SEMI-QUANTITATIVE STABILITY ANALYSIS CONSTRAINS SATURATION LEVELS IN METABOLIC NETWORKS

H. Koeppl[1], M. Hafner[1] and R. Steuer[2,3]

[1]School of Computer and Communication Sciences, EPFL, Switzerland
[2]Institute for Theoretical Biology, Humboldt University Berlin, Germany
[3]Manchester Interdisciplinary Biocentre, The University of Manchester, UK

ABSTRACT

Recently structural kinetic modeling has been proposed as an intermediary approach between a full kinetic description of metabolic networks and a static constrained-based analysis of them. It extends the null-space analysis by a local stability analysis yielding a parametrization of the Jacobian in terms of saturation levels of the involved reactions with respect to their substrate metabolite concentration. These levels are normalized and stay within well-defined bounds for every reaction. We utilize results from robust control theory to determine subintervals of saturation levels that render the steady state asymptotically stable. In particular we apply Kharitonov's theorem and parametric Lyapunov functions in conjunction with interval computation. A glycolytic pathway model comprising 12 reactions is used to illustrate the methods.

1. INTRODUCTION

Although nearly complete knowledge of the metabolic reactions that take place inside several model organisms is now available, kinetic information about most of these reactions is unavailable. The challenge is to find out how metabolic systems function as integrated wholes, even though our knowledge of them is incomplete.

Mathematical models of metabolism will prove crucial in this regard. In the description of cellular metabolism, models have a long-standing tradition. Among the earliest models are detailed kinetic representations of small metabolic networks, such as models of glycolytic oscillations in yeast. Other prominent examples include models of the red blood cell and of the core-metabolism of *E. coli* (see [1] and the references therein). A detailed kinetic model is a set of differential equations whose right-hand-side reflects a rate law, a functional relation between the metabolic flux within a reaction and the concentrations of metabolites. In principle, such a model can predict the time course of all system variables, such as metabolite concentration changes. Rate laws, however, require kinetic information, and such information has always been scarce. To this end such information depends on many factors such as tissue type or experimental and physiological conditions. Rate law characteristics are obtained *in vitro* and it often remains questionable, whether these characteristics can be applied to *in vivo* situations.

Due to the lack of kinetic information researcher's attention turned to more coarse-grained, structural models

such as flux balance analysis (FBA) [1]. FBA requires only information about reaction stoichiometries and about metabolic demands on a network. Using stoichiometric information FBA identifies steady state fluxes that do not violate the requirement of mass conservation. The downside of FBA is that it can not provide any information about the dynamical properties of the metabolic system. This includes the sometimes overlooked fact, that it does not tell us whether every feasible flux corresponds to a stable steady state. That is, whether the network deliberately set to this flux distribution will remain at this distribution or whether it will swing off to another flux distribution at the tiniest fluctuation.

A recent attempt to bridge the gap between explicit kinetic models and coarse-grained structural modeling approaches such as FBA is the method of structural kinetic modeling (SKM) [2]. This approach starts with the observation that many questions about metabolic systems can be answered without an explicit kinetic model. For example to determine under which conditions a steady state loses its stability, only a local linear model of the metabolic system is required.

We propose an extension of SKM based on robust stability theory that allows to determine stability intervals for saturation levels of a SKM model. This reasoning about entire sets of models heralds a new semi-quantitative approach [3] to the analysis and design of biochemical models.

