INVITED PAPER

Multigraph Conditions for Multistability, Oscillations and Pattern Formation in Biochemical Reaction Networks

Mathematical studies of network properties are being used to model, and predict the behavior, of biological processes such as cell replication and death.

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ABSTRACT | We represent interactions among biochemical species using a directed multigraph, which is a generalization of a more commonly used digraph. We show that network properties that are known to lead to multistability or oscillations, such as the existence of a positive feedback cycle, can be generalized to "critical subnetworks" that can contain several cycles. We also derive corresponding graph-theoretic conditions for pattern formation for the respective reaction-diffusion models. We present as an example a model for cell cycle and apoptosis along with bifurcation diagrams and sample solutions that confirm the predictions obtained with the help of the multigraph network conditions.

KEYWORDS | Biochemical reaction networks; multigraph; multistability; oscillations; pattern formation; positive feedback

I. INTRODUCTION

One of the main topics in systems biology is the connection between network motifs and their corresponding biological functions [1]. Therefore methods that can be used to identify functional motifs are particularly important [1], [2]. Some well known network motifs, such as feedback cycles, are associated with interesting dynamic behavior, such as oscillations or multistability [3]–[8]. In this article we present network conditions for multistability, oscillations and pattern formation that are more general and include the positive (feedback) cycle condition as a special case [6]; the precise definition of positive and negative cycles is given in Section IV.

Biochemical oscillations are common phenomena in living organisms, from the cell cycle [9] to daily periodic changes in protein levels associated with circadian rhythms [10]. Some common biochemical oscillators, such as calcium oscillations [11] and glycolytic oscillations [12] are described in [13].

Another phenomenon that occurs often in models of biological processes is multistability, i.e., the existence of several stable equilibria. Often, multistability is associated with models of biological switches, since in response to some stimulus the system can switch between several steady states. Multistability plays an important role in many biological systems, such as MAPK cascades [14], the *lac* operon [15], and cell differentiation mechanisms [16]. For a review of network conditions for multistability in various types of mathematical models see [17].

In this study we consider mathematical models based on systems of differential equations. Oscillations or multistability are usually the result of changes in the behavior of equilibrium solutions of a dynamical system, associated with the loss of stability under small perturbation. Therefore, we will refer to multistability and oscillations as *instabilities*, whenever we describe a feature of both.

Many biochemical oscillator models contain an autocatalytic loop, which occurs when a biochemical species

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activates its own production [13], [18]. Similarly, many biochemical switch models contain a single positive feedback cycle [8], [19]. For three dimensional and higher dimensional models of biochemical oscillators and switches [9], [14], [20] a more general network condition for instability, referred to as *indirect catalysis*, was derived in [6]. Indirect catalysis corresponds to a positive feedback cycle of which autocatalysis is a particular case. However, many complex biochemical reaction networks involve a large number of species (genes, proteins, metabolites, signaling molecules) and a large number of reactions forming many different cycles [21]. Thus, the intuitive analysis of which cycle or cycles is the cause for instability becomes difficult, and a generalization of the indirect catalysis rule is needed. Recent work on biochemical reaction models with mass action kinetics [22] describes network conditions for instabilities that are more general, showing that instabilities can be the result of a subnetwork of non-intersecting cycles rather than a single cycle [23]–[25]. In what follows we will describe some of these methods and their generalizations to other types of kinetics, such as Michaelis-Menten or Hill type kinetics. Other general methods for studying chemical dynamics are the Deficiency Theory of Feinberg, Horn, and Jackson [26] and the Monotone Systems approach of Angeli, De Leenheer, and Sontag [7].

The interactions among the different species in a biochemical reaction network can be represented formally as a bipartite graph. In [23], [24] the bipartite *SR graph* (species-reactions graph) is used, and similarly a bipartite graph is used in [27] to represent a biochemical reaction network. These graphs have two types of vertices, species vertices and reaction vertices, and each edge connects a species vertex to a reaction vertex. SR graph criteria apply for mass action kinetics, and can be used if detailed stoichiometric information is available [23]–[25].

However, detailed stoichiometry of biochemical reaction networks is not always known, which makes it necessary to use non-mass-action kinetics. In this article we describe network conditions for models with non-mass-action kinetics, such as Michaelis-Menten or Hill type kinetics [13], [29]. Our network representation is a *directed multigraph* [30] derived from the Jacobian matrix associated with the ordinary differential equations model. The vertices of the multigraph are the species involved in the reactions, and the directed edges represent interactions among species. A positive or negative sign is associated with each edge, representing activation or inhibition, respectively. In the simplest cases, between any two species there is at most one directed edge in each direction, resulting in a digraph (directed graph) representation [30]. However, in many cases a species can influence the production or inhibition of another species in two or more ways, and at different rates (see [14], [31]). Therefore, it becomes convenient to use a directed multigraph, where there can be more than one directed edge in the same direction between any two vertices. This directed multigraph is called the species multigraph or *S-multigraph* of the reaction network.

We present graph-theoretic conditions for instabilities, which generalize the positive cycle condition; they require the existence of a special subgraph of the S-multigraph which has an odd number of positive cycles. Since these graph-theoretic conditions are only necessary, sufficient conditions for instability may depend also on the precise values of the parameters present in the model.

In this article we discuss oscillations due to positive feedback and its generalizations. There are also oscillations due to negative feedback [10]; in that case, a similar analysis is more difficult and we leave it for future study.

