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ORIGINAL PAPER

Oscillations in non-mass action kinetics models of biochemical reaction networks arising from pairs of subnetworks

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Abstract It is well known that oscillations in models of biochemical reaction networks can arise as a result of a single negative cycle. On the other hand, methods for finding general network conditions for potential oscillations in large biochemical reaction networks containing many cycles are not well developed. A biochemical reaction network with any number of species is represented by a simple digraph and is modeled by an ordinary differential equation (ODE) system with non-mass action kinetics. The obtained graph-theoretic condition generalizes the negative cycle condition for oscillations in ODE models to the existence of a pair of subnetworks, where each subnetwork contains an even number of positive cycles. The technique is illustrated with a model of genetic regulation.

Keywords Biochemical reaction networks · Non-mass action kinetics · Oscillations · Negative feedback cycle

1 Introduction

Modeling oscillations in biochemical models usually involves the analysis of a system of ordinary differential equations (ODE) with non-mass action kinetics [8,11,13,14,33,34,37,39]. Many of the classical models of biochemical reaction networks showing oscillations contain a single negative cycle [11,13,14,37,38]. Since realistic biochemical networks contain a large number of cycles, models incorporating several negative cycles have started to appear in the literature [8,33]. The aim of this paper is to provide a general graph-theoretic condition for oscillations which is applicable to

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biochemical reaction networks with a large number of cycles, and which generalizes the negative cycle condition for oscillations.

A major topic of systems biology is the connection between small recurring network motifs and their corresponding biological function [16,19,26]. Some motifs, such as negative or positive cycles are shown to be responsible for oscillations or multistability in biochemical reaction networks [3,4,12,29,34,39]. In this article we show that more complex combinations of positive or negative cycles, such as pairs of negative cycles, or even pairs of subnetworks with an even number of positive cycles, can be responsible for oscillations.

Biochemical reaction networks are often represented by different types of graphs [5,7,10,29,40]. We study ODE models of biochemical reaction networks with non-mass action kinetics, where the networks are represented as simple digraphs [15]. The mass action kinetics counterpart to this problem where the network is represented by a bipartite graph [15] is studied in [27]. The critical pair of fragments condition for oscillations includes the negative cycle condition for oscillations as a special case [27]. In this work a similar graph-theoretic condition for oscillations in ODE models with non-mass action kinetics is obtained.

The eigenvalues of the Jacobian matrix associated with an ODE system determine the stability of its equilibria. If an equilibrium solution of an ODE system becomes unstable via a single pair of complex conjugated eigenvalues, then simple Hopf bifurcation and oscillations occur. In this paper we will study oscillations arising from Hopf bifurcation only.

Inspection of the digraph of a biochemical reaction network for pairs of subnetworks that can be responsible for oscillations is only part of the process. Once a structure in the digraph that can lead to oscillations is identified, it should be verified that the Jacobian matrix satisfies additional algebraic conditions.

The preliminaries on the ODE model of a biochemical reaction network are explained in Sect. 2. The main idea of the graph-theoretic analysis is introduced in Sect. 3. Using the new graph-theoretic condition, an example of oscillations in a genetic regulation model [23] is studied in Sect. 4.

2 Preliminaries

In this section we describe the ODE model of a biochemical reaction network with non-mass action kinetics. The Jacobian matrix associated with the ODE model and its characteristic polynomial are introduced along with some basic assumptions.

The rate of change of the concentration of any biochemical species depends on the rates of the reactions that produce and consume it. We study biochemical reaction networks with n biochemical species $A_1, A_2, \ldots, A_n$, and m reactions which are not necessarily elementary. 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. The rate functions are usually given by mass action, Michaelis–Menten or Hill type kinetics [18]. For example, the function

$$g_1(u) = \frac{k_1 u}{k_2 + u}, \tag{1}$$

where u is the concentration of a substrate and $k_1 > 0, k_2 > 0$ are kinetic parameters is of Michaelis–Menten type. The function $g_1(u)$ is called activating since $g_1'(u) > 0$. Examples of inhibiting Michaelis–Menten functions are given in [18]. An example of an inhibiting Hill type function is

$$g_2(u) = \frac{k_1}{k_2 + u^h}, \tag{2}$$

where $k_1 > 0, k_2 > 0$ are kinetic parameters, and $h > 0$ is referred to as a Hill coefficient. Note that $g_2'(u) < 0$ for the inhibiting rate function (2)

The ODE system

$$\frac{du_k}{dt} = f_k(\mathbf{u}), \quad k = 1, \ldots, n \tag{3}$$

represents the time evolution of the concentrations $u_k(t)$ of the biochemical species A_k, where $f_k(\mathbf{u})$ are continuously differentiable functions. We assume that $f_k(\mathbf{u})$ is the sum of rate functions, such as Michaelis–Menten type (1), Hill type (2) or mass action kinetics type, multiplied by the stoichiometric coefficients of the reaction.

