Stability Analysis of Biological Systems with Real Solution Classification

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ABSTRACT

This paper presents a new and general approach for analyzing the stability of a large class of biological networks, modeled as autonomous systems of differential equations, using real solving and solution classification. The proposed approach, based on the classical technique of linearization from the qualitative theory of ordinary differential equations yet with exact symbolic computation, is applied to analyzing the local stability of the Cdc2-cyclin B/Wee1 system and the Mos/MEK/p42 MAPK cascade, two well-known models for cell and protein signaling that have been studied extensively in the literature. We provide rigorous proofs and generalizations for some of the previous results established experimentally and report our new findings.

Categories and Subject Descriptors

I.1 [**Symbolic and Algebraic Manipulation**]: Applications, Algorithms; J.3 [**Life and Medical Sciences**]: Biology and Genetics

General Terms

Algorithms

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Biological network, differential equations, equilibrium, stability, polynomial system, real root, solution classification, Cdc2-cyclin B/Wee1, Mos/MEK/p42 MAPK cascade

1. INTRODUCTION

Computational studies of biological systems, also called *bioinformatics*, have emerged as a major area of research on the frontiers of mathematics, biology, and computer science in the last decade. Biological networks may be modeled mathematically by dynamical systems. The analysis of the local and global behaviors of such systems is crucial and challenging. It is extremely difficult to detect and analyze equilibria, stability, bifurcations, and chaos of biological dynamical systems using standard mathematical methods.

Consider, for example, the class of plane autonomous polynomial differential systems, which are the simplest continuous dynamical systems. The problem of determining the number of limit cycles and their relative configurations is the second part of Hilbert's 16th problem, which was posed 105 years ago and on which there is little progress. Therefore, the analysis of stability remains a challenge even for polynomial differential systems. In the research of biological systems, stability behaviors and bifurcations are often analyzed by means of numerical simulation and visualization. It is desirable that the results of such numerical analysis may be confirmed by formal mathematical reasoning.

In this paper, we consider biological networks that may be modeled by autonomous systems of differential equations of the form

$$\begin{cases} \dot{x}_1 = F_1(\boldsymbol{u}, x_1, \ldots, x_n), \\ \dot{x}_2 = F_2(\boldsymbol{u}, x_1, \ldots, x_n), \\ \quad \cdots\cdots \\ \dot{x}_n = F_n(\boldsymbol{u}, x_1, \ldots, x_n), \end{cases} \tag{1}$$

where $F_1, \ldots, F_n$ are rational functions of $\boldsymbol{u}, x_1, \ldots, x_n$ with real coefficients and $\boldsymbol{u}$ is one or several real parameters. As usual, $x_i = x_i(t)$, $\dot{x}_i = \mathrm{d}\,x_i/\mathrm{d}\,t$, and the parameters $\boldsymbol{u}$ are independent of the derivation variable t. For differential systems (1), we propose a new and general approach to detect their real equilibria by solving the rational-function equations $F_1 = 0, \ldots, F_n = 0$ symbolically and to analyze the stability of the equilibria by means of linearization and real solution classification. All the involved computations are performed symbolically and we are not aware of any existing work in this direction. The class of biological networks we consider is large enough to cover many complex systems, including biological positive-feedback loops for cell and protein signaling. We will use the well-known Cdc2-cyclin B/Wee1 system [11, 12] and the Mos/MEK/p42 MAPK cascade [1, 6], which have been studied extensively and experimentally in the literature, to illustrate the features, originality, and performance of our approach. We will also report on our computational results and new findings. The detection of bistability or multistability of such systems is an essential step for understanding how the systems function. Systems are bistable when they toggle between two discrete, alter-

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native stable steady states without being able to rest in intermediate states. We refer to [1, 12] for technical discussions on the behavior and importance of bistability and multistability in the biological context.

The rest of the paper is structured as follows. In the next section, we shall recall some preliminary notions and results from the qualitative theory of ordinary differential equations and present the linearization technique and some stability criteria for our purpose. In Section 3 is provided a brief review on the existing methods dealing with systems of polynomial equations and inequalities. Emphasis will be placed on the techniques that will be used in later sections of this paper. In Section 4, we will present the details of the stability analysis for a simple yet nontrivial example: the Cdc2-cyclin B/Wee1 system. It will show how our method works step by step with symbolic computation. Our general approach to stability analysis for the considered class of biological systems will be described in Section 5. The practical value and potential of our approach will be demonstrated by several experimental results reported in Section 6: some of the previous results established with numerical simulation and visualization may be rigorously proved, refined, and generalized by our program using real solution classification. The paper will be concluded with a few remarks on the novelty, applicability, and limitation of our approach, the encountered computational difficulties, and our future work.

A classical and widely used method for analyzing the stability of biological systems is based on phase plane or space diagrams, which plot the trajectories of the differential system around equilibria by numerical computation [1, 11]. This method is limited to plane and spatial differential systems. A more powerful and theoretical approach for analyzing stability behaviors together with a simple graphical method for deducing bifurcation diagrams for biological positive-feedback systems is described in [1]. The visualization technique is very useful in practice, but its theoretical rigor cannot be easily guaranteed. Our symbolic approach provides a mathematically rigorous framework for the stability analysis of a large class of biological systems of arbitrary dimension. The effectiveness of this approach comes from the novelty of using advanced techniques of symbolic real solving and real solution classification.

2. EQUILIBRIA AND STABILITY OF DIFFERENTIAL EQUATIONS

Let $\mathcal{R}$ denote the field of real numbers. For any real parametric value $\bar{\boldsymbol{u}}$ of $\boldsymbol{u}$, a point $\bar{\boldsymbol{x}} = (\bar{x}_1, \ldots, \bar{x}_n)$ in the n-dimensional real Euclidean space $\mathcal{R}^n$ is called an *equilibrium* (or a *singular point*, *critical point*, or *steady state*) of a system of differential equations of the form (1) if

$$F_1(\bar{\boldsymbol{u}}, \bar{\boldsymbol{x}}) = \cdots = F_n(\bar{\boldsymbol{u}}, \bar{\boldsymbol{x}}) = 0.$$

Therefore, the problem of computing equilibria amounts to finding the real solutions of the system of rational-function equations $F_1 = 0, \ldots, F_n = 0$ depending on the parameters $\boldsymbol{u}$; the latter may be reduced to solving a system of n polynomial equations in n unknowns $x_1, \ldots, x_n$. We shall discuss how to solve such systems of equations as well as the involved computational difficulties in the following section.