Turing instability or diffusion-driven instability is associated with models for the formation of some biological structure or *pattern* [18], [32]. In the most well known cases pattern formation is the result of a competition between an activator and an inhibitor that diffuse at different rates [18]. Systems that exhibit oscillations from autocatalysis often also generate Turing instability. For example, famous two-dimensional systems called the *Brusselator* [33], and the *Belousov-Zhabotinsky reactions* [34], [35] generate Turing instability by autocatalysis.

In a recent article we reviewed graph-theoretic conditions for Turing instabilities for n-dimensional mass action reaction-diffusion systems [36], first derived in [27]. Here we present similar graph-theoretic conditions for Turing instability in terms of the S-multigraph, that apply without the assumption of mass action kinetics.

In Section II we review the usual mathematical formulation of biochemical dynamics models. In Section III we review some algebraic requirements for multistability and oscillations. Then, in Section IV we introduce the S-multigraph and the graph-theoretic conditions for multistability and oscillations. Finally, in Section V we present graph-theoretic conditions for pattern formation.

II. MATHEMATICAL MODELS

We assume that for each reaction in a biochemical network there exists a rate function, which may include kinetic parameters. The rate function is usually mass-action, *Michaelis-Menten* or *Hill* type and is a monotone function of its variables [13], [29]. For example, the usual Michaelis-Menten rate function derived from steady-state approximation is

$$v(u) = V_{max}\frac{u}{K_M + u} \tag{1}$$

where u is the concentration of a substrate and V_{max}, K_M are positive kinetic parameters. Since $v'(u) > 0$ the substrate is considered to be an activator. Another type

of Michaelis-Menten rate function is given by the mixed inhibition and activation function

$$v_1(u,y) = \frac{V_{max}u}{K_m(1+y/K_f)+u} \tag{2}$$

where u is the concentration of the activator and y is the concentration of the inhibitor and V_{max}, K_M, K_f are positive kinetic parameters. Similarly, an example of Hill type kinetics function is

$$g(u) = \frac{k_1}{k_2+u^h} \tag{3}$$

where $h>0$ is the Hill coefficient and u is the concentration of a repressor, since $g'(u)<0$. An example of activating Hill type kinetic function is

$$g_1(u) = \frac{k_1u^h}{k_2+u^h}. \tag{4}$$

The kinetic parameters k_i, $i=1,2$ involved in the rate functions (3), (4) are positive. For more on Michaelis-Menten and Hill type functions and their derivation from steady-state approximations see [13], [29].

The rate of change in the concentration of any species depends on the rates of the reactions that produce and consume it. We assume that the chemical species are $A_1, A_2, , \ldots, A_n$, and that there are m reactions among them, which are not necessarily elementary reactions. Let the concentration of A_k be denoted by u_k, $k=1,\ldots,n$, and let $\mathbf{u}=(u_1,\ldots,u_n)$ be the vector of all concentrations.[1] The associated ordinary differential equation system is

$$\frac{du_k}{dt} = \sum_{i=1}^{m} \gamma_{ki} w_i(\mathbf{u}), \quad k=1,\ldots,n \tag{5}$$

where w_i is the reaction rate of the i^{th} reaction, and γ_{ki} are usually small integers. The reaction rates w_i are usually given by mass-action, Michaelis-Menten or Hill type kinetics. Let $\mathbf{p}$ be the vector of kinetic parameters contained in the rates $w_i(\mathbf{u})$ for all i. An equilibrium solution $\mathbf{u}^*$ to (5) depends on the parameter values $\mathbf{u}^*=\mathbf{u}^*(\mathbf{p})$. We will be interested in positive equilibria, i.e., equilibria for which all $u_i^*>0$.

Example 2.1: Apoptosis, one of the main types of programmed cell death, is a biological process that plays an important role in the development and growth of cells and tissues. Improper function of the apoptosis mechanism has been connected to various diseases: excessive apoptosis causes atrophy, and insufficient apoptosis may result in uncontrolled cell proliferation, such as in cancer tumors. In [37], [38] an implementation of a simple modular model of interaction between cell cycle and apoptosis pathways is studied in detail.

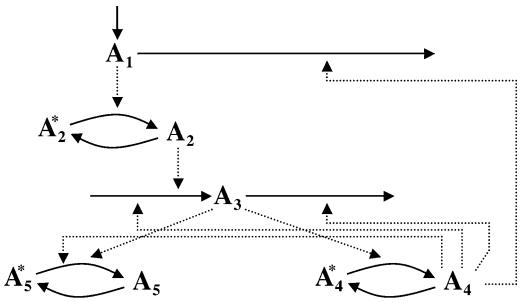

Fig. 1. *Cell cycle and apoptosis network studied in [37], [38]. Thick arrows denote mass transfer, and dotted arrows denote an influence of a species on a reaction, without loss of mass for that species.*