Let $\mathbf{p} > \mathbf{0}$ be the vector of kinetic parameters contained in $f_k(\mathbf{u})$, $k = 1, \ldots, n$. We assume that the ODE system (3) has at least one positive equilibrium solution $\mathbf{u}^*(\mathbf{p}) > 0$, which depends continuously on the parameter values $\mathbf{p}$.

The Jacobian matrix $J = [J_{kl}]$ of the right-hand side of the ODE system (3) plays an important role in determining the stability of the equilibrium solutions of (3) and the possible bifurcations [20]. Let the (k, l) entry of the Jacobian matrix of the right-hand side of (3) be

$$J_{kl}(\mathbf{p}) = \frac{\partial f_k(\mathbf{u})}{\partial u_l}, \tag{4}$$

where it is assumed that (4) is evaluated at a positive equilibrium $\mathbf{u} = \mathbf{u}^*(\mathbf{p})$ of (3). The Jacobian (4) depends continuously on the parameters $\mathbf{p}$, since $\mathbf{u}^*(\mathbf{p})$ depends continuously on $\mathbf{p}$. Also, the following important assumption, which is usually satisfied for ODE models such as (3), will be made from now on:

(A1) Every non-zero entry $J_{kl}(\mathbf{p})$ of the Jacobian matrix (4) is either positive or negative for all parameter values $\mathbf{p}$. In addition, every diagonal entry $J_{kk}(\mathbf{p})$, $k = 1, \ldots, n$ of (4) is negative for all parameter values $\mathbf{p}$.

The eigenvalues of the Jacobian matrix (4) determine the stability of an equilibrium solution $u^*(\mathbf{p})$ of the ODE system (3) and can be computed as the roots of the corresponding characteristic polynomial

$$s(\lambda) = \det(\lambda I - J(\mathbf{p})) = \lambda^n + a_1(\mathbf{p})\lambda^{n-1} + \cdots + a_n(\mathbf{p}). \tag{5}$$

Since the entries $J_{kl}(\mathbf{p})$ of the Jacobian (4) depend continuously on $\mathbf{p}$, it follows that the coefficients $a_k(\mathbf{p})$ of (5) depend continuously on $\mathbf{p}$. For large ODE systems with many parameters, such as (3), finding the roots of the corresponding characteristic

polynomial (5) symbolically is not always possible. Instead, we will use the coefficients $a_k(\mathbf{p})$ of the characteristic polynomial (5) to show the existence of roots with a zero real part which will indicate possible instability and bifurcations.

Any coefficient $a_k(\mathbf{p})$ of (5) is the sum of all principal minors $M(-J(\mathbf{p}))(I_k)$ of the negative Jacobian (4), where $I_k = \{i_1, \ldots, i_k\}$ is a subset of $I_n = \{1, \ldots, n\}$, [21]

$$a_k(\mathbf{p}) = \sum_{I_k \subseteq I_n} M(-J(\mathbf{p}))(I_k), \quad k = 1, \ldots, n. \tag{6}$$

Let $\lambda_i, i = 1, \ldots, n$ be the eigenvalues of the Jacobian (4) and let their corresponding real parts be denoted by $\Re(\lambda_i)$. Since the coefficients $a_k(\mathbf{p})$ of (5) depend continuously on $\mathbf{p}$, it follows that the eigenvalues $\lambda_i(\mathbf{p})$ of (4) depend continuously on $\mathbf{p}$.

We define the open set of parameters

$$S = \{\mathbf{p} \mid \Re(\lambda_i(\mathbf{p})) < 0 \, , i = 1, \ldots, n\}, \tag{7}$$

which is assumed to be non-empty, bounded and connected in order to simplify the analysis. Let the closure of the set S be denoted by $\bar{S}$. We say that the Jacobian matrix $J(\mathbf{p})$ is a *stable* matrix if and only if $\mathbf{p} \in S$. The Jacobian matrix $J(\mathbf{p})$ has an eigenvalue with a zero real part if and only if $\mathbf{p} \in \partial S$, where ∂S is the boundary of the set S.

