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Ensemble Modeling for Robustness Analysis in engineering non-native metabolic pathways

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ABSTRACT

Metabolic pathways in cells must be sufficiently robust to tolerate fluctuations in expression levels and changes in environmental conditions. Perturbations in expression levels may lead to system failure due to the disappearance of a stable steady state. Increasing evidence has suggested that biological networks have evolved such that they are intrinsically robust in their network structure. In this article, we presented Ensemble Modeling for Robustness Analysis (EMRA), which combines a continuation method with the Ensemble Modeling approach, for investigating the robustness issue of non-native pathways. EMRA investigates a large ensemble of reference models with different parameters, and determines the effects of parameter drifting until a bifurcation point, beyond which a stable steady state disappears and system failure occurs. A pathway is considered to have high bifurcational robustness if the probability of system failure is low in the ensemble. To demonstrate the utility of EMRA, we investigate the bifurcational robustness of two synthetic central metabolic pathways that achieve carbon conservation: non-oxidative glycolysis and reverse glyoxylate cycle. With EMRA, we determined the probability of system failure of each design and demonstrated that alternative designs of these pathways indeed display varying degrees of bifurcational robustness. Furthermore, we demonstrated that target selection for flux improvement should consider the trade-offs between robustness and performance.

1. Introduction

Metabolic engineering has advanced from minor alterations of existing pathways to significant re-routing of the metabolic path for better utilization of substrates (Bogorad et al., 2013; Huo et al., 2011; Zhang et al., 1995) or formation of non-native products (Atsumi et al., 2008; Choi and Lee, 2013; Dellomonaco et al., 2011; Ingram et al., 1987; Steen et al., 2010; Zhang et al., 2008, 2010). With a notable exception (Fung et al., 2005), all metabolic engineering efforts aim to achieve a steady state or quasi-steady state. Non-steady states in metabolic engineering typically result in accumulation or disappearance of intermediate metabolites. Since the engineered pathways are not tuned through evolution, non-steady states may occur because the expression levels of the pathway genes may drift outside the working range as the physiological conditions change. Drifting of expression levels or other kinetic parameters may lead to gradual deterioration of performance or sudden system failure characterized by the disappearance of a stable steady state. While the deterioration of performance is undesirable, the occurrence of system failure could be catastrophic for the cell. Thus, a robust pathway design should first focus on avoiding system failure before attempting to improve performance.

Through evolution, native metabolic pathways apparently have solved the robustness problem by selecting a robust network structure such that the feasible range of each parameter is sufficiently large (Alon et al., 1999; Barkai and Leibler, 1997; Stelling et al., 2004a, 2004b). In addition, various regulatory mechanisms are in place to dynamically control the kinetic parameters under various physiological conditions. In contrast, non-native or metabolically engineered native pathways are potentially prone to system failure, when a kinetic parameter moves away from the initially designed level, causing accumulation or depletion of metabolites and the disappearance of a stable steady state. Since a stable steady state disappears after bifurcation occurs, the bifurcational robustness should therefore be an important criterion for designing non-native pathways. Even with an artificial dynamic controller (Dahl et al., 2013; Farmer and Liao, 2000; Zhang et al., 2012) designed for the non-native pathway, it is desirable to choose network configurations or parameter ranges that are inherently robust to bifurcation.

The robustness problem calls for a modeling approach that integrates kinetic parameters with systems performance. Kinetic

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parameters are perturbed in such models to examine the consequences of drifting. Unfortunately, key kinetic parameters (*e.g.*, V_{max}'s) are system-dependent and usually unknown. Previous efforts have addressed the uncertainty of metabolic parameters through the random sampling of parameters (Steuer et al., 2006; Tran et al., 2008; Wang et al., 2004) to form an ensemble of models. Various approaches are then used to extract useful information from the ensemble upon large parameter changes (Rizk and Liao, 2009; Tan and Liao, 2012), or infinitesimal perturbations that define control coefficients (Alves and Savageau, 2000; Schwacke and Voit, 2004; Wang et al., 2004). Since non-native pathway design normally starts with little knowledge of kinetic parameters, it is sensible to investigate bifurcational robustness for an ensemble of models and to quantify it using the probability of system failure.

Here we combine a continuation method (Allgower and Georg, 2003) with the previously developed Ensemble Modeling (EM) technique (Tan and Liao, 2012; Tran et al., 2008) to evaluate the bifurcational robustness of non-native metabolic pathways. This hybrid approach, termed Ensemble Modeling for Robustness Analysis (EMRA), allows the investigation of large parameter changes in an ensemble of models. For each model, the continuation method enables rapid determination of the steady-state solution as single or multiple parameters are altered up or down from the baseline. The continuation of steady-state solution proceeds along either direction until a bifurcation point, beyond which a stable steady state loses its stability. In metabolic systems, the disappearance of a stable steady state may or may not be accompanied by the emergence of sustained oscillations (Chandra et al., 2011; Danø et al., 1999). Instead, accumulation and depletion of intermediate metabolites are far more common, which lead to system failure. Even if metabolic oscillations are functional or beneficial in certain conditions, an oscillatory metabolic system is by no means ideal for maintaining a consistent supply of pathway products. Thus, non-native pathway design should consider bifurcational robustness, which can be quantified by the probability of system failure in an ensemble of models.

