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Optimization of metabolic pathways under stability considerations

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Abstract

Metabolic networks are often approached through steady-state optimization formulations that are solved to interpret and predict the behavior of the network subject to changes in external fluxes or internal enzyme activity. The major question addressed in this paper is how to ensure that solutions to these steady-state optimization models for metabolic networks are implementable from a stability point of view. The stability of a dynamic system is closely related to matrix stability. Hence, it can be determined through the computation of the largest eigenvalue of a coefficient matrix. While it is straightforward to analyze the stability of a given system, the challenge is to redesign a metabolic network in a way that guarantees that the system will be stable around the new steady-state. For this purpose, we propose to model metabolic networks through classical optimization formulations, such as the classical *S*-system representation, with an additional constraint to enforce stability within a prespecified neighborhood of the solution point. The proposed formulation is a bilevel optimization problem that is very difficult to solve. We develop a suitable global optimization algorithm to solve this problem after transforming it to a semi-infinite optimization problem. Computational results are presented, including application to tryptophan biosynthesis in bacteria and anaerobic fermentation in *Saccharomyces cerevisiae*.

Keywords: Metabolic engineering; Dynamic stability; Semi-infinite programming

1. Introduction

Systematic tools from the mathematical theory of optimization have been used extensively over the last two decades for the analysis and manipulation of metabolic networks. For example, linear programming techniques have been used to calculate fluxes, determine whether hypothesized objective functions can be used to interpret the behavior of metabolic networks, and find conditions under which the production of certain metabolites is minimized or maximized (Majewski & Domach, 1990; Papoutsakis, 1984; Savinell & Palsson, 1992a, 1992b). Integer programming techniques have been introduced in order to model the effect of gene additions and deletions (Burgard & Maranas, 2001; Hatzimanikatis, Floudas, & Bailey, 1996a, 1996b). Finally, nonlinear and mixed-integer nonlinear optimization models have been developed to optimize nonlinear objectives, such as selectivity, as well as capture the effect of mechanistic kinetic relationships, nonlinear dynamics, and uncertainty (Dean & Dervakos, 1996; Hatzimanikatis, 1997; Petkov & Maranas, 1997).

One standard approach to the directed improvement of cellular properties begins with the assumption that metabolic transients and dynamics are much faster than bigger changes, such as volume expansion by growth and, therefore, the underlying metabolic network may be assumed to be at steady-state. One would then like to find a solution to the usually underdetermined network mass balances in a way that optimizes a certain objective. The solution can then be used to set external fluxes in a way that directs the network to a favorable operating condition. The salient assumption behind such an approach is that the dynamic system can be steered to the new operating condition and that this operating condition is a stable steady-state of the system under possible external disturbances.

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In Section 2, we present a small example that demonstrates that the standard approach to optimize metabolic networks may lead to an unstable operating condition. We also demonstrate that there exist other solutions that are stable and improve the underlying objective. In Sections 3–5, we present a systematic way for searching for such solutions. First, in Section 3, a natural bilevel programming formulation is obtained through the addition of an optimization problem as one of the constraints of standard optimization formulations of biochemical networks. In Section 4, the model is transformed into a semi-infinite optimization problem. A suitable global optimization solution algorithm is developed in Section 5. Finally, in Sections 6–9, the proposed approach is illustrated through several examples, including tryptophan biosynthesis in bacteria and anaerobic fermentation in *Saccharomyces cerevisiae*.

2. Motivating example

Consider the network of Fig. 1 with two dependent variables, two feedback activation loops, one feedforward activation loop, and the following dynamics:

$$\frac{dX_1}{dt} = X_3X_1^2X_2^2 - X_4X_1 \tag{1}$$

$$\frac{dX_2}{dt} = X_4X_1 - X_5X_1^2X_2, \tag{2}$$

where X_1 and X_2 denote metabolite concentrations, while X_3, X_4, and X_5 denote enzyme activities.

