corresponding author.
E-mail address: steffen.waldherr@ovgu.de (S. Waldherr).

http://dx.doi.org/10.1016/j.jtbi.2014.10.035
0022-5193/

1. Introduction

A key aspect of cellular dynamics is the ability to adapt metabolic activity to changing environments. This involves a dynamic re-organization of enzyme expression levels, in order to accommodate for variability in nutrient abundance and environmental shocks that have a deleterious impact on growth. These adaptations emerge from a complex array of regulatory interactions between metabolism and the genetic machinery. Since many of these interactions are unknown or incompletely understood, a fully mechanistic grasp of how they control metabolic adaptations is currently beyond our reach. Moreover, the analysis of large-scale mechanistic models is typically hampered by the high number of molecular species and parameters involved.

An alternative approach to predict metabolic adaptations is to assume an underlying optimality principle (Watson, 1986; Fell and Small, 1986; Varma and Palsson, 1994a). Numerous studies have considered metabolic adaptations in microbes by computing optimal metabolic fluxes in a stoichiometric model under a suitable objective function (van Riel et al., 2000; Covert and Palsson, 2002; Meadows et al., 2010; Steuer et al., 2012). Stoichiometric models are a structural description of a metabolic network and cannot provide information on the enzyme concentrations. Several approaches have attempted to overcome this by integrating gene regulation with stoichiometric models, either by modeling enzyme expression qualitatively with Boolean variables describing regulatory effects (Covert et al., 2001; Covert and Palsson, 2002), or by explicitly including enzyme capacity constraints in the optimization problems (Goelzer et al., 2011). An alternative approach are the cybernetic models (Ramkrishna and Song, 2012), where regulation is explicitly modeled and assumed to optimize a cellular objective. While the classical cybernetic approach explicitly includes reaction kinetics in the model, a hybrid approach has been suggested (Kim et al., 2008), where the rates are determined from flux balance analysis.

Models that integrate metabolism and gene expression can potentially yield better predictions than those focused on metabolism in isolation. This can be particularly helpful in metabolic adaptations caused by environmental fluctuations. To capture the dynamics of biomass and gene expression linked to metabolic activity, previous studies have mostly used ad-hoc combinations of various modeling frameworks. Examples are combinations of constraint-based steady state models with ordinary differential equations or Boolean regulatory logic (Varma and Palsson, 1994a; Covert and Palsson, 2002; Covert et al., 2008). This combination approach has been successful in proposing integrated models up to whole-cell dynamics (Karr et al., 2012).

In this paper, we propose a dynamic modeling framework for metabolic networks coupled with gene expression of enzymes and production of other macromolecules. We develop an optimization algorithm to predict optimal time courses for nutrient uptake, metabolic fluxes, and gene expression rates in such networks.

The classical approach to constraint-based optimization of metabolic fluxes, commonly called flux balance analysis (FBA), relies on an optimization problem with algebraic constraints stemming from a steady state restriction (Varma and Palsson, 1994b; Reed and Palsson, 2003; Orth et al., 2010). Mathematically, the FBA approach in the simplest form leads to a linear program of the form:

$$\max_{v}\{b^{\mathrm{T}}v \mid Sv = 0,\ v_{\min} \le v \le v_{\max}\}, \tag{1}$$

where v is the reaction flux vector, b a biomass weighting vector, S the stoichiometric matrix, and $v_{\min}$, $v_{\max}$ are lower and upper component-wise bounds on the fluxes, respectively. While the most common optimization objective is the maximization of biomass production, an experimental evaluation also highlighted additional biologically relevant objectives (Schuetz et al., 2007).

One point of critique to FBA is its coarse description of the biomass composition. While growth-dependent changes in the biomass composition have been taken into account in the past (Pramanik and Keasling, 1997), constraints related to the actual biomass composition by enzymes or other cellular macromolecules are usually not considered. At least on the level of individual metabolic pathways, there is good evidence that the enzyme production cost is an important factor in the regulation of these pathways (Wessely et al., 2011). Thus, it seems plausible that the inclusion of biomass composition and enzyme costs in metabolic optimization can potentially improve the quality of its predictions. As an extension to FBA in this direction, the resource balance analysis (RBA) approach has been proposed (Goelzer et al., 2011). This includes the conversion of metabolites into specific enzymes and other proteins in the network, and adds the enzymatic capacity as constraint on metabolic fluxes for the optimization. RBA yields a linear optimization problem and can intrinsically describe changes in both the growth rate and biomass composition due to environmental changes from an optimization principle alone. A conceptually equivalent approach has been proposed independently by Lerman et al. (2012) under the term ME (metabolism and macromolecular expression) model. Both approaches are however limited to situations of steady exponential growth.

For batch processes or in changing environments, the model needs to go beyond the stationary approach and account for dynamic changes in metabolic activity. FBA has been used to predict dynamic changes in biomass and nutrients using iterative approaches (Varma and Palsson, 1994a). However, the iterative optimization uses a steady state constraint and does not account for the model dynamics, and thus the predictions may not be optimal in changing environments. Dynamic effects are physiologically important, as is evidenced by the experimental observation that even in steady state, cells would show flux distributions which are slightly suboptimal, but which allow for easier transitions to other environmental conditions (Schuetz et al., 2012). Also, the numerical accuracy of the iterative approach can at best be evaluated heuristically or by numerical experimentation, unless specialized numerical algorithms are applied (Höffner et al., 2013).

By formulating an appropriate dynamic optimization problem, it is possible to compute optimal fluxes over the whole time range of interest. This approach has been proposed in dynamic flux balance analysis (dFBA) (Mahadevan et al., 2002). In dFBA, one can distinguish between a “static optimization approach (SOA)”, similar to the previously used iterative FBA (Varma and Palsson, 1994a), and a “dynamic optimization approach (DOA)”. The static approach is useful to get feasible nutrient and biomass dynamics under metabolic constraints, but it cannot resolve the optimization problem over the complete timescale of interest. The dynamic approach DOA directly considers an objective function which depends on the dynamics over the complete timescale, potentially under dynamic metabolic constraints, and thus provides a consistent solution to the dynamic optimization problem. However, in the same way as classical FBA, dynamic FBA uses only a coarse description of biomass composition. Biomass is captured only as one component, and different allocations of biomass to different metabolic tasks, such as considered in RBA, cannot be represented.

In the study described here, we developed a mathematical framework for dynamic models of coupled metabolism and gene expression. We denote such models with the term *metabolic–genetic networks*. From a rigorous timescale separation, we approximate this model by a quasi-steady state, constraint-based part for the intracellular metabolism, and a dynamic part for the evolution of biomass and substrate concentrations. For this model class, we