The system studied in [38] is shown in Fig. 1. Here A_1 is a signaling molecule, A_2 is an active signaling protein, A_3 is a control node of transcription factors, A_4 is an active cell cycle marker (for initiation of S-phase) and A_5 is an active apoptosis marker; also, A_i^* is an inactive form of A_i, and we assume that the total concentration of active and inactive A_i is 1. The following differential equation model is obtained:

$$\frac{du_1}{dt} = k_1 - k_2u_1 - k_3u_4u_1 \tag{6}$$

$$\frac{du_2}{dt} = \frac{k_4u_1(1-u_2)}{K_1+(1-u_2)} - \frac{k_5u_2}{K_2+u_2} \tag{7}$$

$$\frac{du_3}{dt} = k_6u_2 + k_7u_4 - k_8u_3 - k_9u_4u_3 \tag{8}$$

$$\frac{du_4}{dt} = \frac{k_{10}u_3(1-u_4)}{K_3+(1-u_4)} - \frac{k_{11}u_4}{K_4+u_4} \tag{9}$$

$$\frac{du_5}{dt} = \frac{k_{12}u_3(1-u_5)}{K_5+(1-u_5)} + \frac{k_{13}u_4(1-u_5)}{K_6+(1-u_5)} - \frac{k_{14}u_5}{K_7+u_5} \tag{10}$$

The chemical interactions in this model are either of Michaelis-Menten or mass-action type. In Section IV we will show that this model can give rise to oscillations which originate from a positive feedback cycle. We will also see that a simplified version of the same model can give rise to multistability, originating from a set of feedback cycles, one of them positive as well.

III. ALGEBRAIC CONDITIONS FOR INSTABILITIES

In this section we describe necessary conditions for instabilities, associated with the Jacobian of the right-hand side

[1]We denote vectors by bold letters.

of (5). In the following section these algebraic conditions for instabilities are translated into graph-theoretic conditions, associated with the S-multigraph.

The Jacobian matrix $J = (J_{kl})$ of the right-hand side of (5) plays an important role in determining algebraic conditions for instabilities. We write the Jacobian matrix in a convenient form as

$$J_{kl}(\mathbf{p}) = \sum_{i=1}^{m} \gamma_{ki} \frac{\partial w_i(\mathbf{u})}{\partial u_l} \tag{11}$$

where it is assumed that (11) is evaluated at some positive equilibrium $\mathbf{u} = \mathbf{u}^*(\mathbf{p})$ of (5). Note that finding a positive equilibrium of (5) symbolically is difficult, even for relatively small systems, due to the nonlinearities and the many unknown parameters; therefore, we will work with the formal expressions (11).

Rather than finding the eigenvalues of J, it is easier to analyze the coefficients of the characteristic polynomial of J:

$$p(\lambda) = \det(\lambda I - J) = \lambda^n + a_1\lambda^{n-1} + \ldots + a_n. \tag{12}$$

This will provide information on the types of eigenvalues and the signs of their real parts. It is important to note that each coefficient $a_k = a_k(\mathbf{p})$ is the sum of all principal minors of order k of the matrix $-J$ (see [39]). Also, each $a_k = a_k(\mathbf{p})$ is a continuous function of $\mathbf{p}$.

Usually multistability and oscillations occur as a result of saddle-node and Hopf bifurcation, respectively. These are the only two types of bifurcations which occur in generic one-parameter families of dynamical systems [40]. For the rest of this paper we will focus on instabilities that originate from these two types of bifurcations. A necessary condition for a saddle-node bifurcation is a zero root of (12), and for Hopf bifurcation is a pair of pure imaginary roots of (12), see [40].

The polynomial (2) has a zero root if and only if

$$a_n(\mathbf{p}) = \det(-J(\mathbf{p})) = 0. \tag{13}$$

Using algebraic geometry methods, a necessary and sufficient condition for the existence of a pair of pure imaginary roots is described in [40]. However, this condition leads to very complicated computations, and seems difficult to relate to the network structure. Instead, we use Orlando's formula [39] for the Hurwitz determinant

$$H_{n-1} = \prod_{1 \le i < k \le n} (-1)^{\frac{n(n-1)}{2}} (\lambda_i + \lambda_k) \tag{14}$$

where $\lambda_i = \lambda_i(\mathbf{p})$, $i = 1, \ldots, n$ are the roots of (12). Therefore by (14) a necessary condition for the existence of a pair of pure imaginary roots is $H_{n-1}(\mathbf{p}) = 0$ for some parameter value $\mathbf{p}$. If a parameter value $\mathbf{p}$ has the property that all eigenvalues of $J(\mathbf{p})$ have non-positive real parts then the Hurwitz determinant H_{n-1} satisfies Hadamard's inequality [41]:

$$0 \le H_{n-1}(\mathbf{p}) \le a_1(\mathbf{p}) \ldots a_{n-1}(\mathbf{p}).$$

It follows that, if $a_k(\mathbf{p}) = 0$ for some $k < n$ and for such $\mathbf{p} = \mathbf{p}_0$, then there exists some parameter value $\mathbf{p} = \mathbf{p}_1$ such that $H_{n-1}(\mathbf{p}_1) = 0$. Therefore we use the simpler condition $a_k(\mathbf{p}) = 0$, $k < n$ for some $\mathbf{p} = \mathbf{p}_0$ as evidence for possible Hopf bifurcation. For a more detailed discussion of the relationship between the condition $a_k(\mathbf{p}) = 0$, $k < n$ and $H_{n-1}(\mathbf{p}) = 0$ see [42].

If the system (5) satisfies some conservation relations, then the rank of J equals $n - r$, for some $r > 0$. In that case $a_{n-r+1}(\mathbf{p}) \equiv 0, \ldots, a_n(\mathbf{p}) \equiv 0$. Then, a necessary condition for saddle-node bifurcation is $a_{n-r}(\mathbf{p}) = 0$, and a necessary condition for Hopf bifurcation is $H_{n-r-1}(\mathbf{p}) = 0$.