3 Main results

First we introduce a sufficient algebraic condition for the model ODE system (3) to have a Jacobian matrix (4) with a single pair of purely imaginary eigenvalues. This condition leads to a sufficient condition for Hopf bifurcation and oscillations using a theorem from [22]. Then the digraph associated with a biochemical reaction network is defined, as well as structures in the digraph, such as cycles and subfactors that are necessary for the graph-theoretic analysis. The graph-theoretic condition for oscillations is based on a sufficient condition for zero Hurwitz determinant of $(n-1)$st order which is introduced next.

The Hurwitz matrix of kth order where $a_j(\mathbf{p})$ are the coefficients of (5) is defined by

$$M_k(\mathbf{p}) = \begin{pmatrix} a_1(\mathbf{p}) & a_3(\mathbf{p}) & a_5(\mathbf{p}) & a_7(\mathbf{p}) & \ldots \\ 1 & a_2(\mathbf{p}) & a_4(\mathbf{p}) & a_6(\mathbf{p}) & \ldots \\ 0 & a_1(\mathbf{p}) & a_3(\mathbf{p}) & a_5(\mathbf{p}) & \ldots \\ 0 & 1 & a_2(\mathbf{p}) & a_4(\mathbf{p}) & \ldots \\ \ldots & \ldots & \ldots & \ldots & \ldots \\ 0 & \ldots & \ldots & a_{k-1}(\mathbf{p}) & a_{k+1}(\mathbf{p}) \\ 0 & \ldots & \ldots & a_{k-2}(\mathbf{p}) & a_k(\mathbf{p}) \end{pmatrix}. \tag{8}$$

The corresponding Hurwitz determinants of kth order will be denoted as $H_k(\mathbf{p}) = \det(M_k(\mathbf{p}))$, $k = 1, \ldots, n$, [9]. In the next theorem, we will use two submatrices of the $(n-1)$st order Hurwitz matrix (8) which are defined next. The submatrix

$$M_{n-1,i}(\mathbf{p}), \quad i = 1, 2 \tag{9}$$

of (8) is obtained by deleting rows $(n-1)$ and $(n-2)$, and columns $(n-1)$ and $(n-1-i)$, respectively.

A sufficient condition for a single pair of purely imaginary eigenvalues of a matrix is obtained in [35]. The next theorem is a slight modification of [35, Theorem 2.1] since the Hurwitz matrix (8) of $(n-1)$st order $M_{n-1}(\mathbf{p})$ is used instead of the Sylvester matrix. This can be done because $M_{n-1}(\mathbf{p})$ can be obtained from the Sylvester matrix by row and column reordering. The condition of a positive coefficient $a_n(\mathbf{p}) = \det(-J(\mathbf{p}))$ of (5) where $\mathbf{p} \in \bar{S}$ is added to exclude the possibility for a zero eigenvalue of the Jacobian matrix (4) and to guarantee that all other eigenvalues have negative real parts.

Theorem 1 *If $H_{n-1}(\mathbf{p}_0) = 0$, $\det(M_{n-1,1}(\mathbf{p}_0))\det(M_{n-1,2}(\mathbf{p}_0)) > 0$ and $a_n(\mathbf{p}_0) > 0$ are satisfied at some parameter value $\mathbf{p}_0 \in \bar{S}$, then the Jacobian matrix $J(\mathbf{p}_0)$ has exactly one pair of purely imaginary eigenvalues and all other eigenvalues have negative real parts.*

Proof Let $\lambda_i(\mathbf{p}), i = 1, \ldots, n$ be the eigenvalues of the Jacobian $J(\mathbf{p})$ defined in (4). Since $\mathbf{p}_0 \in \bar{S}$, it follows that the real parts of the eigenvalues of the Jacobian $\Re(\lambda_i(\mathbf{p}_0)) \leq 0$ for all i. Since $a_n(\mathbf{p}_0) > 0$, the Jacobian $J(\mathbf{p}_0)$ has no zero eigenvalues. By [35, Theorem 2.1], the first two conditions of the theorem guarantee that the Jacobian $J(\mathbf{p}_0)$ has a single pair of purely imaginary eigenvalues. Since $\mathbf{p}_0 \in \bar{S}$, it follows that all other eigenvalues have negative real parts. □

The condition $a_n(\mathbf{p}) > 0$ is easily satisfied if $a_n(\mathbf{p})$ consists of positive summands. The same is true, if both $\det(M_{n-1,1}(\mathbf{p}))$ and $\det(M_{n-1,2}(\mathbf{p}))$ consist of summands of the same sign.