For an arbitrary but fixed real value $\bar{\boldsymbol{u}}$ of $\boldsymbol{u}$, let $\bar{\boldsymbol{x}}$ be an equilibrium of (1). We want to analyze the stability of $\bar{\boldsymbol{x}}$. To do so, we use Lyapunov's first method with the technique of linearization, that is, by considering the Jacobian matrix

$$\boldsymbol{J} = \begin{pmatrix} \frac{\partial F_1}{\partial x_1} & \frac{\partial F_1}{\partial x_2} & \cdots & \frac{\partial F_1}{\partial x_n} \\ \frac{\partial F_2}{\partial x_1} & \frac{\partial F_2}{\partial x_2} & \cdots & \frac{\partial F_2}{\partial x_n} \\ \vdots & \vdots & & \vdots \\ \frac{\partial F_n}{\partial x_1} & \frac{\partial F_n}{\partial x_2} & \cdots & \frac{\partial F_n}{\partial x_n} \end{pmatrix}.$$

Then system (1) may be written in the following matrix form:

$$\dot{\boldsymbol{x}}^{\mathrm{T}} = \boldsymbol{J}(\bar{\boldsymbol{u}}, \bar{\boldsymbol{x}})(\boldsymbol{x} - \bar{\boldsymbol{x}})^{\mathrm{T}} + \boldsymbol{G},$$

where the superscript T denotes matrix transpose and

$$\boldsymbol{G} = [F_1(\bar{\boldsymbol{u}}, \boldsymbol{x}), \ldots, F_n(\bar{\boldsymbol{u}}, \boldsymbol{x})]^{\mathrm{T}} - \boldsymbol{J}(\bar{\boldsymbol{u}}, \bar{\boldsymbol{x}})(\boldsymbol{x} - \bar{\boldsymbol{x}})^{\mathrm{T}}$$

is $o(|\boldsymbol{x} - \bar{\boldsymbol{x}}|)$ as $\boldsymbol{x} \to \bar{\boldsymbol{x}}$. The following theorem serves to determine the stability of the equilibrium $\bar{\boldsymbol{x}}$.

THEOREM 1. (*a*) *If all the eigenvalues of the matrix* $\boldsymbol{J}(\bar{\boldsymbol{u}}, \bar{\boldsymbol{x}})$ *have negative real parts, then* $\bar{\boldsymbol{x}}$ *is asymptotically stable.*
(*b*) *If the matrix* $\boldsymbol{J}(\bar{\boldsymbol{u}}, \bar{\boldsymbol{x}})$ *has at least one eigenvalue with positive real part, then* $\bar{\boldsymbol{x}}$ *is unstable.*

It is more difficult to determine the stability of $\bar{\boldsymbol{x}}$ when some of the eigenvalues of $\boldsymbol{J}(\bar{\boldsymbol{u}}, \bar{\boldsymbol{x}})$ have zero real parts, but none of them has positive real part. In this case, if the eigenvalues with zero real parts correspond to a simple zero of the characteristic polynomial of $\boldsymbol{J}(\bar{\boldsymbol{u}}, \bar{\boldsymbol{x}})$, then $\bar{\boldsymbol{x}}$ is stable; otherwise, it may be unstable.

In the case $n = 2$, we may have a more precise classification of the equilibria. Let the Jacobian matrix evaluated at $(\bar{\boldsymbol{u}}, \bar{\boldsymbol{x}})$ for $n = 2$ be

$$\boldsymbol{J}_2(\bar{\boldsymbol{u}}, \bar{\boldsymbol{x}}) = \begin{pmatrix} a & b \\ c & d \end{pmatrix}$$

and λ_1, λ_2 the two eigenvalues of $\boldsymbol{J}_2(\bar{\boldsymbol{u}}, \bar{\boldsymbol{x}})$. More concretely, λ_1, λ_2 are the two roots of the characteristic polynomial

$$\begin{vmatrix} a - \lambda & b \\ c & d - \lambda \end{vmatrix} = \lambda^2 + p\lambda + q,$$

where $p = -(a + d)$ and $q = ad - bc$. Set $\Delta = p^2 - 4\,q$. We have the following criteria:

C1. when $q > 0$, $p > 0$, and $\Delta \geq 0$ (in this case, λ_1, λ_2 are real and $\lambda_1 < 0, \lambda_2 < 0$), $\bar{\boldsymbol{x}}$ is a *stable node*;

C2. when $q > 0$, $p < 0$, and $\Delta \geq 0$ (in this case, λ_1, λ_2 are real and $\lambda_1 > 0, \lambda_2 > 0$), $\bar{\boldsymbol{x}}$ is an *unstable node*;

C3. when $q < 0$ (in this case, λ_1, λ_2 are real and $\lambda_1 \lambda_2 < 0$), $\bar{\boldsymbol{x}}$ is an (unstable) *saddle*;

C4. when $q > 0$, $p > 0$, and $\Delta < 0$ (in this case, λ_1, λ_2 are complex conjugates and $\mathrm{Re}\,\lambda_1 = \mathrm{Re}\,\lambda_2 < 0$, where Re denotes the *real part*), $\bar{\boldsymbol{x}}$ is a *stable focus* (or *spiral*);

C5. when $q > 0$, $p < 0$, and $\Delta < 0$ (in this case, λ_1, λ_2 are complex conjugates and $\mathrm{Re}\,\lambda_1 = \mathrm{Re}\,\lambda_2 > 0$), $\bar{\boldsymbol{x}}$ is an *unstable focus*;