2. STRUCTURAL KINETIC MODELING

SKM highlights the fact that even in the absence of detailed kinetic information questions like the stability and its margin of the steady state operating point can be addressed. Questions, classical steady state level analyses, such as metabolic flux balance analysis can not address. As outlined in the following, it does so by introducing a direct parametrization of the Jacobian matrix that governs local stability of the network. The classical formulation for metabolic networks read

$$\frac{d\mathbf{S}}{dt} \equiv \dot{\mathbf{S}} = \mathbf{N}\mathbf{v}(\mathbf{S}, \mathbf{k}) \qquad (1)$$

with $\mathbf{S}$ the vector of concentration of all involved species, $\mathbf{N}$ the $N \times L$ stoichiometric matrix, $\mathbf{v}(\mathbf{S}, \mathbf{k})$ the vector of fluxes and $\mathbf{k}$ a vector comprising the parameters of all the rate laws. If we assume that the network has at least one

non-zero steady state at concentration $\mathbf{S}^0$ (does not need to be stable) we can equivalently write

$$\frac{\dot{S}_i}{S_i^0} = \sum_{j=1}^{R} N_{ij} \frac{v_j(\mathbf{S}^0)}{S_i^0} \frac{v_j(\mathbf{S})}{v_j(\mathbf{S}^0)}. \tag{2}$$

Introducing concentrations that are normalized by the steady state concentration $x_i = \frac{S_i}{S_i^0}$ one obtains

$$\dot{\mathbf{x}} = \mathbf{\Lambda}\boldsymbol{\mu}(\mathbf{x}), \tag{3}$$

with the constant matrix $\Lambda_{ij} \equiv N_{ij}\frac{v_j(\mathbf{S}^0)}{S_i^0}$ and the vector of normalized fluxes $\mu_j(\mathbf{x}) \equiv \frac{v_j(\mathbf{S})}{v_j(\mathbf{S}^0)}$. A linearization of the system at the steady state $\mathbf{x} = \mathbf{1}$ yields with $\mathbf{\Lambda}\boldsymbol{\mu}(\mathbf{1}) = \mathbf{0}$

$$\dot{z}_i = \sum_{j=1}^{R} \sum_{k=1}^{N} \Lambda_{ij} \left.\frac{\partial \mu_j(\mathbf{z})}{\partial z_k}\right|_{\mathbf{z}=\mathbf{1}} (z_k - 1). \tag{4}$$

Introducing the matrix $\theta_{z_k}^{\mu_j} \equiv \left.\frac{\partial \mu_j(\mathbf{z})}{\partial z_k}\right|_{\mathbf{z}=\mathbf{1}}$ we obtain the local linear model

$$\dot{\mathbf{z}} = \mathbf{\Lambda}\boldsymbol{\theta}_{\mathbf{z}}^{\mu}(\mathbf{z} - \mathbf{1}). \tag{5}$$

The stability of the nonlinear system (3) at $\mathbf{x} = \mathbf{1}$ is thus determined by the eigenvalues of the matrix $\mathbf{\Lambda}\boldsymbol{\theta}_{\mathbf{z}}^{\mu}$. The matrix $\mathbf{\Lambda}$ contains the topological as well as the steady state information, while every entry of the matrix $\boldsymbol{\theta}_{\mathbf{z}}^{\mu}$ can be interpreted as the relative saturation level of one particular reaction with respect to one particular substrate concentration.

3. ROBUST STABILITY

We can now apply the ideas of robustness analysis for linear systems to the Jacobian matrix $\mathbf{J} \equiv \mathbf{\Lambda}\boldsymbol{\theta}_{\mathbf{z}}^{\mu}$ of our linearized metabolic network. Allowing uncertainty in the kinetic rate law, corresponds here to an uncertainty about the saturation matrix $\boldsymbol{\theta}_{\mathbf{x}}^{\mu}$. Thus we define the interval Jacobian as

$$\mathbf{J}(\mathcal{B}_{\boldsymbol{\theta}}) = \left\{\mathbf{J} \,|\, \mathbf{J} = \mathbf{\Lambda}\boldsymbol{\theta}_{\mathbf{x}}^{\mu}, \, \boldsymbol{\theta}_{\mathbf{x}}^{\mu} \in \mathcal{B}_{\boldsymbol{\theta}} \subset \mathbb{R}^{L \times N}\right\}, \tag{6}$$

with the hyperbox

$$\mathcal{B}_{\boldsymbol{\theta}} \equiv \left\{\boldsymbol{\theta} \,|\, \theta_{x_j}^{\mu_i} \in [\underline{\theta}_{x_j}^{\mu_i}, \bar{\theta}_{x_j}^{\mu_i}], \, \underline{\theta}_{x_j}^{\mu_i} \leq \bar{\theta}_{x_j}^{\mu_i}, \, \forall(i,j)\right\}.$$

In the following, methods from robust control theory to deal with such an *interval matrix* (6) are introduced and extended.