The next corollary follows by the criterion for simple Hopf bifurcation (Liu's theorem) obtained in [22] under the assumption that all parameters in $\mathbf{p}$ except one are fixed. The condition for a single pair of purely imaginary eigenvalues obtained in Theorem 1 is equivalent to condition (CH1) from Liu's theorem [22].

Corollary 1 *If the conditions of Theorem 1 are satisfied, and if there exists a smooth curve of equilibria $(\mathbf{p}, \mathbf{u}^*(\mathbf{p}))$ for the ODE system (3), and if $\frac{\partial H_{n-1}}{\partial \mathbf{p}}(\mathbf{p}_0) \neq \mathbf{0}$, then a simple Hopf bifurcation exists.*

By Corollary 1 oscillations arising from Hopf bifurcation exist, if the additional condition for non-zero derivative of the $(n-1)$st Hurwitz determinant at the point $\mathbf{p_0} \in \bar{S}$ is satisfied. In fact, if in a neighborhood of $\mathbf{p_0} \in \bar{S}$, Hopf bifurcation exists, it follows that $\mathbf{p_0} \in \partial S$ where ∂S is the boundary of S.

A necessary condition for the existence of a pair of purely imaginary eigenvalues of the Jacobian matrix (4) is $H_{n-1}(\mathbf{p}) = 0$ for some values of the parameters $\mathbf{p}$, which follows by Orlando's formula [9]. Therefore, the determining condition for the existence of a pair of purely imaginary eigenvalues in Theorem 1, on which the graph-theoretic condition will be based, is $H_{n-1}(\mathbf{p}) = 0$.

The product $a_1(\mathbf{p}) \ldots a_{n-1}(\mathbf{p})$ of diagonal entries in the Hurwitz determinant $H_{n-1}(\mathbf{p})$ contains at least one positive summand corresponding to a product of diagonal entries of the negative Jacobian (4) by assumption **(A1)**. If there exists a negative

summand in a product $\pm a_{i_1}(\mathbf{p})\dots a_{i_{n-1}}(\mathbf{p})$, $i_1+i_2+\dots+i_{n-1}=(n-1)n/2$ of the Hurwitz determinant $H_{n-1}(\mathbf{p})$, then there may exist a parameter value $\mathbf{p}$, such that $H_{n-1}(\mathbf{p})=0$ by continuity. Therefore, obtaining a graph-theoretic condition leading to $H_{n-1}(\mathbf{p})=0$ for some parameter values of $\mathbf{p}$ involves finding structures in the digraph of a biochemical reaction network that correspond to negative summands in the Hurwitz determinant $H_{n-1}(\mathbf{p})$. In general, this is an extremely difficult problem, since the Hurwitz determinant $H_{n-1}(\mathbf{p})$ contains a large number of summands even for relatively small n. However, a simpler sufficient algebraic condition for $H_{n-1}(\mathbf{p})=0$, which is formulated in Theorem 2, will lead to a simpler graph-theoretic condition.

If $\mathbf{p}\in\bar{S}$, the inequality

$$0\le H_{n-1}(\mathbf{p})\le a_1(\mathbf{p})\dots a_{k-1}(\mathbf{p})a_k(\mathbf{p})a_{k+1}(\mathbf{p})\dots a_{n-1}(\mathbf{p}) \tag{10}$$

can be used to show that $H_{n-1}(\mathbf{p})=0$ for some $\mathbf{p}\in\bar{S}$. A graph-theoretic condition for oscillations which generalizes the positive cycle condition for oscillations based on the inequality (10) is obtained in [29]. However, if all coefficients $a_k(\mathbf{p})>0$ of (5) for all $\mathbf{p}\in\bar{S}$ are not sufficiently small, then (10) can not imply $H_{n-1}(\mathbf{p})=0$ for any $\mathbf{p}\in\bar{S}$. In the next lemma, a more general inequality than (10) that can lead to $H_{n-1}(\mathbf{p})=0$ for some $\mathbf{p}\in\bar{S}$ is obtained.

Lemma 1 *Let $\mathbf{p}\in\bar{S}$. If $H_{n-1}(\mathbf{p})$ is the $(n-1)$st Hurwitz determinant, then*

$$0\le H_{n-1}(\mathbf{p})\le a_1(\mathbf{p})\dots a_{k-2}(\mathbf{p})h_k(\mathbf{p})a_{k+1}(\mathbf{p})\dots a_{n-1}(\mathbf{p}), \tag{11}$$

where $a_j(\mathbf{p})\ge 0$, $j=1,\dots,n$ are the coefficients of the characteristic polynomial (5) and

$$h_k(\mathbf{p})=a_{k-1}(\mathbf{p})a_k(\mathbf{p})-a_{k-2}(\mathbf{p})a_{k+1}(\mathbf{p})\ge 0,\quad k=2,\dots,n-1 \tag{12}$$

is a principal minor of order two of the Hurwitz matrix (8).