C6. when $q > 0$ and $p = 0$ (in this case, λ_1, λ_2 are complex conjugates and $\mathrm{Re}\,\lambda_1 = \mathrm{Re}\,\lambda_2 = 0$), $\bar{\boldsymbol{x}}$ is a *center* of $\dot{\boldsymbol{x}}^{\mathrm{T}} = \boldsymbol{J}_2(\bar{\boldsymbol{u}}, \bar{\boldsymbol{x}})(\boldsymbol{x} - \bar{\boldsymbol{x}})^{\mathrm{T}}$ and the stability of the equilibrium $\bar{\boldsymbol{x}}$ of (1) depends on $\boldsymbol{G}$ (higher-order approximation);

C7. when $q = 0$, the Jacobian matrix $\boldsymbol{J}_2(\bar{\boldsymbol{u}}, \bar{\boldsymbol{x}})$ is singular and the first approximation $\dot{\boldsymbol{x}}^{\mathrm{T}} = \boldsymbol{J}_2(\bar{\boldsymbol{u}}, \bar{\boldsymbol{x}})(\boldsymbol{x} - \bar{\boldsymbol{x}})^{\mathrm{T}}$ of (1) has infinitely many critical points.

For $n > 2$, whether all the eigenvalues of the Jacobian matrix $\boldsymbol{J}(\bar{\boldsymbol{u}}, \bar{\boldsymbol{x}})$ have negative real parts can be determined according to Routh–Hurwitz's criterion. This will be explained at the end of Section 5.

The method presented above is classical in the qualitative theory of ordinary differential equations and may be found in standard textbooks (e.g., [10, 23]). However, for practical application, there are several difficulties one must overcome. First of all, one has to compute the equilibria of the differential system (1), which are real solutions of a system of polynomial equations. Even in the simpler case when the parameters $\boldsymbol{u}$ are not present, in general the real solutions may not be given analytically. The components of the solutions are usually determined as real roots of some polynomials with rational coefficients. The approximate values of such real roots may be computed by numerical methods. Symbolically, the real roots may be isolated by rational intervals of arbitrarily small width, but real solution isolation for general systems of polynomial equations is still an outstanding problem of research in symbolic computation.

In the presence of parameters $\boldsymbol{u}$, one has to identify for what parametric values $\bar{\boldsymbol{u}}$ of $\boldsymbol{u}$ the system of equations

$$F_1(\bar{\boldsymbol{u}}, \boldsymbol{x}) = 0, \ldots, F_n(\bar{\boldsymbol{u}}, \boldsymbol{x}) = 0$$

has real solutions, and how many, for the unknowns $\boldsymbol{x}$. How to describe or represent the real solutions in terms of the parameters $\boldsymbol{u}$? These are simple questions that cannot be easily answered. In order to decide the stability of the equilibria, one also has to determine rigorously the signs of the real parts of the eigenvalues of the Jacobian matrix at these equilibria. For instance, in the case $n = 2$, one needs to determine the signs of q, p, and Δ in the above criteria. Even for a given value $\bar{\boldsymbol{u}}$ of $\boldsymbol{u}$, as the equilibrium $\bar{\boldsymbol{x}}$ may be determined only as real roots of polynomials and represented symbolically by means of isolating intervals, the sign determination is computationally nontrivial. It is clearly more difficult to derive the conditions on the parameters $\boldsymbol{u}$ for the real parts of the eigenvalues to have prescribed signs.

The approach proposed in the present paper may solve most of these problems. We will present the details in the following sections.

3. REAL SOLVING AND SOLUTION CLASSIFICATION OF POLYNOMIAL SYSTEMS

Solving systems of polynomial equations has been one of the central topics in computer algebra. It is required and used in many scientific and engineering applications. There are several methods based on resultants, Gröbner bases [3], and triangular sets [7, 8, 13, 17] which may be used to transform such systems into certain triangular form. What we need here is to find exact (number of) real solutions of polynomial systems and to establish conditions for parametric polynomial systems to have a prescribed number of real solutions, which are computationally difficult. There exist general methods such as the method of cylindrical algebraic decomposition (CAD) [4, 5] that may be applied to some of our problems in principle. However, it is well known that the CAD method and its variants have high computational complexity. Much of the recent research on real solving has focused on using the techniques of triangular sets and Gröbner bases to transform the involved systems of polynomial equations and introducing specialized devices to handle the projection and lifting process for restricted classes of systems of polynomial equations and inequalities. Extensive work in this direction includes [2, 9, 18, 21].

The authors of this paper have been much involved in the design and implementation of triangular-set-based decomposition algorithms [13, 14, 15] and real solving based on discrimination systems [18, 19, 21], and we have the necessary software packages in hand for different kinds of experiments. Therefore, we base this initial analysis of stability of biological systems on the work using triangular sets and discrimination systems [14, 15, 19, 21]. It is apparent that other methods such as the CAD method [4, 5], quadratic quantifier elimination [16], and the method using Gröbner bases and discriminant varieties [9] for real solving may also be applied to the same problem of stability analysis according to our general approach. We hope to explore such application and observe and compare the performances of different methods in the near future.

In what follows, we present very briefly the method we will use for symbolic real solving and solution classification of (parametric) polynomial systems. Consider the following system of n equations

$$P_1(\boldsymbol{u}, \boldsymbol{x}) = 0, P_2(\boldsymbol{u}, \boldsymbol{x}) = 0, \ldots, P_n(\boldsymbol{u}, \boldsymbol{x}) = 0, \qquad (2)$$

where $P_1, \ldots, P_n$ are polynomials in $\boldsymbol{u}$ and $\boldsymbol{x} = (x_1, \ldots, x_n)$ with rational coefficients and $\boldsymbol{u}$ is one or several real parameters. Let P be any polynomial in $\boldsymbol{u}$ and $\boldsymbol{x}$ with rational coefficients. We are concerned with the following problems.

P1. Assume that the parameters $\boldsymbol{u}$ are not present. Determine the number of real solutions of (2) for $\boldsymbol{x}$ and isolate all the isolated real solutions of (2) by rational intervals.

P2. For any integer $s \geq 0$, determine the conditions on $\boldsymbol{u}$ for system (2) to have exactly s distinct real solutions for $\boldsymbol{x}$.

P3. Assume that the parameters $\boldsymbol{u}$ are not present. Determine the sign of P at each isolated real solution of (2).

