Computational methods for analyzing bistability in biochemical reaction networks

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***Abstract*—Bistability plays a key role in important biological processes, such as cell division, differentiation, and apoptosis. Examples show that there is a very delicate relationship between the structure of a reaction network and its capacity for bistable behavior. We describe mathematical methods that discriminate between networks that have the capacity for bistability and those that do not, as well as algorithms derived from these methods. We have implemented some of these algorithms in the software package BioNetX. We present results obtained by using this package to analyze random samples from a comprehensive database of reaction networks.**

I. Introduction

A chemical reaction network is usually given by a finite list of reactions that involve a finite set of chemical species. As an example, consider the reaction network with two species A_1 and A_2 schematically given in the diagram (1).

$$2A_1 \rightleftharpoons A_1 + A_2 \rightleftharpoons 2A_2 \tag{1}$$

To keep track of the temporal variation of the state of this chemical system, we define the functions $c_{A_1}(t)$ and $c_{A_2}(t)$ to be the molar concentrations of the species A_1, A_2 at time t. The chemical reactions in the network are responsible for changes in the concentrations; for instance, whenever the reaction $A_1 + A_2 \to 2A_1$ occurs, there is a net gain of a molecule of A_1, whereas one molecule of A_2 is lost. Similarly, the reaction $2A_2 \to 2A_1$ results in the the creation of two molecules of A_1 and the loss of two molecules of A_2.

We will assume that the rate of change of the concentration of each species is governed by *mass-action kinetics* [12], i.e., that each reaction takes place at a rate that is proportional to the product of the concentrations of the species being consumed in that reaction. For example, under the mass-action kinetics assumption, the contribution of the reaction $A_1 + A_2 \to 2A_1$ to the rate of change of c_{A_1} has the form $k_{A_1+A_2\to 2A_1} c_{A_1} c_{A_2}$, where $k_{A_1+A_2\to 2A_1}$ is a positive number called *reaction rate constant*. Collecting these contributions from all the reactions, we obtain the following system of differential equations:

$$\begin{aligned}
\dot{c}_{A_1} = \; & -k_{2A_1\to A_1+A_2} c_{A_1}^2 + k_{A_1+A_2\to 2A_1} c_{A_1} c_{A_2} + \\
& + k_{2A_2\to A_1+A_2} c_{A_2}^2 - 2k_{2A_1\to 2A_2} c_{A_1}^2 + 2k_{2A_2\to 2A_1} c_{A_2}^2 \\
\dot{c}_{A_2} = \; & k_{2A_1\to A_1+A_2} c_{A_1}^2 - k_{A_1+A_2\to 2A_1} c_{A_1} c_{A_2} - \\
& - k_{2A_2\to A_1+A_2} c_{A_2}^2 + 2k_{2A_1\to 2A_2} c_{A_1}^2 - 2k_{2A_2\to 2A_1} c_{A_2}^2
\end{aligned} \tag{2}$$

We now introduce the standard terminology of Chemical Reaction Network Theory (see [12], [5] for more details). We denote by $\mathbb{R}$ the set of real numbers, by $\mathbb{R}_+$ the set of strictly positive real numbers, and by $\bar{\mathbb{R}}_+$ the set of nonnegative real numbers. For an arbitrary finite set I we denote by $\mathbb{R}^I$, $\mathbb{R}^I_+$ and $\bar{\mathbb{R}}^I_+$ the sets of formal sums $\alpha = \sum_{i\in I} \alpha_i i$ with $\alpha_i \in \mathbb{R}$, $\alpha_i \in \mathbb{R}_+$ and $\alpha_i \in \bar{\mathbb{R}}_+$ respectively. The *support* of an element $\alpha \in \mathbb{R}^I$ is $supp(\alpha) = \{i \in I : \alpha_i \neq 0\}$.