IV. MULTIGRAPH, DETERMINANT AND GRAPH-THEORETIC CONDITIONS FOR INSTABILITIES

In this section we explain how the algebraic conditions for instabilities discussed in the previous section can be interpreted in graph-theoretic terms. Given a reaction network with Jacobian matrix J as in (11) we associate to it the S-multigraph $D(J)$.

In order to define $D(J)$ we will first define the digraph $D(M)$ of an $n \times n$ matrix M [45]. The vertex set of $D(M)$ is $V = \{1, \ldots, n\}$ and there is a directed edge from l to k if and only if $M_{kl} \neq 0$. A *weight* M_{kl} is assigned to each directed edge from l to k. Also, instead of the weight M_{kl}, only its *sign* may be assigned to the corresponding directed edge.

However, in general an entry J_{kl} of the Jacobian J is a sum of m terms (some of which could be zero), each corresponding to some reaction rate. Since the sum can have undetermined sign we represent each non-zero summand by a distinct directed edge. Then the digraph should be replaced by a multigraph [30], because there can be up to m directed edges between any two vertices. More precisely, if

$$J_{kl}(\mathbf{p}) = \sum_{j=1}^{m} \gamma_{kj} \frac{\partial w_j}{\partial u_l} \tag{15}$$

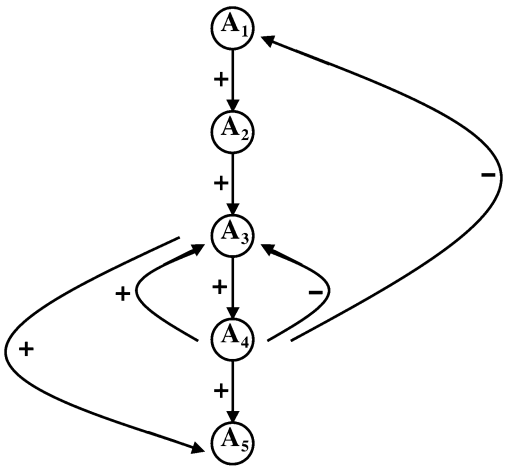

Fig. 2. *The S-multigraph of the reaction network in Example 2.1.*

then for each non-zero summand

$$F_{lk}^{(j)}(\mathbf{p}) = \gamma_{kj}\frac{\partial w_j}{\partial u_l}, \quad j = 1, \ldots, m \tag{16}$$

we draw a directed edge from l to k; also, we assign the sign of $F_{lk}^{(j)}(\mathbf{p})$ to it. For reasons that will become clear later, we do not draw any negative weight edges from a vertex to itself.

For example, Fig. 2 shows the S-multigraph associated to the reaction network in Example 2.1; note that there are two different edges from the vertex A_4 to vertex A_3. This occurs because

$$J_{34}(\mathbf{p}) = \frac{\partial}{\partial u_4}(k_6u_2 + k_7u_4 - k_8u_3 - k_9u_4u_3) = k_7 - k_9u_3$$

then, the positive edge from A_4 to A_3 is due to the term $F_{43}^{(7)}(\mathbf{p}) = k_7$, and the negative edge from A_4 to A_3 is due to the term $F_{43}^{(9)}(\mathbf{p}) = -k_9u_3$.

Next we introduce several definitions from graph theory that will be useful in the following discussion [30]. A *walk* $(i_1, e_{j_1}, i_2, e_{j_2}, i_3 \ldots, i_{k-1}, e_{j_{k-1}}, i_k)$ in a directed (multi)graph D is an alternating sequence of vertices i_n and directed edges e_{j_n}, such that $e_{j_n} = (i_n, i_{n+1})$. A walk $(i_1, e_{j_1}, i_2, e_{j_2}, i_3 \ldots, i_{k-1}, e_{j_{k-1}}, i_k)$ with distinct vertices $i_1, i_2, \ldots, i_k$ is called a *path* of D. If $e_{j_k} = (i_k, i_1)$ is also a directed edge then the path $c = (i_1, e_{j_1}, i_2, e_{j_2}, i_3, \ldots, i_{k-1}, e_{j_{k-1}}, i_k, i_1)$ is called a *cycle* of D. Note that a *loop* from k to k, i.e., $c = (k, e, k)$ is also a cycle. If D is a multigraph, it is possible to have more than one cycle through the same vertices, since there can be more than one directed edge between any two vertices.

For the multigraph $D(J)$, if $c = (i_1, e_{j_1}, i_2, e_{j_2}, i_3, \ldots, i_{k-1}, e_{j_{k-1}}, i_k, e_{j_k}, i_1)$ is a cycle in $D(J)$ let

$$J[c] = F_{i_1i_2}^{(j_1)}(\mathbf{p})F_{i_2i_3}^{(j_2)}(\mathbf{p})\ldots F_{i_ki_1}^{(j_k)}(\mathbf{p})$$

be the corresponding product of weights (16). If $c = (k, e_j, k)$ is a loop of $D(J)$ then $J[c] = F_{kk}^{(j)}(\mathbf{p})$ for some j. If $J[c] > 0$ then c is called a *positive cycle* and if $J[c] < 0$ then c is called a *negative cycle*. Note that in the first case $J[c]$ contains an even number of negative entries $F_{lk}^{(j)}(\mathbf{p})$ and in the second case $J[c]$ contains an odd number of negative entries $F_{lk}^{(j)}(\mathbf{p})$ for some indices k, l, j. We say that a pair of cycles is *disjoint if their vertex sets are disjoint*.