P4. Determine the conditions on $\boldsymbol{u}$ for P to be 0, positive, or negative at the isolated real solutions of (2).

The method for solving these problems works by first transforming the set $\mathbb{P} = \{P_1, \ldots, P_n\}$ of polynomials into finitely many *regular sets* $\mathbb{T}_1, \ldots, \mathbb{T}_e$ [7, 14, 22] such that

$$\mathrm{Zero}(\mathbb{P}) = \bigcup_{i=1}^{e} \mathrm{Zero}(\mathbb{T}_i / J_i), \qquad (3)$$

where $\mathrm{Zero}(\mathbb{P})$ denotes the set of all common zeros (in some extension of the field of rational numbers) of $P_1, \ldots, P_n$,

$\text{Zero}(\mathbb{T}_i/J_i) = \text{Zero}(\mathbb{T}_i) \setminus \text{Zero}(\{J_i\})$, and J_i is the product of *initials* of the polynomials in $\mathbb{T}_i$ for each i (see [15] for the definition and computation of regular sets and other relevant concepts). Furthermore, we may assume that each $\mathbb{T}_i$ is *simplicial* [22] with respect to P (i.e., P is either zero at all the real zeros of $\mathbb{T}_i$, or nonzero at every real zero of $\mathbb{T}_i$) and no two of the regular sets have common zeros [21].

Without loss of generality, we only consider one regular set

$$\mathbb{T} = \mathbb{T}_i = [T_1(\boldsymbol{u}, x_1), \ldots, T_n(\boldsymbol{u}, x_1, \ldots, x_n)].$$

If the parameters $\boldsymbol{u}$ are not present, we first isolate the real roots of T_1 for x_1 by rational intervals. For each isolating interval I, substitute the two endpoints for x_1 in T_2, respectively, and obtain two polynomials in x_2. From these two polynomials, one can obtain the isolating intervals for the real zeros of $[T_1, T_2]$ when x_1 is on I, provided that I is sufficiently small. In this way, we can isolate all the real zeros of $\mathbb{T}$. To determine the sign of P at each isolated real zero of $\mathbb{T}$, one substitutes the intervals for the variables in P and determines the sign of P by interval arithmetic. If the algorithm proposed in [18] is used, we only need to take a point in the isolating interval arbitrarily and to determine the sign of P at this point. For algorithmic details, we refer to [18].

If the parameters $\boldsymbol{u}$ are present, we compute a so-called *border polynomial* $B(\boldsymbol{u})$ of (2) according to the regular sets $\mathbb{T}_i$ [20, 21]. For each regular set $\mathbb{T}_i = [T_1, \ldots, T_n]$, the border polynomial contains the resultant of the leading coefficient of T_j and $[T_1, \ldots, T_{j-1}]$ as well as the resultant of the discriminant of T_j and $[T_1, \ldots, T_{j-1}]$ as its factors. If the sign of P is to be determined, then $B(\boldsymbol{u})$ also contains the resultants of P and $\mathbb{T}_i$. The border polynomial has the property that the number of distinct real solutions of the system (2) is invariant in each connected component (called *cell*) of the complement of $B(\boldsymbol{u}) = 0$ in the parametric space. It is closely related to the notion of *discriminant variety* introduced by Lazard and Rouillier [9]. Thus, to determine the number of distinct real solutions of (2) in each cell, it suffices to check the situation at one point of this cell. So one may take a sample point from each cell and isolate the real solutions of (2) at the sample point. Finally, the signs of the factors of $B(\boldsymbol{u})$ together with the numbers of real solutions of (2) at the sample points form a real solution classification of (2), from which the conditions for problem P4 above may be easily obtained. The interested reader may consult [20, 21] for details.

4. STABILITY ANALYSIS OF THE CDC2-CYCLIN B/WEE1 SYSTEM

As an example to illustrate our general approach, in this section we analyze the stability of the Cdc2-cyclin B/Wee1 system. It is a bivariate system that describes the interplay between two proteins: the Cdc2-cyclin B complex and the Wee1 protein (see [1, 11, 12] and Figure 1). Its stability behavior may be determined numerically by the classical phase plane analysis [11] and another graphical method proposed by Angeli and others in [1].

We refer to [1] for the setting details of this example. Under certain assumptions, the system of differential equations that model the two-component, mutually inhibitory feedback loop is reduced to the following form

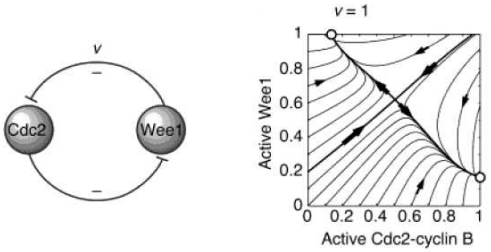

Figure 1: The Cdc2-cyclin B/Wee1 system and its phase plane diagram under unitary feedback ($v = 1$), reproduced from [1]

$$\begin{cases} \dot{x}_1 = \alpha_1(1 - x_1) - \dfrac{\beta_1 x_1 (v y_1)^{\gamma_1}}{K_1 + (v y_1)^{\gamma_1}}, \\ \dot{y}_1 = \alpha_2(1 - y_1) - \dfrac{\beta_2 y_1 x_1^{\gamma_2}}{K_2 + x_1^{\gamma_2}}, \end{cases} \tag{4}$$

where $\alpha_1, \alpha_2, \beta_1, \beta_2$ are rate constants, K_1, K_2 are Michaelis (saturation) constants, γ_1, γ_2 are Hill coefficients, and v is a coefficient (feedback) that reflects the strength of the influence of Wee1 on Cdc2-cyclin B. For easy reference and comparison, we take the same numerical values for the biological constants as in [1]:

$$\gamma_1 = \gamma_2 = 4, \quad \alpha_1 = \alpha_2 = 1,$$
$$\beta_1 = 200, \ \beta_2 = 10, \quad K_1 = 30, \ K_2 = 1.$$

For simplicity of notation, let $x = x_1$ and $y = y_1$. Then system (4) becomes

$$\dot{x} = \frac{P}{30 + v^4 y^4}, \quad \dot{y} = \frac{Q}{1 + x^4}, \tag{5}$$

where

$$P = 30 - 30\,x + v^4(1 - 201\,x)\,y^4,$$
$$Q = 1 + x^4 - (1 + 11\,x^4)\,y,$$

and v is a real parameter. Our problem is to detect the stability of (5). In particular, we want to know for what parametric value of v bistability may arise in this system, i.e., for what value of v system (5) may have two stable steady states. This amounts to determining the number of equilibria of (5), or equivalently, finding the number of real solutions of $P = 0, Q = 0$ for x, y in terms of v.