A *chemical reaction network* is a triple $(\mathcal{S}, \mathcal{C}, \mathcal{R})$, where $\mathcal{S}$ is the set of chemical *species*, $\mathcal{C} \subseteq \bar{\mathbb{R}}^{\mathcal{S}}_+$ is the set of *complexes* (i.e., the objects on the left or right side of reaction arrows), and $\mathcal{R}$ is a relation on $\mathcal{C}$, denoted $y \to y'$ and represents the set of *reactions* in the network. Moreover, the set $\mathcal{R}$ must satisfy the following three conditions: (i) it cannot contain elements of the form $y \to y$; (ii) for any $y \in \mathcal{C}$ there exists some $y' \in \mathcal{C}$ such that either $y \to y'$ or $y' \to y$; (iii) the union of the supports of all $y \in \mathcal{C}$ is $\mathcal{S}$.

For the network (1), the set of species is $\mathcal{S} = \{A_1, A_2\}$, the set of complexes is $\mathcal{C} = \{2A_1, A_1 + A_2, 2A_2\}$ and the set of reactions is $\mathcal{R} = \{2A_1 \rightleftharpoons A_1 + A_2, 2A_2 \rightleftharpoons 2A_1, 2A_2 \to A_1 + A_2\}$, and consists of 5 reactions, represented as two reversible reactions and one irreversible reaction.

Note that we regard the complexes as non-negative linear combinations of the species; on the other hand, it will be useful to also think of the complexes as (column) vectors of dimension equal to the number of elements of $\mathcal{S}$, via an identification given by a fixed ordering of the species. For example, the complexes above are

$$2A_1 = \begin{bmatrix} 2 \\ 0 \end{bmatrix}, A_1 + A_2 = \begin{bmatrix} 1 \\ 1 \end{bmatrix}, \text{ and } 2A_2 = \begin{bmatrix} 0 \\ 2 \end{bmatrix}.$$

This slight abuse of notation provides a convenient way of representing the *reaction vectors* $y' - y$ for all $y \to y' \in \mathcal{R}$.

We may now define mass-action systems and the set of differential equations that governs mass-action kinetics. First we introduce a useful notation. Given two vectors $u = \sum_{s\in\mathcal{S}} u_s s$ and $v = \sum_{s\in\mathcal{S}} v_s s$ in $\bar{\mathbb{R}}^{\mathcal{S}}_+$, we denote $u^v = \prod_{s\in\mathcal{S}} (u_s)^{v_s}$, with the convention $0^0 = 1$.

A *mass-action system* is a quadruple $(\mathcal{S}, \mathcal{C}, \mathcal{R}, k)$, where $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ is a chemical reaction network and $k \in \mathbb{R}^{\mathcal{R}}_+$, where $k_{y\to y'}$ is the *reaction rate constant* of the reaction $y \to y' \in \mathcal{R}$. The *system of differential equations* for the mass-action

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system $(\mathcal{S},\mathcal{C},\mathcal{R},k)$ is

$$\dot{c}(t) = \sum_{y\to y'} k_{y\to y'} c(t)^y (y'-y) \tag{3}$$

where $c \in \mathbb{R}^{\mathcal{S}}$ is the nonnegative vector of species concentrations. For example, the ODE system (2) may be written using the vector-based formula (3), as shown in equation (4) below.

$$\begin{aligned}\begin{bmatrix}\dot{c}_{A_1}\\ \dot{c}_{A_2}\end{bmatrix} &= k_{2A_1\to A_1+A_2} c^{2A_1}\begin{bmatrix}-1\\1\end{bmatrix} \\ &+k_{A_1+A_2\to 2A_1} c^{A_1+A_2}\begin{bmatrix}1\\-1\end{bmatrix} + k_{2A_2\to A_1+A_2} c^{2A_2}\begin{bmatrix}1\\-1\end{bmatrix} \\ &+k_{2A_1\to 2A_2} c^{2A_1}\begin{bmatrix}-2\\2\end{bmatrix} + k_{2A_2\to 2A_1} c^{2A_2}\begin{bmatrix}2\\-2\end{bmatrix}.\end{aligned} \tag{4}$$