A set $g = \{c_1, c_2, \ldots, c_p\}$, consisting of pairwise disjoint cycles c_j is called a *subfactor* of $D(J)$. If a subfactor g contains k vertices then we say that it is of *order* k and denote it by g_k. We define

$$J[g_k] = \prod_{j=1}^{p} J[c_j]$$

as a product over all corresponding weights (16) associated with the subfactor g_k. In the multigraph $D(J)$ two different subfactors can have the same set of vertices, but not the same set of edges.

We are interested in the coefficient $a_n(\mathbf{p}) = \det(-J)$ [see (11)] which can be represented as follows

$$\begin{aligned} a_n(\mathbf{p}) &= \det(-J) \\ &= \sum_{g_n \in D(J)} (-1)^{|g_n|} J[g_n] \\ &= \sum_{g_n \in D(J)} (-1)^{|g_n|} \prod_{c \in g_n} J[c] \end{aligned} \tag{17}$$

where $|g_n|$ is the number of cycles in g_n, and the sum is over all subfactors of order n in $D(J)$. Each term

$$(-1)^{|g_n|} \prod_{c \in g_n} J[c] \tag{18}$$

in the right-hand side of (17) is a non-zero term in the expansion of $\det(-J)$, and is in one-to-one correspondence with a subfactor $g_n \in D(J)$. For a detailed explanation and derivation of this formula in the case of the digraph see [25], [43], [45]. The terms $J[c]$ corresponding to negative cycles c contribute positive terms to the product in (18), since each one of them is multiplied by a negative sign. Therefore the sign of each product term (18) is determined

by the number of positive cycles in g_n. In particular, note that negative loops do not influence the sign of (18). This is the reason why we do not draw the negative loops in the S-multigraph.

The expansion (17) of $\det(-J)$ contains a negative term if and only if there is a subfactor g_n with an odd number of positive cycles. For biochemical reaction models there is almost always a positive term in $\det(-J)$, corresponding to the product of negative decay terms from each J_{kk}. For example, this is always the case if each species is consumed by at least one reaction.

Since any coefficient $a_k(\mathbf{p})$ of (12) is a sum of all principal minors of order k of the matrix $-J$, formula (17) can be applied for $a_k(\mathbf{p})$ as well:

$$a_k(\mathbf{p}) = \sum_{g_k \in D(J)} (-1)^{|g_k|} J[g_k] = \sum_{g_k \in D(J)} (-1)^{|g_k|} \prod_{c \in g_k} J[c], \quad k = 1, \ldots, n \qquad (19)$$

where $|g_k|$ is the number of cycles in a subfactor g_k and the sum is over all possible subfactors of order k. For more details see [25]. As before, each non-zero summand in the expansion (19) of $a_k(\mathbf{p})$ is in one-to-one correspondence with a subfactor $g_k \in D(J)$. Similarly, the coefficient $a_k(\mathbf{p})$ contains a negative term if and only if there exists a subfactor $g_k \in D(J)$ with an odd number of positive cycles.

We define a *critical subfactor* to be a subfactor that contains an odd number of positive cycles. Therefore a critical subfactor g_n in $D(J)$ may cause multistability that originates from a saddle-node bifurcation. Similarly, a critical subfactor g_k in $D(J)$ with $k < n$ may cause oscillations that originate from a Hopf bifurcation. If the system (5) has r conservation relations then n is replaced by $n - r$ in the previous statement.

Remark: Note that in the case of mass-action kinetics it was shown in [23] that $\det(J) = 0$ is a necessary condition for multistability, and not just for the existence of a saddle-node bifurcation. This holds despite Pinchuk's counter-example [44] to the real Jacobian conjecture (more details about the relationship with the real Jacobian conjecture are available in [23]). Also in [23] it was shown that, under some additional assumptions, $\det(J) = 0$ becomes a sufficient condition for multistability.

Example 4.1: In [46], [47] a model of two-gene switch is given by

$$\frac{du_1}{dt} = \frac{k_1}{1 + u_2^h} - \mu_1 u_1 \qquad (20)$$

$$\frac{du_2}{dt} = \frac{k_2}{1 + u_1^h} - \mu_2 u_2 \qquad (21)$$

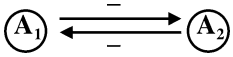

Fig. 3. *The S-multigraph for a two-gene switch model.*

with Hill type kinetics, i.e., $h \geq 1$. The variables u_1 and u_2 are the concentrations of two repressor proteins and both of the Hill functions are inhibiting. For certain parameter values the model (20), (21) shows bistability; more precisely, one unstable and two stable positive equilibria exist. The model (20), (21) is used to construct a synthetic, bistable gene network in *Escherichia coli*, which consists of two repressible promoters arranged in a mutually inhibitory network [47].

The Jacobian matrix of the system (20), (21) is

$$J = \begin{pmatrix} -\mu_1 & -\frac{k_1 h u_2^{h-1}}{\left(1+u_2^h\right)^2} \\ -\frac{k_2 h u_1^{h-1}}{\left(1+u_1^h\right)^2} & -\mu_2. \end{pmatrix}.$$

The S-multigraph in Fig. 3 consists of two vertices connected in a positive cycle, which is a necessary condition for multistability. If Michaelis-Menten rates are used (i.e., $h = 1$) the S-multigraph remains the same. However, in [46], [47] it is shown that if $h = 1$ then there is no multistability for any values of the kinetic parameters. Therefore the graph-theoretic condition for multistability is necessary, but not sufficient in this case. In [48], [49] it is shown that the addition of stochastic effects to the genetic switch model gives rise to multistability even for $h = 1$ in (20), (21). Moreover, if the degradation rates in (20), (21) are nonconstant and nonlinear: $\mu_i = d + (d_r k_0/(1 + k_0 u_i))$, where $d > 0$, $d_r > 0$ and $k_0 > 0$ are constants, then multistability arises even without stochasticity.