In this article, we demonstrate the utility of EMRA by comparing possible designs of two non-native pathways: non-oxidative glycolysis (NOG) (Bogorad et al., 2013) and reverse glyoxylate cycle (rGC) (Mainguet et al., 2013). In each design, we determined probabilities of system failure and identified targets that might improve performance. With both results, EMRA allows the selection of targets for flux improvement by considering both performance and robustness.

2. Materials and methods

2.1. Dynamic, kinetics-based model

A generic expression of kinetic parameter-based models for metabolic pathways is

$$\frac{d\mathbf{x}}{dt} = \mathbf{F}(\mathbf{x}, \mathbf{p}) = \mathbf{S} \cdot \mathbf{v}(\mathbf{x}, \mathbf{p}) \tag{1}$$

Here, the time derivative of metabolite concentrations in vector form ($\mathbf{x}$) is represented as the product of $\mathbf{S}$, the stoichiometric matrix, and $\mathbf{v}$, the vector of reaction fluxes. Since each reaction flux is also a function of kinetic parameters ($\mathbf{p}$), such models are useful for studying the effect of parameter drifting on system performance. One way to accomplish this is to alter the parameter of interest and then solve the ordinary differential equation (ODE)-based model to a new steady state (Fig. 1a). This time-domain approach, although straightforward, is computationally expensive if one needs to analyze varying degrees of perturbation for a large number of models. Most importantly, the time-domain approach is inadequate for detecting the loss of a stable steady state.

2.2. Continuation method

Here we adopt a computationally efficient and scalable continuation method (Allgower and Georg, 2003) to investigate the effect of parameter drifting. This method aims to find a connected path of steady-state solutions ($\mathbf{x}_{SS}$) to the following equation:

$$\frac{d\mathbf{x}}{dt} = \mathbf{F}(\mathbf{x}_{SS}, \mathbf{p}) = 0. \tag{2}$$

Since $\mathbf{F}(\mathbf{x}_{SS},\mathbf{p})$ is equal to zero, it follows that the total derivative of $\mathbf{F}(\mathbf{x}_{SS},\mathbf{p})$ with respect to $\mathbf{p}$ is also zero

$$\frac{d\mathbf{F}(\mathbf{x}_{SS}, \mathbf{p})}{d\mathbf{p}} = \frac{\partial \mathbf{F}}{\partial \mathbf{x}_{SS}} \frac{d\mathbf{x}_{SS}}{d\mathbf{p}} + \frac{\partial \mathbf{F}}{\partial \mathbf{p}} = \mathbf{0}. \tag{3}$$

Further rearrangement of Eq. (3) yields Eq. (4)

$$\frac{d\mathbf{x}_{SS}}{d\mathbf{p}} = -\left(\frac{\partial \mathbf{F}}{\partial \mathbf{x}_{SS}}\right)^{-1} \frac{\partial \mathbf{F}}{\partial \mathbf{p}}, \tag{4}$$

which specifies the derivatives of steady-state concentrations with respect to kinetic parameters. Starting from a stable steady state, we can solve Eq. (4) along the direction where a kinetic parameter (usually the activity of a particular enzyme) is drifted up or down from the baseline (Fig. 1b). The corresponding solution, which traces a trajectory in the $\mathbf{x}_{SS}$–$\mathbf{p}$ space, will then characterize how the steady state responds to the drifting of single or multiple parameters. It should be noted that solving Eq. (4) is technically equivalent to solving the steady-state first-order sensitivity equations. Therefore, the sensitivity profile of metabolite concentrations with respect to parameters is being updated regularly as the algorithm (*i.e.*, the differential equation solver) proceeds along the parametric domain.

Given that the calculation of the inverse of the Jacobian matrix ($\partial\mathbf{F}/\partial\mathbf{x}_{SS}$) is required for solving Eq. (4), it is crucial to detect the point where the Jacobian matrix becomes singular. Interestingly, this point is also the bifurcation point: beyond this point the system no longer reaches a stable steady state. Thus, this point defines the parameter space where a system is functional with a stable steady state. In practice, the Jacobian almost always becomes badly conditioned when the system is approaching a bifurcation point. We consider such an edge case a bifurcation point. Additionally, due to the nature of numerical integration, it is possible to "jump" over the region of singularity. To account for this, we routinely check if any of the eigenvalues of the Jacobian matrix has crossed the zero line to detect if the system has passed through a bifurcation point.

2.3. System failure

Since a stable steady state is required for proper functioning of the metabolic system for metabolite production, but nonetheless disappears beyond the bifurcation point, any parameter drifting that crosses the bifurcation point can be considered as entering the region of system failure (Fig. 1b). The current algorithm does not specifically flag the oscillatory behaviors if they are damped in nature, but such features can be added if necessary. Sustained oscillation or oscillation with ever-increasing amplitude could only occur beyond the bifurcation point (*i.e.*, after the real part of an eigenvalue becomes zero or positive) and therefore have already been excluded by the algorithm. Such systems may be of interest for special purposes, but are generally not the goal of metabolic engineering for metabolite production. Overall, the continuation method allows both the investigation of parametric sensitivity and the detection of system failure due to disappearance of a stable steady state.

2.4. Ensemble Modeling

The construction of dynamic metabolic models in the form of Eq. (1) is based on both network stoichiometry, which is usually