The special structure of the differential equation system (3) constrains its solution to an affine subspace of $\mathbb{R}^{\mathcal{S}}$. Indeed, let S denote the *stoichiometric subspace* of the chemical reaction network $(\mathcal{S},\mathcal{C},\mathcal{R})$, i.e., the span of its reaction vectors, $S = span\{y'-y \mid y\to y' \in \mathcal{R}\}$. For any initial condition c^0, integrating (3) yields

$$c(t) = c^0 + \sum_{y\to y'}\left(\int_0^t \kappa_{y\to y'} c(s)^y ds\right)(y'-y),$$

and therefore $c(t) \in c^0 + S$ for all times t. The invariant set $(c^0+S)\cap\mathbb{R}^{\mathcal{S}}_+$ is called the *stoichiometric compatibility class* for initial condition c_0.

If we view a reaction network $(\mathcal{S},\mathcal{C},\mathcal{R})$ as a directed graph Γ with vertices given by the complexes in $\mathcal{C}$ and edges given by the reactions in $\mathcal{R}$, then we say it is *weakly reversible* if this directed graph Γ has strongly connected components [12].

II. The Jacobian Method

Let $(\mathcal{S},\mathcal{C},\mathcal{R},k)$ be a mass-action system where all the species have corresponding outflow reactions, i.e., there is an "outflow" or "degradation" reaction $s\to 0$ for all $s\in\mathcal{S}$. The Jacobian Method ([5]) gives a necessary condition for existence of multiple positive equilibria for the system of differential equations (3).

We say that a biochemical reaction network $(\mathcal{S},\mathcal{C},\mathcal{R})$ *does not have the capacity for bistability* if for any choice of reaction rate constants $k\in\mathbb{R}^{\mathcal{R}}_+$ the corresponding mass-action system $(\mathcal{S},\mathcal{C},\mathcal{R},k)$ gives rise to differential equations (3) that have at most one positive equilibrium.

Let $p(c,k)$ be the negative vector of polynomials on the right hand side of (3), or more precisely,

$$p(c,k) = -\sum_{y\to y'} k_{y\to y'} c(t)^y (y'-y).$$

Then $(\mathcal{S},\mathcal{C},\mathcal{R})$ *does have the capacity for bistability* iff for some positive vector $k\in\mathbb{R}^{\mathcal{R}}_+$ of the reaction rate constants, there exist two distinct vectors of concentration values c and $\tilde{c}$ such that $p(c,k)=p(\tilde{c},k)=0$. Therefore, if $p(\cdot,k)$ *is* injective for all positive choices of the reaction rate constants vector k, then $(\mathcal{S},\mathcal{C},\mathcal{R})$ does *not* have the capacity for bistability. The Jacobian Method investigates the injectivity of the polynomial function $p(\cdot,k)$ and relates it to the positivity of coefficients in the expansion of the determinant of the Jacobian of $p(c,k)$, regarded as a function of c, and with fixed but unspecified parameter vector k. The main result is the following theorem ([5, Theorem 3.3]):

Theorem II.1. *Let $(\mathcal{S},\mathcal{C},\mathcal{R})$ be a reaction network that contains outflow or degradations reactions for all species. If all the coefficients in the expansion of*

$$\det\left(\frac{\partial p}{\partial c}(c,k)\right) \tag{5}$$

are positive, then $(\mathcal{S},\mathcal{C},\mathcal{R})$ does not have the capacity for bistability.