Example 2.1 (Continued): In order to obtain a multistable system, we will assume that the variable u_1 is a constant (i.e., make $k_1 = k_2 = k_3 = 0$), reducing the dimension of the system to four; moreover, note that the concentration of the apoptosis marker A_5 does not influence the dynamics of concentrations of the other species A_2, A_3, A_4, so we can focus on the three-dimensional system given by (7)–(9). Using parameter values given in [38] we obtain the bifurcation diagram shown in Fig. 4. Multistability in the u_2, u_3, u_4 subsystem is due to a critical subfactor of order three, $g_3 = \{(A_2, e_5, A_2), (A_3, e_{10}, A_4, e_7, A_3)\}$, which contains one positive cycle.

On the other hand, if we reintroduce the equation for variable u_1, the bifurcation curve in Fig. 4 becomes an S-shaped nullcline in a reduced two-dimensional model of (6)–(9). Hysteretic oscillations arise in this model, as

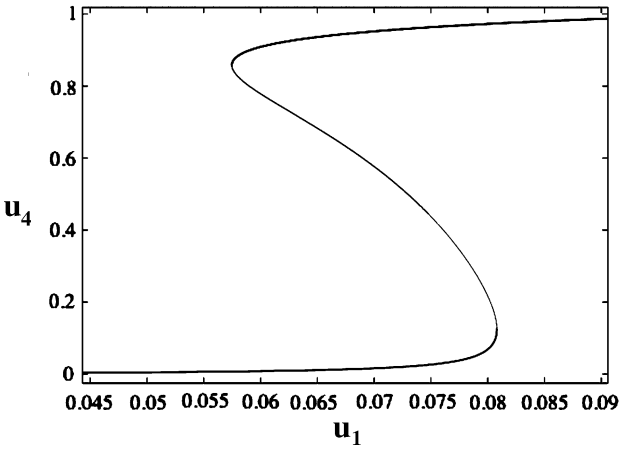

Fig. 4. *Bifurcation diagram for the variable u_4 with respect to parameter u_1; thick curves indicate stable equilibria, and thin curves indicate unstable equilibria.*

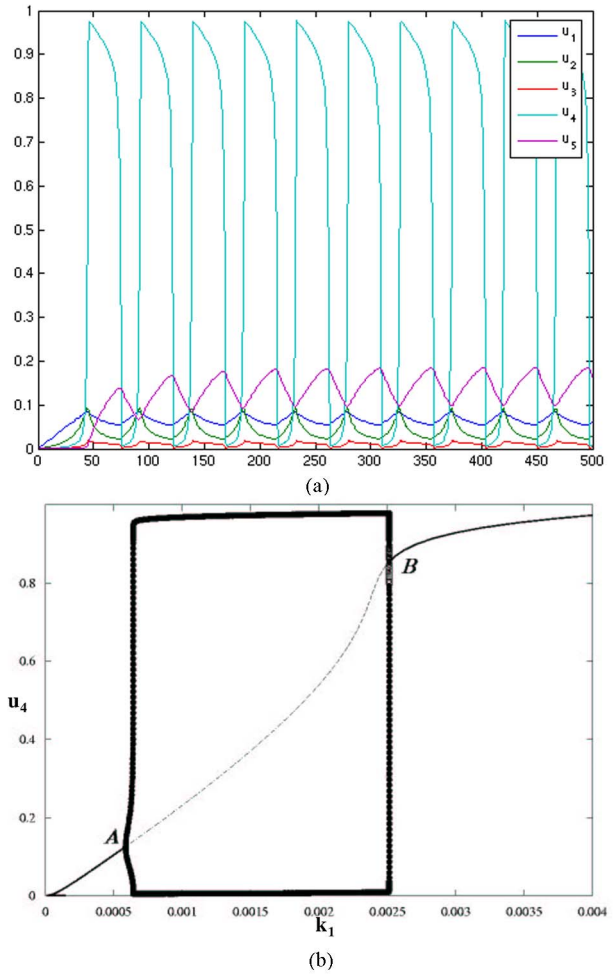

Fig. 5. *(a) Oscillations and (b) bifurcation diagram for the variable u_4 with respect to parameter k_1; the points A and B indicate Hopf bifurcations.*

explained in [38] (also, see Fig. 5). Moreover, if the positive feedback cycle between the nodes A_3 and A_4 is disrupted (see Fig. 2), then these oscillations disappear (see [38]). The positive cycle responsible for the oscillations is $c = (A_3, e_{10}, A_4, e_7, A_3)$ in Fig. 2, where the edge e_{10} corresponds to the rate function w_{10} that contains the kinetic parameter k_{10}, and the edge e_7 corresponds to w_7, which contains k_7. Note that this positive cycle c is also a critical subfactor of order 2 in an S-multigraph with 4 vertices.