3.1. Kharitonov polynomials

Given a Jacobian of a linearized dynamics, stability can be determined by checking the Hurwitz property, i.e., whether all eigenvalues have negative real part. But how to test stability of a whole family of Jacobians specified by an interval matrix? One important theorem here is the following due to Kharitonov [4].

Theorem 1. *Every polynomial*

$$p(\lambda, \mathbf{c}) = c_0 + c_1\lambda + \cdots + c_{n-1}\lambda^{n-1} + c_n\lambda^n \tag{7}$$

of degree n which is an instance of the polynomial set $p(\lambda, \mathcal{B}_{\mathbf{c}}) = \{p(\lambda, \mathbf{c}) \,|\, \mathbf{c} \in \mathcal{B}_{\mathbf{c}}\}$ and $c_n > 0$ is a Hurwitz polynomial, if and only if the associated following four Kharitonov polynomials

$$p^{+-}(\lambda, \mathbf{c}) = \bar{c}_0 + \underline{c}_1\lambda + \underline{c}_2\lambda^2 + \bar{c}_3\lambda^3 + \bar{c}_4\lambda^4 + \underline{c}_5\lambda^5 + \cdots$$
$$p^{++}(\lambda, \mathbf{c}) = \bar{c}_0 + \bar{c}_1\lambda + \underline{c}_2\lambda^2 + \underline{c}_3\lambda^3 + \bar{c}_4\lambda^4 + \bar{c}_5\lambda^5 + \cdots$$
$$p^{-+}(\lambda, \mathbf{c}) = \underline{c}_0 + \bar{c}_1\lambda + \bar{c}_2\lambda^2 + \underline{c}_3\lambda^3 + \underline{c}_4\lambda^4 + \bar{c}_5\lambda^5 + \cdots$$
$$p^{--}(\lambda, \mathbf{c}) = \underline{c}_0 + \underline{c}_1\lambda + \bar{c}_2\lambda^2 + \bar{c}_3\lambda^3 + \underline{c}_4\lambda^4 + \underline{c}_5\lambda^5 + \cdots$$
$$\tag{8}$$

are Hurwitz polynomials.

The result looks for very promising because it gives a necessary and sufficient condition for stability. However, the necessity will be lost if the coefficients $\mathbf{c}$ are not independent as it is the case for the characteristic polynomial $p(\lambda) = \det(\mathbf{J} - \lambda\mathbf{I})$. Thus, for our case the theorem just provides a sufficient condition, i.e., with this method a family of stable Jacobians can be erroneously classified as unstable (but not vice-versa). One way to compute the characteristic polynomial and the coefficient interval $\mathcal{B}_{\mathbf{c}}$ induced by the interval Jacobian is to use interval arithmetic [5]. Besides the above sufficiency, the use of interval arithmetic introduces a second source of conservatism.

3.2. Quadratic Stability

Quadratic stability of a linear interval system is defined that for each member of this interval family one can use the same quadratic Lyapunov function to determine stability. With that, quadratic stability is stronger than testing the Hurwitz stability of each of the members. Thus for a interval system that is quadratically stable all members are stable, but a system that is not quadratically stable can still be stable for all members. Quadratic stability thus gives us just another way to obtain conservative stability bounds. However, quadratic stability can be determined without conservatism with a finite number of tests. An $L \times N$ interval matrix can be thought of describing a hyperbox in $\mathbb{R}^{LN}$. The vertices of this box are associated with all the $K = 2^{LN}$ possible combinations of interval boundaries.