At steady-state, (1)–(2) become:

$$X_3X_1^2X_2^2 - X_4X_1 = 0 \tag{3}$$

$$X_4X_1 - X_5X_1^2X_2 = 0. \tag{4}$$

Let us assume that the network originally operates at $X_i = 1$, for $i = 1, \ldots, 5$. Let us also assume that physiological considerations require metabolite concentrations and enzyme activities to stay within the region $S = \{X \in \mathbb{R}^5_+ : 0.9 \le X_1 \le 1.1, 0.9 \le X_2 \le 1.2, 0.1 \le X_3 \le 10, 0.1 \le X_4 \le 20, 0.1 \le X_5 \le 10\}$.

The system (3)–(4) has three degrees of freedom. One can then set the values of the three enzyme activities in a way that the resulting solution in terms of metabolite concentrations optimizes some desired objective. Let us consider the problem of maximizing the flux V_2 from X_2:

$$\begin{aligned} \max\ & V_2 = X_5X_1^2X_2 \\ \text{s.t.}\ & X_3X_1^2X_2^2 - X_4X_1 = 0 \\ & X_4X_1 - X_5X_1^2X_2 = 0 \\ & X \in S. \end{aligned}$$

Solving this problem as is, which has been the traditional approach of optimizing a biochemical network, is easy and becomes a lot easier when we realize that this problem can be transformed into a linear program by taking the logarithm of the variables. The optimal solution gives $V_2 = 14.52$, which is attained at $X = (1.1, 1.2, 8.33, 13.2, 10)$ and corresponds to an over 14-fold increase of V_2 compared to the nominal system. This solution suggests that the flux from X_2 can be maximized if enzyme activities are set to $X_3 = 8.33$, $X_4 = 13.2$, and $X_5 = 10$.

Let us assume that the above optimal solution is implemented and that the metabolite concentrations reach the desired levels of $X_1 = 1.1$ and $X_2 = 1.2$. Consider now a small external perturbation to the system. In particular, assume that, at time $t = 1$, X_3 is slightly increased to 8.5 and, at time $t = 1.02$, it is decreased back to its desired value of 8.33. This is a very small (less than 2%) perturbation that lasts for a very narrow time window only. Fig. 2 shows the solution of the dynamic system (1)–(2) after these changes. Clearly, the system becomes unstable and violates the physiological condition $X \in S$, thus suggesting that the results of the classical optimization formulation should not be implemented as they would make the system vulnerable to external perturbations that could become lethal.

For the same pathway example, consider now the following redesign: $X = (1.03, 0.97, 8, 7.75, 7.76)$. Under the same disturbance as before, the dynamic behavior of this design is shown in Fig. 3. This design improves the flux V_2 only eight-fold compared to the nominal steady-state. How-

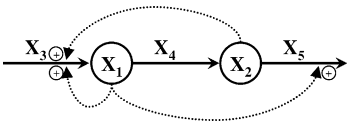

Fig. 1. Pathway for motivating example.

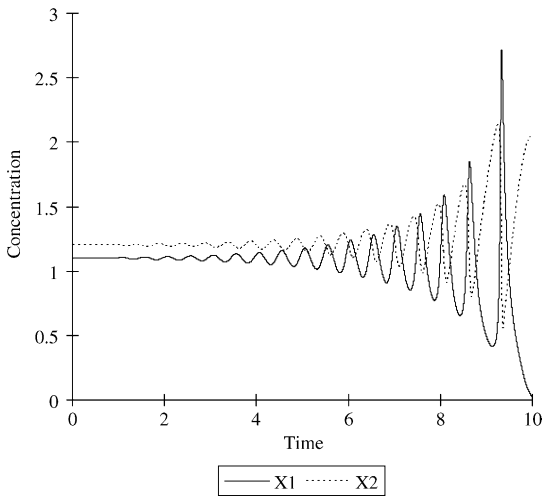

Fig. 2. System response to 2% external perturbation.