V. TURING INSTABILITY

In this section we present similar graph-theoretic conditions for detecting Turing instability in biochemical reaction networks. We will use the same network representation, i.e., the S-multigraph. As in the previous section, these graph-theoretic conditions for Turing instability are applicable to reaction networks with any number of biochemical species.

Finding Turing instability in biochemical network models involves the analysis of a system of reaction-diffusion equations. If the concentrations $u_k = u_k(\mathbf{x}, t)$ are spatially non-homogeneous functions in $\mathbf{x} = (x_1, \ldots, x_p)^T$, the reaction-diffusion system corresponding to (5) with initial condition $\mathbf{u}^0(\mathbf{x})$ and no-flux boundary condition is

$$\frac{\partial u_k}{\partial t} = d_k \Delta u_k + \sum_{i=1}^{m} \gamma_{ki} w_i(\mathbf{u}), \quad \mathbf{x} \in \Omega, \quad 0 < t < T; \tag{22}$$

$$u_k(\mathbf{x}, 0) = u_k^0(\mathbf{x}), \quad \mathbf{x} \in \Omega; \tag{23}$$

$$\frac{\partial u_k}{\partial \boldsymbol{\nu}}(\mathbf{x}, t) = 0, \quad \mathbf{x} \in \partial\Omega, \quad 0 < t < T \tag{24}$$

for $k = 1, \ldots, n$ [50]. The diffusion coefficients d_k are positive constants, and Δ denotes the Laplacian in (22). The directional derivative normal to the boundary $\partial\Omega$ is denoted by $\partial/\partial\boldsymbol{\nu}$ in (24). The initial value functions satisfy $u_k^0(\mathbf{x}) \geq 0$ for all $k = 1, \ldots, n$ and $\mathbf{x} \in \Omega$, since they represent species concentrations. The no-flux boundary condition (24) is considered standard for systems studied for Turing instabilities [18], meaning that there is no inflow or outflow of reactant concentrations through the side boundary. Later we will use the diagonal matrix $D = diag(d_1, \ldots, d_n)$, which has the diffusion coefficients $d_k > 0$ on its main diagonal.

Notice that any constant equilibrium solution $\mathbf{u}^*(\mathbf{p})$ of the ODE system (5) is also a solution of the reaction-diffusion system (22). We say that Turing instability occurs if a constant equilibrium solution $\mathbf{u}^*(\mathbf{p})$ is linearly asymptotically stable as a solution to the ODE system (5), but is unstable as a solution to the reaction-diffusion system (22) (see [18]).

The problem of diffusion-driven instability can be reduced to the problem of stability of the matrix $J(\mathbf{p}) - \mu D$ where $J(\mathbf{p})$ is the Jacobian (11) of the ordinary differential equation system (5) and $\mu \geq 0$ is an eigenvalue of the negative Laplacian. Turing instability occurs if, for some values of $\mathbf{p}$ and D, all eigenvalues of the matrix $J(\mathbf{p})$ have negative real parts, and the matrix $J(\mathbf{p}) - \mu D$ has an eigenvalue with a positive real part [18], [36]. In particular, Turing instability can arise from saddle-node bifurcation of constant equilibrium solutions, and the appearance of new spatially non-homogeneous steady-state solutions. The existence of these new spatially non-homogeneous steady states is usually referred to as "pattern formation" (see [18]). Turing instability arising from Hopf bifurcation is also possible, and this would be an interesting topic for future research.

A necessary condition for a saddle-node bifurcation is $\det(J(\mathbf{p}) - \mu D) = 0$, i.e., the existence of a zero root of the characteristic polynomial of $J(\mathbf{p}) - \mu D$ for some $\mu > 0$. However, $\det(J(\mathbf{p}) - \mu D) = 0$ if and only if $\det(\mu I - J(\mathbf{p})D^{-1}) = 0$, where the matrix $D^{-1} = diag(1/d_1, \ldots, 1/d_n)$ is the inverse of D. In other words the matrix $J(\mathbf{p})D^{-1}$ must have a positive eigenvalue μ for some values of $\mathbf{p}$ and D. The matrix $J(\mathbf{p})D^{-1}$ is the Jacobian (11) of the corresponding ODE system (5) with the k-th column $J_k(\mathbf{p})$ multiplied by d_k^{-1} for $k = 1, \ldots, n$. The analogous of (19) for the coefficients of the characteristic polynomial of $J(\mathbf{p})D^{-1}$:

$$\begin{aligned}\tilde{p}(\mu) &= \det\left(\mu I - J(\mathbf{p})D^{-1}\right) \\ &= \mu^n + \tilde{a}_1\mu^{n-1} + \ldots + \tilde{a}_n = 0\end{aligned} \tag{25}$$

is

$$\tilde{a}_k(\mathbf{p}, \mathbf{d}) = \sum_{g_k \in D(J)} (-1)^{|g_k|} \frac{J[g_k]}{d_{i_1} \ldots d_{i_k}}, \quad k = 1, \ldots, n \tag{26}$$

where $\mathbf{d} = (d_1, \ldots, d_n)$. Note that the diffusion coefficients $d_{i_1}, \ldots, d_{i_k}$ in (26) correspond to the vertices $\{i_1, \ldots, i_k\}$ of the subfactor g_k.