Theorem 2. *A linear interval system is quadratically stable if and only if all its vertex systems are stable.*

It remains to find a common Lyapunov function for all vertex systems. The proof of the theorem is based on the observation that every point in the hyperbox is an element of the convex hull of vertex points

$$\mathbf{J}(\boldsymbol{\alpha}) = \sum_{k=1}^{K} \alpha_k \mathbf{J}_k \quad \text{with} \quad \sum_{k=1}^{K} \alpha_k = 1 \quad \text{and} \quad \alpha_k \geq 0, \tag{9}$$

where $\mathbf{J}_k$ denote the vertex matrices. Quadratic stability corresponds to finding one common Lyapunov function $\mathbf{x}^T\mathbf{P}\mathbf{x}$ for all vertex matrices $\mathbf{J}_k$. Thus we have to solve the simultaneous set of Lyapunov equations

$$\mathbf{J}_i^T\mathbf{P} + \mathbf{P}\mathbf{J}_i \prec \mathbf{0} \tag{10}$$

for $i = 1, \ldots, K$ and $\mathbf{P} \succ 0$, the common positive-definite Lyapunov matrix.

3.3. Affine Quadratic Stability

In contrast to classical quadratic stability (Section 3.2), affine quadratic stability searches for quadratic parameter-dependent Lyapunov function, where the dependency is assumed to be affine. Writing it in terms of polytopes for the family of Jacobian

$$\mathbf{J}(\boldsymbol{\alpha}) = \sum_{k=1}^{K} \alpha_k \mathbf{J}_k \quad \text{with} \quad \sum_{k=1}^{K} \alpha_k = 1 \quad \text{and} \quad \alpha_k \geq 0,$$

(11)

with $\mathbf{J}_k$ the vertex matrices, we seek for a Lyapunov matrix of the form

$$\mathbf{P}(\boldsymbol{\alpha}) = \sum_{k=1}^{K} \alpha_k \mathbf{P}_k,$$

(12)

where the matrices $\mathbf{P}_k$ have to be found. Thus we obtain the Lyapunov equation,

$$\mathbf{J}(\boldsymbol{\alpha})^T \mathbf{P}(\boldsymbol{\alpha}) + \mathbf{P}(\boldsymbol{\alpha})\mathbf{J}(\boldsymbol{\alpha}) \prec \mathbf{0}$$

(13)

that has to hold for any $\boldsymbol{\alpha}$ in the convex combination. In general affine quadratic stability leads to bilinear matrix inequalities that are difficult to solve. However, forcing another constraint on the Lyapunov function, namely multi-convexity [6] one arrives at vertex conditions similar to the one in Section 3.2.

4. GLYCOLYTIC PATHWAYS

To illustrate our approach we present a minimal model of glycolysis in Section 4.1 and medium sized model in 4.2.

4.1. Minimal Model

A minimal model of the glycolytic pathway is depicted in Fig. 1. where A, B, X_0, S and X_1 corresponds to the
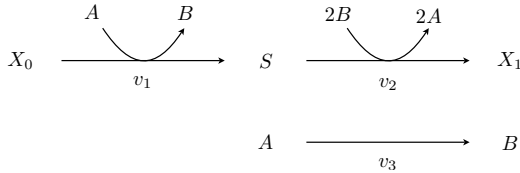
Figure 1. Minimal model of the glycolytic pathway

concentrations of ATP, ADP, Glucose, $_{1,3}P_2G$ and Pyruvate, respectively. The corresponding differential equations read

$$\frac{\mathrm{d}}{\mathrm{d}t} \begin{pmatrix} S \\ A \\ B \end{pmatrix} = \begin{pmatrix} +1 & -1 & 0 \\ -1 & +2 & -1 \\ +1 & -2 & +1 \end{pmatrix} \begin{pmatrix} v_1(A) \\ v_2(S, B) \\ v_3(A) \end{pmatrix}, \quad (14)$$

where we assume the following kinetic rate laws

$$v_1(A) = \frac{k_1 A}{1 + \left(\frac{A}{K_i}\right)^n}$$

(15)

$$v_2(S, B) = k_2 S B$$

(16)