Example. Consider the reaction network

$$X \rightleftharpoons A+C \to B \to A+Y \tag{6}$$

augmented to include the outflow reactions $A\to 0, B\to 0, C\to 0, X\to 0$ and $Y\to 0$ (and may also include some inflow reactions $0\to A, 0\to B$, etc). The expansion of the determinant of the Jacobian (5) for the corresponding mass-action system is $k_2k_4k_9k_7k_8c_C + k_2k_6k_9k_7k_8c_C + k_3k_6k_9k_7k_1c_C + k_4k_5k_9k_2k_8c_A + k_4k_5k_9k_3k_1c_A + k_4k_5k_9k_7k_1 + k_5k_6k_9k_2k_8c_A + k_5k_6k_9k_3k_1c_A + k_5k_6k_9k_7k_1 + k_3k_6k_9k_7k_8c_C + k_4k_5k_9k_3k_8c_A + k_4k_5k_9k_7k_8 + k_5k_6k_9k_3k_8c_A + k_5k_6k_9k_7k_8$, where we denoted $k_1 = k_{X\to A+C},\ k_2 = k_{A+c\to X},\ k_3 = k_{A+C\to B},\ k_4 = k_{B\to A+Y},\ k_5 = k_{A\to 0},\ k_6 = k_{B\to 0},\ k_7 = k_{C\to 0},\ k_8 = k_{X\to 0},\ k_9 = k_{Y\to 0}$. All the coefficients of this multivariate polynomial happen to be positive (actually equal to 1). Therefore Theorem II.1 applies, and the mass-action system (6) does not have the capacity for bistability.

Note that we required that $\mathcal{R}$ contains the outflow reactions $s\to 0$ for all species $s\in\mathcal{S}$. This guarantees that the stoichiometric compatibility class of any positive initial condition is the whole positive orthant, $\mathbb{R}^{\mathcal{S}}_+$. This is not necessarily the case if some species are not in the outflow (since there might be conserved quantities, e.g., mass conservation).

In that case, the formulation of the bistability question changes significantly. The relevant question becomes: can there be bistability *within the same stoichiometric compatibility class*? It turns out that, given a reaction network $(\mathcal{S},\mathcal{C},\mathcal{R})$ which may have some "entrapped" species (i.e., species that are not in the outflow and do not decay) it may still be possible to extract information about its capacity for bistability by applying the Jacobian Method to the augmented network $(\mathcal{S},\tilde{\mathcal{C}},\tilde{\mathcal{R}})$ obtained from $(\mathcal{S},\mathcal{C},\mathcal{R})$ by adding all the (missing) outflow reactions. Indeed, according to [7], even if some or all species are not in the outflow, the same Jacobian Method described above (applied for the augmented network $(\mathcal{S},\tilde{\mathcal{C}},\tilde{\mathcal{R}})$) still implies that $(\mathcal{S},\mathcal{C},\mathcal{R})$ does not have the capacity for multiple *nondegenerate* equilibria within any stoichiometric compatibility class. Moreover, under some mild additional assumptions, the nondegeneracy restriction may be removed completely [9]. For example, if $(\mathcal{S},\mathcal{C},\mathcal{R})$ is weakly reversible, then the Jacobian Method can be applied to rule out the capacity for bistability within any stoichiometric compatibility class, even if $\mathcal{R}$ contains only some or none of the outflow reactions ([9]). It is actually enough to check that a

properly chosen projected subnetwork is weakly reversible ([9, Theorem 8.2]) or at least is "normal" (see [9] for definitions and examples).

III. The Determinant Optimization Method

If some of the coefficients in the expansion of the determinant (5) are negative, then the Jacobian Method is inconclusive. In that case one can use the Determinant Optimization Method ([5, Theorem 4.1]):

Theorem III.1. *Consider some reaction network $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ (augmented to include the set of inflow and outflow reactions for all species). For $\eta \in \mathbb{R}^{\mathcal{R}}_+$ let $T_\eta : \mathbb{R}^{\mathcal{S}} \to \mathbb{R}^{\mathcal{S}}$ be defined by $T_\eta(\delta) = \sum_{y \to y' \in \mathcal{R}} \eta_{y \to y'}(y \cdot \delta)(y - y')$, and let $f(\eta) = \det(T_\eta)$. Suppose that for some $\eta^* \in \mathbb{R}^{\mathcal{R}}_+$ we have*

$$f(\eta^*) \quad < \quad 0, \tag{7}$$

$$\sum_{y \to y' \in \mathcal{R}} \eta^*_{y \to y'}(y - y') \quad \in \quad \mathbb{R}^{\mathcal{S}}_+. \tag{8}$$

Then $\mathcal{N}$ does have the capacity for bistability.