In general, one can apply the methods of triangular sets, Gröbner bases, and resultants to triangularize the polynomial system in equation. However, the system $P = 0, Q = 0$ for this example is too simple for any of these methods and the triangularization process is trivial: solving $Q = 0$ for y and substituting the solution to P, we obtain an irreducible polynomial of degree 17 in x with parameter v:

$$\begin{aligned} H = {} & (439230 + 201\,v^4)\,x^{17} - (439230 + v^4)\,x^{16} \\ & + (159720 + 804\,v^4)\,x^{13} - (159720 + 4\,v^4)\,x^{12} \\ & + (21780 + 1206\,v^4)\,x^9 - (21780 + 6\,v^4)\,x^8 \\ & + (1320 + 804\,v^4)\,x^5 - (1320 + 4\,v^4)\,x^4 \\ & + (30 + 201\,v^4)\,x - 30 - v^4. \end{aligned}$$

Now the problem is to derive the conditions on v for H to have $0, 1, 2, \ldots$ real roots for x. This can be done by using the

method of real root classification sketched in the previous section. Our implementation of the method allows us to establish the conditions automatically. More concretely, the program may find a polynomial $R = v\bar{R}$ (which in fact is the discriminant of H with respect to x), where $\bar{R}$ is of degree 32 in v and has 9 terms and 4 real roots and is a little bit too large to be reproduced here. Denote the 5 real roots of R by $\bar{v}_2 < \bar{v}_1 < v_0 = 0 < v_1 < v_2$ (where $v_1 = -\bar{v}_1 \approx 0.8315735076$, $v_2 = -\bar{v}_2 \approx 1.796868764$), which may be isolated as follows:

$$\left[-2, -\frac{3}{2}\right], \ \left[-1, -\frac{1}{2}\right], \ [0, 0], \ \left[\frac{1}{2}, 1\right], \ \left[\frac{3}{2}, 2\right].$$

These intervals can be made arbitrarily small if we wish. As v is for the strength of the influence of Wee1 on Cdc2-cyclin B (or in view of the symmetry), we may assume that $v \geq 0$. From the output of the program, we get directly the following results:

E1. when $0 < v < v_1$ or $v_2 < v < +\infty$, system (5) has only one steady state (or equilibrium);

E2. when $v_1 < v < v_2$, system (5) has three steady states;

E3. when $v = 0$, system (5) has a unique steady state;

E4. when $v = v_1$ or $v = v_2$, system (5) has two steady states.

Finally, we need to determine the stability of the steady states, i.e., to determine whether the steady states are stable or not. To this end, we consider the Jacobian matrix of (5), whose entries are the partial derivatives of

$$F = \frac{P}{30 + v^4 y^4}, \quad G = \frac{Q}{1 + x^4}$$

with respect to x and y, i.e.,

$$a = \frac{\partial F}{\partial x} = -\frac{3\,(10 + 67\,v^4 y^4)}{30 + v^4 y^4}, \quad b = \frac{\partial F}{\partial y} = -\frac{24000\,v^4 x y^3}{(30 + v^4 y^4)^2},$$

$$c = \frac{\partial G}{\partial x} = -\frac{40\,x^3 y}{(1 + x^4)^2}, \qquad d = \frac{\partial G}{\partial y} = -\frac{1 + 11x^4}{1 + x^4}.$$

Let

$$p = -(a + d) = \frac{2\,\bar{p}}{(30 + v^4 y^4)\,(1 + x^4)},$$

$$q = ad - bc = \frac{3\,\bar{q}}{(30 + v^4 y^4)^2\,(1 + x^4)^2},$$

$$\Delta = p^2 - 4\,q = \frac{100\,\bar{\Delta}}{(30 + v^4 y^4)^2\,(1 + x^4)^2},$$

where

$$\begin{aligned}
\bar{p} &= 30 + 180\,x^4 + 101\,v^4 y^4 + 106\,v^4 x^4 y^4, \\
\bar{q} &= 67\,y^8\,(1 + 11\,x^4)\,(1 + x^4)\,v^8 \\
&\quad + 20\,y^4\,(101 - 14788\,x^4 + 1111\,x^8)\,v^4 \\
&\quad + 300\,(1 + 11\,x^4)\,(1 + x^4), \\
\bar{\Delta} &= x^8\,(19\,v^4 y^4 - 30)^2 + 40\,v^4 x^4\,(930 + 19\,v^4 y^4)\,y^4 \\
&\quad + 400\,v^8 y^8.
\end{aligned}$$

It is easy to see that $a < 0$, $d < 0$, $p > 0$, $\Delta \geq 0$ always hold. As output, our program gives the following results:

S1. when $0 < v < v_1$ or $v_2 < v < +\infty$, $q > 0$ and $\Delta > 0$ hold at the only steady state, so this steady state is a stable node;

S2. when $v_1 < v < v_2$, one of the three steady states, at which $q < 0$, is an (unstable) saddle and the other two steady states (at which $q > 0$ and $\Delta > 0$) are stable nodes;

S3. when $v = 0$, $p > 0$, $q > 0$, and $\Delta > 0$ hold at the unique steady state, so this steady state is a stable node;

S4. when $v = v_1$ or $v = v_2$, $q > 0$ and $\Delta > 0$ hold at one of the two steady states, so this steady state is a stable node, and $q = 0, a < 0, d < 0$, and $bc > 0$ hold at the other steady state. In the latter case, because $q = 0$ (i.e., the Jacobian matrix of (5) is singular), the method of linearization is inapplicable, but it is not difficult to see that the steady state in this case is unstable.