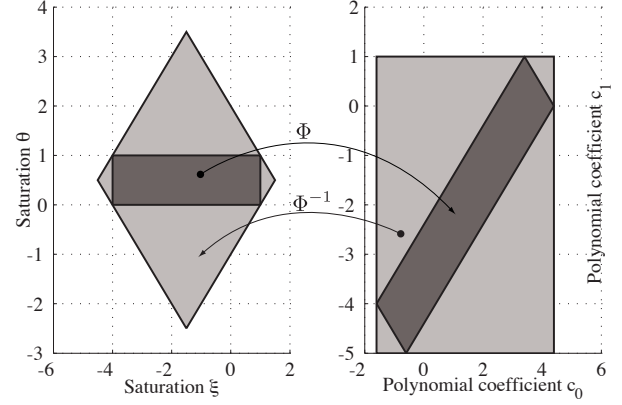$$v_3(A) = \frac{k_3 A}{K_m + A}$$

(17)

Figure 2. Affine parameter transform introducing conservatism; uncertainty-rectangle in parameter space and its image (dark gray) under the affine mapping Φ, the bounding rectangle from interval arithmetic in coefficients space and its image in the parameter space (light gray).

With $A + B = \mathrm{const}$ it can be reduced to a 2-dimensional system, whereas the Jacobian matrix reads

$$\mathbf{J} = \begin{pmatrix} -\alpha_1 & \alpha_1(\xi + \alpha_2) \\ +2 & -\xi - \theta - 2\alpha_2 \end{pmatrix}, \quad (18)$$

with $\alpha_1 \equiv \frac{A^0}{S^0}$ and $\alpha_2 = \frac{A^0}{B^0}$. The characteristic polynomial is then

$$p(\lambda, \xi, \theta) = \lambda^2 + \lambda(\xi + \alpha_1 + 2\alpha_2 + \theta) + (\theta - \xi),$$

with $\theta \in [\underline{\theta}, \bar{\theta}]$ and $\xi \in [\underline{\xi}, \bar{\xi}]$. For Kharitonov analysis we apply interval arithmetic to the affine transform Φ that maps the parameters to the coefficients of the polynomial. The parameter rectangle maps under this transform to a parallelotope. To obtain the interval coefficient of the polynomial one has to bound the parallelotope with another rectangle. In the original parameter space that bounding rectangle corresponds to a larger region of parameter space. That steps corresponds exactly to the conservatism hidden in the Kharitonov test. To illustrate this we show the result of that mapping and its inverse for this 2-dimensional model in Fig. 2. Fig. 3 shows the real stability region in parameter space (gray) and the inscribed stability rectangles determine by quadratic stability, affine quadratic stability and Kharitonov's method. Kharitonov's method as well as affine quadratic stability do not show any conservatism but quadratic stability returns a more conservative result. The proposed general algorithms to determine the stability intervals for the three methods of Section 3 is iterative and proceeds by a multi-dimensional bisection. The bisection uses the supplied nominal stable parameter set (indicated in Fig. 3) as an expansion point. Due to the dependency of the bisection on this nominal values and on its particular expansion policy, we can not guarantee that the final stability rectangle is the one of maximum volume possible for a particular method.