Remark. Note that if some vector $\eta^* \in \mathbb{R}^{\mathcal{R}}_+$ satisfies (7) and (8), then $\lambda\eta^*$ also satisfies (7) and (8) for any positive number λ. Therefore, if there is some η^* that satisfies (7) and (8) and has all coordinates positive, then there is some η^{**} that satisfies (7) and (8) and has all coordinates positive *and of total sum* 1. Then the criterion in Theorem III.1 can be implemented by solving the polynomial optimization problem (9)–(12), with linear constraints on a compact domain:

$$\text{minimize} f(\eta) \tag{9}$$

subject to the constraints

$$\eta_{y \to y'} \geq \varepsilon \quad \forall y \to y' \in \mathcal{R}, \tag{10}$$

$$\sum_{y \to y' \in \mathcal{R}} \eta_{y \to y'} = 1, \tag{11}$$

$$\sum_{y \to y' \in \mathcal{R}} \eta_{y \to y'}(y_s - y'_s) \geq \varepsilon, \quad \forall s \in \mathcal{S}, \tag{12}$$

where ε is some very small positive number. Note that, from the point of view of applying Theorem III.1, it is enough to find *some* vector η^* satisfying (10)–(12) and such that $f(\eta^*) < 0$ (i.e., we don't need to find the global minimum, as we are just interested in knowing if the minimum is negative).

An alternative to solving this nonlinear optimization problem is given by the following linear optimization criterion ([5, Theorem 4.2]):

Theorem III.2. *Consider some reaction network $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ (augmented to include the inflow and outflow reactions). Suppose that there is a set of n reactions $\{y_1 \to y'_1, \ldots, y_n \to y'_n\}$ (where n is the number of species) such that*

$$\det(y_1, \ldots, y_n) \det(y_1 - y'_1, \ldots, y_n - y'_n) < 0, \tag{13}$$

and there exist positive numbers $\eta_1, \ldots, \eta_n$ such that

$$\sum_{i=1}^{n} \eta_i(y_i - y'_i) \in \mathbb{R}^{\mathcal{S}}_+. \tag{14}$$

		Number of reactions						
		1	**2**	**3**	**4**	**5**	**6**	**7**
Number of species	**2**	83.3%	58.3%	34.5%	18.8%	8.3%	3.6%	1%
	3	100%	71%	47.2%	28.6%	14.6%	7.7%	
	4	100%	85%	60.5%	38.6%	21.1%		
	5		95.6%	70.7%	48.6%			
	6		100%	82.8%	56.8%			
	7		100%	91.6%	67.3%			
	8			97%	78.6%			
	9			100%	88%			
	10			100%				

Fig. 1. Percentages of reaction networks enumerated in [11], which were found to *not* have capacity for bistability by using the Jacobian Method, even if we augment the network with inflow and outflow reactions for *all* species.

Then $\mathcal{N}$ does have the capacity for bistability.

Note that there is a direct connection between the inequality (13) and the Jacobian Method computation: each number on the left-hand side of (13) must equal the coefficient of a monomial in the expansion of the determinant (5) (see [5] for more details). Therefore, a simple way to check if this inequality can be satisfied is to compute the determinant (5).

Example. If we apply the Determinant Optimization Method to the network

$$S + E \rightleftharpoons SE \rightleftharpoons S + P, \quad 2S \rightleftharpoons P + R, \tag{15}$$

we notice that there are several negative monomials. Each such monomial corresponds to a set of $n = 5$ reactions that satisfy (13). The linear inequalities (14) are satisfied for one of these sets, and we conclude that the network (15), augmented with inflow and outflow reactions for all species, does have the capacity for bistability.