Therefore, it is rigorously proved that the system exhibits bistability when $v_1 < v < v_2$. This completes our analysis of the stability of (5).

5. DESCRIPTION OF THE GENERAL APPROACH

In this section, we provide a short description of the algorithmic steps of our general approach for the stability analysis of biological systems. It is a formulation of the main steps shown in the above illustrative example.

We are given an autonomous system of ordinary differential equations of the form (1) that models a biological network. In the case when the parameters $\boldsymbol{u}$ are not present, our problem is to compute the real steady states of the system symbolically, in the sense that the coordinates of each steady state may be given as rational intervals of *arbitrarily* small width, and to determine the stability of each steady state. In the presence of parameters $\boldsymbol{u}$, our problem is to determine the conditions on $\boldsymbol{u}$ for the system to have prescribed numbers of stable and unstable steady states. The following steps provide solutions to these problems.

M1. Equate the numerators of the rational functions on the right-hand side of (1) to 0, yielding a system of polynomial equations of the form (2). In view of the background of the problem, there may be additional constraints on the system. For example, the denominators of the rational functions on the right-hand side of (1) should be nonzero and some variables may be positive.

M2. Decompose the polynomial set $\mathbb{P} = \{P_1, \ldots, P_n\}$ into regular sets $\mathbb{T}_1, \ldots, \mathbb{T}_e$ using any of the algorithms described in [7, 14, 22] (or more concretely, using the function RegSer available in the Epsilon library [15]), such that (3) holds. Make $\mathbb{T}_i$ and $\mathbb{T}_j$ have no common zero for all $i \neq j$ and simplicial with respect to all constraints according to [21, 22].

M3. If the parameters $\boldsymbol{u}$ are present, then go to step M4. Otherwise, isolate the real zeros of each $\mathbb{T}_i$ according to the algorithms presented in [18] (see the second paragraph from the end of Section 3). These real zeros are

all the steady states of (1). Take any value for $\bar{\boldsymbol{u}}$ (as $\boldsymbol{u}$ are not present) and go to step M7.

M4. Compute the *border polynomial* $B(\boldsymbol{u})$ of system (2) with all possible constraints (from $\mathbb{T}_1, \ldots, \mathbb{T}_e$) according to the algorithm proposed in [21]. The real part of the hypersurface $B(\boldsymbol{u}) = 0$ gives a topological decomposition of the parametric space. If, for example, $\boldsymbol{u}$ is a single parameter, then all the real numbers are decomposed into a finite number of open intervals and points by the real roots of $B(\boldsymbol{u})$. Any two points in one such interval have the following property: the number and the stability of the steady states of (1) are invariant respectively at these two points.

M5. Take a sample point from each connected component (cell) of the complement of $B(\boldsymbol{u}) = 0$ in the parametric space. This can be done by applying a partial CAD algorithm. If $\boldsymbol{u}$ is a single parameter, one only needs to take a sample point between u_i and u_{i+1} for all i $(0 \leq i \leq r)$ by isolating all the distinct real roots $u_1, \ldots, u_r$ of $B(\boldsymbol{u})$, where $u_0 = -\infty$ and $u_{r+1} = \infty$.

M6. For each sample point $\bar{\boldsymbol{u}}$, substitute $\bar{\boldsymbol{u}}$ for $\boldsymbol{u}$ in $\mathbb{T}_i$ (noting that $\bar{\boldsymbol{u}}$ does not make the vanishing of the initial of any polynomial in $\mathbb{T}_i$) and isolate the real solutions of the resulting regular sets, yielding the steady states of system (1) at $\boldsymbol{u} = \bar{\boldsymbol{u}}$. Because of the property of $B(\boldsymbol{u})$, the number of steady states of (1) at $\boldsymbol{u} = \bar{\boldsymbol{u}}$ is also the number of steady states of (1) when $\boldsymbol{u}$ is in the same cell.

At the same time, one may obtain the signs of the factors of $B(\boldsymbol{u})$ at $\bar{\boldsymbol{u}}$. If the conditions on $\boldsymbol{u}$ for system (1) to have a prescribed number of steady states are desired, we output the signs of the factors of $B(\boldsymbol{u})$ at the sample points of those cells in which the system has exactly the prescribed number of steady states.

M7. Suppose that $\bar{\boldsymbol{x}}$ is a steady state of system (1) at $\boldsymbol{u} = \bar{\boldsymbol{u}}$. We want to determine the stability of (1) at $\bar{\boldsymbol{x}}$. By the technique of linearization explained in Section 2, we compute the Jacobian matrix $\boldsymbol{J}(\boldsymbol{u}, \boldsymbol{x})$ of (1) and its characteristic polynomial $H(\boldsymbol{u}, \boldsymbol{x}, \lambda)$. Now the problem is reduced to determining the *stability* of $H(\bar{\boldsymbol{u}}, \bar{\boldsymbol{x}}, \lambda)$, which can be done by computing the signs of the Hurwitz determinants of $H(\bar{\boldsymbol{u}}, \bar{\boldsymbol{x}}, \lambda)$ according to the method described below. Finally, we output the signs of those cells in which system (1) has exactly the number of stable (or unstable) steady states as desired.

A standard method to determine the stability of a polynomial is Routh–Hurwitz's criterion [10, pp. 184–186]. Let

$$P = a_0\lambda^m + b_0\lambda^{m-1} + a_1\lambda^{m-2} + b_1\lambda^{m-3} + \cdots \quad (a_0 \neq 0)$$

be a real polynomial in λ and consider the $m \times m$ matrix

$$\boldsymbol{P} = \begin{pmatrix} b_0 & b_1 & b_2 & \cdots & b_{m-1} \\ a_0 & a_1 & a_2 & \cdots & a_{m-1} \\ 0 & b_0 & b_1 & \cdots & b_{m-2} \\ 0 & a_0 & a_1 & \cdots & a_{m-2} \\ 0 & 0 & b_0 & \cdots & b_{m-3} \\ \vdots & \vdots & \vdots & & \vdots \end{pmatrix},$$

where we take $a_i = 0$ when $i > m/2$, and $b_j = 0$ when $j \geq m/2$. The *Hurwitz determinants* $\Gamma_1, \ldots, \Gamma_m$ of P are defined to be the minors of $\boldsymbol{P}$. According to the Routh–Hurwitz criterion, the real parts of all the roots of P are negative if and only if $\mathrm{V}(a_0, \Gamma_1, \Gamma_3, \ldots) = \mathrm{V}(1, \Gamma_2, \Gamma_4, \ldots) = 0$, where $\mathrm{V}(\ldots)$ means the number of sign changes of the sequence.