The specific policy applied here is that all parameter dimensions are expanded simultaneously at an rate that is
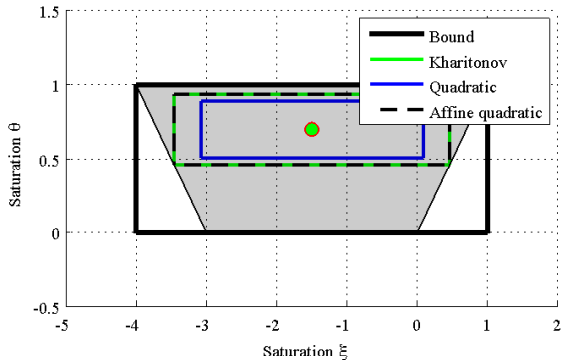
Figure 3. Stability region in terms of the saturation parameters θ and ξ for a steady state flux $v = 1$ and the steady state concentrations $A^0 = 1$, $B^0 = 1$ and $S^0 = 1$ obtained stability boxes for the three different methods.

proportional to the size of the initial bounding box. If a proposal box in unfeasible we sequentially test every dimension separately whether it can be expanded. The dimensions that can be expanded further are then simultaneously expanded. Alternatively, algorithms based on the branch-and-bound method may be used.

4.2. Medium-Scale Model

The second considered model of the glycolytic pathway is depicted in Fig. 4. We have 12 unknown saturation parameters of the involved reactions which are just known to reside within particular bounding interval (by knowing the cooperativity of the reactions). The stability of the system is sensitive to the strength of the inhibitory feedback $\xi \in [0, n]$ and with it, the different levels of saturation $\theta_{ATP}^{\mu_1} = 1 - \xi$ (reaction Glc $\rightarrow$ FBP), where n is the estimated upper bound for the effective kinetic order of the inhibition. To perform our stability analysis we make the assumption that all saturation parameters are within $\theta_s^\mu \in (0, 1]$, except $\theta_{ATP}^{\mu_1}$ with $\xi \in [0, 2]$. Michaelis-Menten kinetics leads to an interval $(0, 1]$. Furthermore, we assume to be given a stable nominal parameter set $\tilde{\theta}_s^\mu = 0.8$ and $\tilde{\xi} = 1$. The stability hyperbox is expanded around the nominal set taking into account the following constraint to simplify the bisection method. The side lengths of the box are kept equal for all parameters, except for $\theta_{ATP}^{\mu_1}$, that was varied individually. The 12-dimensional stability region obtained via Karithonov's method is shown in Fig. 5. The stability region may be ex-
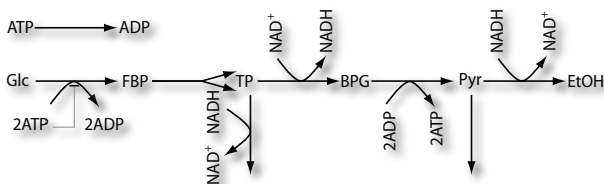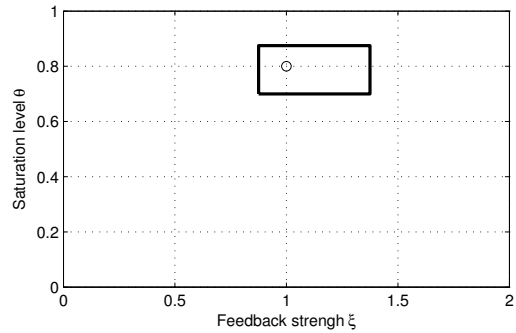

Figure 5. Hyperbox describing a guaranteed region for asymptotic stability in parameter space based on Karithonov's method; each of the saturation parameters can vary independently within that interval and result in a stable system.

panded further by decoupling these parameter dimensions in the bisection.

5. CONCLUSION

We incorporated methods from robust control into the framework of structural kinetic modeling. Guaranteed multivariate stability intervals for unknown reaction saturation levels can be determined. Multivariate bisection was used in conjunction with the binary stability tests. Those were based on Kharitonov's theorem, quadratic stability and quadratic affine stability. Two simple models of the glycolytic pathway are used to illustrate the method. The important conceptual advance of the presented approach is that it operates on whole family of models, instead of individual models.

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Figure 4. Medium-scale model of the glycolytic pathway featuring 12 unknown saturation parameters of the reactions.