Since $\mu > 0$ in (25), we must require that at least one $\tilde{a}_k(\mathbf{p}, \mathbf{d}) < 0$ for some values of $\mathbf{p}$ and the diffusion coefficients $\mathbf{d}$, otherwise $\tilde{p}(\mu) > 0$. This means that there is a negative term in at least one $\tilde{a}_k(\mathbf{p}, \mathbf{d})$. Assuming that all eigenvalues of $J(\mathbf{p})$ have negative real parts for some values of $\mathbf{p}$, it follows that $k < n$, because $\tilde{a}_n(\mathbf{p}, \mathbf{d}) = a_n(\mathbf{p})/d_1 \ldots d_n = \det(-J(\mathbf{p}))/d_1 \ldots d_n > 0$. Therefore the existence of a critical subfactor g_k of the multigraph $D(J)$ of order $k < n$ is a necessary condition for Turing instability arising from a saddle-node bifurcation. This critical subfactor condition is a generalization of the autocatalysis condition for Turing instability.

Several other important characteristics of models with Turing instability carry over from the two-dimensional to the n-dimensional case. First, different diffusion coefficients are a necessary condition for Turing instability. Indeed, if all of the diffusion coefficients are equal to $d > 0$ then $\tilde{a}_k(\mathbf{p}, \mathbf{d}) = a_k(\mathbf{p})/d^k > 0$ for all k in (26) and therefore $\tilde{p}(\mu) > 0$ for $\mu > 0$ [see (25)]. By (26) the diffusion constants $d_{i_1}, \ldots, d_{i_k}$ corresponding to the vertices in a critical subfactor g_k should be smaller than the other diffusion coefficients, in order for $\tilde{a}_k(\mathbf{p}, \mathbf{d}) < 0$. This generalizes another well-known property of two-dimensional Turing systems: that the ratio of the diffusion coefficients must be much larger than one for Turing instability to occur.

VI. DISCUSSION

In this article we have presented graph-theoretic conditions for oscillations and multistability for reaction network models with reaction rates that are not necessarily of mass action type. These conditions apply irrespective of the number of species. Moreover, this type of graph-theoretic condition is applicable to reaction-diffusion systems leading to Turing instability and pattern formation. The main extension of some previous work [6], [25] is the use of the S-multigraph instead of the simpler digraph, which allows for multiple rate functions between any two species. The usual condition for multistability or oscillations, i.e., the existence of a positive feedback cycle has been generalized to the existence of a special type of subgraph with an odd number of positive cycles, called a critical subfactor of the S-multigraph.

The idea of relating the dynamic behavior of a chemical reaction model to its network structure goes back to the Stoichiometric Network Analysis theory of Clarke [3], [42], [51]–[53] and the Deficiency Theory of Feinberg, Horn, and Jackson [26]. Clarke studied mainly mass-action kinetics systems, and in particular oscillations in different models of the Belousov-Zhabotinsky reaction [54], [55]. Also Aguda and Clarke [56] studied examples of multistable enzymatic reactions. Based on Clarke's theory, Eiswirth and co-authors classified chemical oscillators arising from Hopf bifurcations [57]. The theory of Clarke was further developed in the works of Ivanova [58], [59] and has been recently used in the construction and analysis of positive feedback oscillations in enzyme reactions by Goldstein and co-workers [60], [61]. While Clarke has focused on instabilities, Feinberg, Horn, and Jackson [26] have studied sufficient conditions for uniqueness of a stable equilibrium. Recently, Craciun and Feinberg [23], [24] and Mincheva and Roussel [25] have described computational and graph-theoretic conditions for uniqueness of positive equilibrium. These works mainly considered mass-action kinetics, while here we study more general systems with rate functions that include mass-action, Michaelis-Menten or Hill type kinetics.

Many other groups have studied the connection between network structure and dynamic instabilities [62]–[66]. R. Thomas has formulated the conjecture that a positive feedback cycle (circuit) is a necessary condition for the existence of multiple steady states, also called multistationarity [63]. A recent proof of the Thomas' conjecture was obtained by C. Soule in [64] with some further developments in [65]. R. Thomas also studied the role of negative feedback cycles in biology, as a cause for oscillations and homeostasis [63].

Angeli, De Leenheer and Sontag have developed the theory of monotone systems with input and output, which they used to analyze biochemical reaction networks as an interconnection of monotone systems [7], [8], [67]. In particular, an analysis of properties of the MAPK cascade is presented in [8].

A version of the theory presented here can be applied also for delay differential equations, as described in [70]. Delay-differential equations are especially important in modeling genetic regulatory systems, since time delays occur due to transcription and translation processes [71]–[73]. Delay-induced instability, i.e., instability caused by the inclusion of delays in the model, is usually associated with Hopf bifurcation and oscillations. In a recent article [70] we obtained graph-theoretic conditions for delay-induced instability, which generalize the negative feedback cycle condition [74]. Similar graph-theoretic conditions for reaction network models can be derived for the S-multigraph, following [70].

In general, with the increase of the number of species in a model the number of critical subfactors in the respective graph should increase as well. Therefore, higher dimensional models may be more robust [68], [69], as they may produce biochemical oscillations or biochemical switches for an extended set of parameters, due to the higher number of critical subfactors.

The feedback cycle conditions can be associated with other biological functions, such as the acceleration or delay of transcriptional processes [75], [76], and the network conditions derived here may be applicable in this case as well.

Since these graph-theoretic conditions are independent of kinetic parameters, they may characterize possible instabilities even if incomplete information about the network model is available [77]. Moreover, in terms of constructing functional synthetic gene networks [47], [78] this theory may predict the behavior of biochemical reaction networks consisting of combinations of simple motifs, such as feedback cycles. ■

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