6. EXPERIMENTS

In this section, we report on some of our experiments with the proposed approach for stability analysis of biological systems. Our experiments have been made on the Cdc2-cyclin B/Wee1 model without taking values for some of the biological constants and on a more complicated modular, five-variable example: the Mos/MEK/p42 MAPK cascade. As in our approach the obtained results are exact and rigorous, the involved symbolic computations are heavy in general. The occurring polynomials may be of very high degree with large integer coefficients. However, as shown by our experiments the symbolic methods underlying our approach are powerful enough as to be used for the stability analysis of reasonably complex biological systems.

The condition on v derived in Section 4 for the Cdc2-cyclin B/Wee1 system to exhibit bistability is for the given values of the biological constants $\alpha_1, \alpha_2, \beta_1, \beta_2, K_1, K_2, \gamma_1, \gamma_2$. Estimation of the constant values are very difficult: some of the values may be determined experimentally and others may be chosen so that the model can simulate the type of biological behavior that is observed or expected. Our symbolic approach allows us to establish conditions on some constant parameters for the system to exhibit certain desired behavior such as bistability or multistability.

To fix the idea, let us consider the Cdc2-cyclin B/Wee1 system as in Section 4, but without taking values for the Michaelis constants K_1, K_2. We want to know for what values of K_1, K_2 and v the system exhibits bistability.

From the meanings of the biological constants and variables, we know that $K_1 > 0$, $K_2 > 0$ and v, x_1, y_1 are nonnegative. Our program may compute a polynomial R_1 of degree 32 in v and degree 8 in either of K_1 and K_2 with 81 terms. Under the above assumption, we have $a < 0, d < 0, p > 0, r \geq 0, b \leq 0, c \leq 0$. Then we can conclude that

N1. when $R_1 < 0$, the system has three steady states, of which two are stable (in this case $q > 0$) and the other is unstable (in this case $q < 0$);

N2. when $R_1 > 0$, the system has only one steady state which is stable.

It follows that the system exhibits bistability if and only if $R_1 < 0$. This generalizes the result given in [1] and Section 4. The computation in the case $R_1 = 0$ is too heavy and could not be completed within three hours in Maple 9 on a notebook computer (Pentium 1.13 Ghz CPU with 256 M memory). From the results in the cases with specialized values of K_1, K_2, we guess that the system has two steady states, of which one is stable and the other is unstable, when $R_1 = 0$.

To determine the range of K_1, K_2 for the system to exhibit bistability, we may compute a polynomial R_2 of K_2:

$$\begin{aligned} R_2 = {} & 1123963607439473175421875\ K_2^4 \\ & - 92447046521175917830905 36\ K_2^3 \\ & - 50888283650649575113263 82\ K_2^2 \\ & - 62301929415679096\ K_2 + 51046875. \end{aligned}$$

Let the two positive real roots of R_2 be $k_1 \approx 0.77 \cdot 10^{-9}$ and $k_2 \approx 8.74$. Our computation shows that the system exhibits bistability for some range of the feedback gain v when $K_1 > 0$ and $k_1 < K_2 < k_2$, or no bistability otherwise. It follows that the system always exhibits bistability for some range of v, no matter what value K_1 takes. This conclusion is related to a question in [1]. The computation of the above results took less than three minutes in Maple 9 on the above-mentioned machine.

Now we discuss our experiments on a three-tier MAPK cascade, based on the Mos/MEK/p42 MAPK cascade present in *Xenopus* oocytes, which has been studied extensively in the literature (see, e.g., [1, 6]). This system is modeled by the following five equations [1]:

$$\begin{cases} \dot{x} = -\dfrac{xV_2}{K_2 + x} + vz_3V_0 + V_1, \\ \dot{y_1} = \dfrac{(1200 - y_1 - y_3)\,V_6}{K_6 + 1200 - y_1 - y_3} - \dfrac{xy_1V_3}{K_3 + y_1}, \\ \dot{y_3} = \dfrac{x(1200 - y_1 - y_3)\,V_4}{K_4 + 1200 - y_1 - y_3} - \dfrac{y_3V_5}{K_5 + y_3}, \\ \dot{z_1} = \dfrac{(300 - z_1 - z_3)\,V_{10}}{K_{10} + 300 - z_1 - z_3} - \dfrac{y_3z_1V_7}{K_7 + z_1}, \\ \dot{z_3} = \dfrac{y_3(300 - z_1 - z_3)\,V_8}{K_8 + 300 - z_1 - z_3} - \dfrac{z_3V_9}{K_9 + z_3}. \end{cases} \tag{6}$$

For this five-dimensional system, the graphical technique of phase plane or space analysis does not work and it is highly nontrivial to detect its multistability. According to [1], we take numerical values for the biological constants as follows:

$$\begin{array}{llll} V_0 = 3/2000, & V_1 = 1/500000, & V_2 = 6/5, & K_2 = 200, \\ V_3 = 8/125, & K_3 = 1200, & V_4 = 8/125, & K_4 = 1200, \\ V_5 = 5, & K_5 = 1200, & V_6 = 5, & K_6 = 1200, \\ V_7 = 3/50, & K_7 = 300, & V_8 = 3/50, & K_8 = 300, \\ V_9 = 5, & K_9 = 300, & V_{10} = 5, & K_{10} = 300. \end{array}$$