The problem of counting the number of negative monomials in these determinant expansions is discussed in detail in [16], [17]. Related bistability criteria based on degree theory are described in [10]. These and other related criteria have been implemented by J. W. Helton and collaborators and are available at `http://www.math.ucsd.edu/~chemcomp/`.

IV. Results

We have implemented the Jacobian Method and the Determinant Optimization Method in the software package BioNetX [19]. We discuss the results obtained by running this code on a random sample of networks from the comprehensive uni- and bi-molecular reaction network databases described in [11]. Empty spaces in the tables in Figures 1 and 2 correspond to cases where either there are no such networks, or the number of such networks is too large to be able to enumerate all of them (see [11] for details). Note that by adding the corresponding percentages in Figures 1 and 2 we can also compute the percentages of reaction networks enumerated in [11] for which neither the Jacobian Method nor the Determinant Optimization Method is conclusive. For example, this percentage is low (3.6%) for the case of 5 species and 2 reactions, but high (33%) for the case of 2 species and 5 reactions. In general, the percentage of reaction networks

Number of species	Number of reactions 1	2	3	4	5	6	7
2	0%	8.4%	24.6%	40.8%	58.9%	71.4%	83.1%
3	0%	4.7%	17.6%	36.9%	54.6%	64.5%	
4	0%	1.9%	13.3%	30.3%	49.1%		
5		0.8%	8.2%	22.8%			
6		0%	4.9%	17%			
7		0%	1.7%	11.7%			
8			.9%	6.9%			
9			0%	3.7%			
10			0%				

Fig. 2. Percentages of reaction networks enumerated in [11], which were found to *have* capacity for bistability by using the Determinant Optimization Method, assuming that *all* species are in the inflow and in the outflow.

for which neither one of these two tests in conclusive is low for "sparse" networks (i.e, networks that have a low ratio of reactions to species), and could be high (20-40%) for "dense" networks (i.e, for networks that have a high ratio of reactions to species). On the other hand, it turns out that a high percentage of the "dense" networks do have capacity for bistability (see Figure 2).

V. Concluding Remarks

We have described some mathematical and computational methods for discriminating biochemical reaction networks that have the capacity for bistability from others that do not. Our main focus was on two such methods, called the Jacobian Method (which can be used to rule out capacity for bistability for some networks) and the Determinant Optimization Method (which can be used to show that some networks do have capacity for bistability) [5]. We are making available a software package that implements both methods [19]. By running this software on large reaction network databases we conclude that the combination of both methods is a powerful tool, and is especially effective in *ruling out* the capacity for bistability for sparse networks. On the other hand, for dense networks we show that a very large percentage of networks *does* have capacity for bistability, if we assume that all species are in the inflow and in the outflow.

Other methods for analyzing bistability (which we have not described here) are based on graph-theoretical, algebraic, or topological tools. For example, a very powerful method is based on *deficiency theory* [12], [13], [14], [18], and is implemented in the Chemical Reaction Network Toolbox [15]. Related results have also been obtained recently for stochastic models [1].

In other recent work, graph-theoretical methods have been developed based on the Jacobian Method [6], [8], or its generalizations [4], [2], [3]. These generalizations apply not only for mass-action systems but also for more general chemical kinetics such as Michaelis-Menten or Hill laws.

Other generalizations of the Jacobian Method have been described in [10], [16], [17]. For example, the *core determinant method* [16] has very interesting connections with the Jacobian Method. This method is applicable to systems that are not necessary mass-action; on the other hand, for mass-action systems the Jacobian Method may be conclusive in cases where the core determinant method is not (network (6) is one such example).

In future work we intend to analyze in more detail the relationships between some of these methods, and to extend our software package BioNetX [19] to include additional algorithms, e.g., ones derived from the graph-theoretic criteria described in [3].

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