Then equating the numerators of the rational functions on the right-hand side of (6) to 0, we obtain a system of five equations

$$\begin{cases} P_1 = 150000\,vz_3 + 750\,vz_3x - 599999\,x + 200 = 0, \\ P_2 = 625\,y_1^2 + 750000\,y_3 + 625\,y_3y_1 + 19200\,xy_1 \\ \qquad - 8\,xy_1^2 - 8\,xy_1y_3 - 900000000 = 0, \\ P_3 = -11520000\,x + 9600\,xy_1 + 8\,xy_1y_3 + 8\,xy_3^2 \\ \qquad + 1500000\,y_3 - 625\,y_3y_1 - 625\,y_3^2 = 0, \\ P_4 = 250\,z_1^2 + 75000\,z_3 + 250\,z_3z_1 + 1800\,y_3z_1 \\ \qquad - 3\,y_3z_1^2 - 3\,y_3z_1z_3 - 22500000 = 0, \\ P_5 = -270000\,y_3 + 900\,y_3z_1 + 3\,y_3z_1z_3 + 3\,y_3z_3^2 \\ \qquad + 150000\,z_3 - 250\,z_3z_1 - 250\,z_3^2 = 0. \end{cases} \tag{7}$$

Because of the background of the problem, the above system should satisfy the following constraints:

$$x \geq 0,\ y_1 \geq 0,\ y_3 \geq 0,\ z_1 \geq 0,\ z_3 \geq 0,$$
$$1200 - y_1 - y_3 \geq 0,\ 300 - z_1 - z_3 \geq 0.$$

Determining the steady states of (6) with the above-specified constant values is equivalent to finding the real solutions of the polynomial system (7) under these constraints.

Table 1: Steady states of the Mos/MEK/p42 MAPK cascade and their stability

labels	1–10	11, 12	13	14–16	17	18	19, 20	21
steady states	1	1	3	3	3	2	2	0
stable states	0	1	0	**2**	0	1	0	0

Our program computed a polynomial B of degree 93 in v (the feedback strength) with 93 terms and 41 distinct real roots. Let v_i $(i = 1, \ldots, 41)$ denote the 41 roots in ascending order, where $v_{21} = 0$. Because v is the feedback strength, we may assume that $v \geq 0$; thus only 21 open intervals $(v_{21}, v_{22}), \ldots, (v_{40}, v_{41}), (v_{41}, +\infty)$ need be considered. Our program has then isolated the real solutions of system (7) in these 21 intervals and returned the following results. In any of the first 12 open intervals, i.e., $(v_{21}, v_{22}), \ldots, (v_{32}, v_{33})$, system (7) has only one steady state. In each of the next five intervals, i.e., $(v_{33}, v_{34}), \ldots, (v_{37}, v_{38})$, the system has three steady states. The system has two steady states in $(v_{38}, v_{39}), (v_{39}, v_{40})$, or (v_{40}, v_{41}) and no steady states in the interval $(v_{41}, +\infty)$.

The characteristic polynomial of the Jacobian matrix of (6) is

$$H = -\lambda^5 + c_4\lambda^4 + c_3\lambda^3 + c_2\lambda^2 + c_1\lambda + c_0,$$

where the c_i are rational functions in v, x, y_1, y_3, z_1, z_3, whose numerators are very large polynomials. For instance, the numerator of c_1 is a polynomial of total degree 16 in v, x, y_1, y_3, z_1, z_3 with 4165 terms. Let $\Gamma_1, \ldots, \Gamma_5$ be the Hurwitz determinants of H. For each of the 21 intervals, we need to check the signs of the Γ_i at each of the steady states. Let the 21 intervals be labeled with 1 through 21 in ascending order. The numbers of (stable) steady states of system (7) determined by our program are shown in Table 1.

Moreover, when

$$v = v_{34} = \frac{14999911}{20025000} \approx 0.749 \quad \text{or} \quad v = v_{37} \approx 2.585,$$

where v_{37} is the unique positive root of

$$\begin{aligned} &4251528000000000000000\,v^3 + 12802676662170000000000\,v^2 \\ &- 2830964863426863817500 0\,v - 85804058978275037002757, \end{aligned}$$

system (7) has three steady states and none of them is stable. Thus, it is rigorously proved that system (7) exhibits bistability when $v_{34} < v < v_{37}$. This confirms the result for unitary feedback (i.e., $v = 1$) given in [1]. However, our result contradicts the conclusion in [1] that the system is bistable for any value of v between ≈ 0.7 and a *very large* real number. The upper bound of v we have obtained for the system to be bistable is only ≈ 2.585, which is not large. We hope that this upper bound can be confirmed by other theoretical and experimental studies.

7. CONCLUDING REMARKS

The general symbolic approach proposed in this paper has been applied to two well-known biological models, the Cdc2-cyclin B/Wee1 system and the Mos/MEK/p42 MAPK cascade. We have rigorously proved and generalized the result given in [1] for the Cdc2-cyclin B/Wee1 system and confirmed some of the results in [1] for the Mos/MEK/p42

MAPK cascade. Moreover, we have found an exact upper bound near 2.585 on the feedback gain v for the MAPK cascade to exhibit bistability that is much smaller than an upper bound which is unknown but was claimed to be very large in the recently published paper [1].

Our approach uses exact symbolic computation and thus ensures that all the results obtained are mathematically rigorous. However, it is limited to the case when the F_i on the right-hand side of (1) are rational functions and it may be inapplicable when the Jacobian matrix is singular or some of its eigenvalues have zero real parts but none of them has positive real part (a much more complicated case in differential equations). The computations involved in our method may also be expensive and some of the polynomials representing the exact real values in our results are very large and cannot be reproduced in the paper. The interested reader may request those polynomials and the isolating intervals of their real roots from the authors.

In this initial study, we have focused our attention mainly to the analysis of local stability for two concrete biological models. Our approach may be applied to many other models in biology and biochemistry. It may also be used and refined for the study of several relevant problems such as global stability and bifurcation of limit cycles. How to solve a large system of (parametric) polynomial equations and inequalities coming from biological networks, represent and classify their exact real solutions, and determine the sign of a rational expression evaluated at these solutions more efficiently, how to generalize our approach for other biological or non-biological systems, and how to develop a practical software tool to automate the process of stability analysis are some examples of questions for our future research. We believe that investigations on these questions will make our symbolic approach a promising and powerful tool for the qualitative study of such biological networks that may be modeled by systems of ordinary differential equations. It may become a good alternative to the experimental approach based on numerical simulation and visualization.

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