The proof of Lemma 1 follows by Fisher's inequality [6] and can be found in [27]. The difference between inequality (10) and inequality (11) is in the kth factor on the right-hand side, where $a_k(\mathbf{p})$ lies on the diagonal of Hurwitz matrix (8) and $h_k(\mathbf{p})$ has a corresponding (2×2) submatrix also on the diagonal of (8).

A sufficient condition for zero Hurwitz determinant of $(n-1)$st order follows directly by the inequality (11) and the properties of the set S defined in (7).

Theorem 2 *If $h_k(\mathbf{p})\ge 0$, where $k\in\{2,\dots,n-1\}$, is sufficiently small for some parameter values $\mathbf{p}\in\bar{S}$, then there exists $\mathbf{p}_0\in\bar{S}$, such that $H_{n-1}(\mathbf{p}_0)=0$.*

The principal minor $h_k(\mathbf{p})$ defined in (12) contains a positive summand in $a_{k-1}(\mathbf{p})a_k(\mathbf{p})$ corresponding to a product of diagonal entries of the negative Jacobian (4). If $h_k(\mathbf{p})$ contains a positive summand in $a_{k-2}(\mathbf{p})a_{k+1}(\mathbf{p})$, which cannot be cancelled by a similar positive summand in $a_{k-1}(\mathbf{p})a_k(\mathbf{p})$ and is sufficiently large with respect to all other positive summands, then $h_k(\mathbf{p})\ge 0$ can be made small for some values $\mathbf{p}\in\bar{S}$.

Therefore, it follows by Theorem 2, that the problem of finding a graph structure in the digraph of a biochemical reaction network that corresponds to a negative summand in $H_{n-1}(\mathbf{p})$ is reduced to the problem of finding a graph structure that corresponds to a negative dominant summand in the principal minor (12).

Next we define the digraph associated with a biochemical reaction network, and cycles and subfactors of the digraph that are needed for the graph-theoretic analysis.

Let $D(J)$ be the digraph of the Jacobian matrix (4) with a node set $V = \{1, \ldots, n\}$, where $k \in V$ corresponds to species A_k of the biochemical reaction network. We draw a directed edge or an arc (l, k) if and only if $J_{kl}(\mathbf{p}) \neq 0$ and denote the set of arcs by E. The weight function $W : E \to [J_{kl}(\mathbf{p})]$ associates to each arc (l, k) the weight $J_{kl}(\mathbf{p}) \neq 0$ of (4) that by assumption (**A1**) is either positive or negative for all $\mathbf{p}$. Note that the weighting $W = W(\mathbf{f})$ of the digraph $D(J)$ depends on the choice of the rate functions in $f_k(\mathbf{u})$, $k = 1, \ldots, n$ from the ODE system (3). Therefore, the digraph $D(J)$ can be defined as the triple $D(J) = \{V, E, W(\mathbf{f})\}$.

We assume that the digraph $D(J)$ of a biochemical reaction network is simple, i.e., there is at most one arc between any two nodes. The theory developed here can be extended to the case of a multigraph, where multiple arcs can exist between any two nodes [15].

We introduce several definitions from graph theory that will be used in the discussion that follows [15]. A *walk* $(i_1, i_2, i_3, \ldots, i_{k-1}, i_k)$ in the digraph $D(J)$ is a sequence of nodes, such that $(i_l, i_{l+1}), l = 1, \ldots, k-1$ is an arc of $D(J)$. A walk $(i_1, i_2, i_3, \ldots, i_{k-1}, i_k)$ with distinct nodes $i_1, i_2, \ldots, i_k$ is called a *path* of $D(J)$. If (i_k, i_1) is also an arc, then the path $c_k = c_k(i_1, i_2, i_3, \ldots, i_{k-1}, i_k)$ is a *cycle of order* k of the digraph $D(J)$.

A *loop* from a node k to k is defined as a cycle of order one. If $c_1 = c_1(k)$ is a loop in the digraph $D(J)$, then $J[c_1] = J_{kk}(\mathbf{p})$ is its corresponding weight. Since the diagonal entries $J_{kk}(\mathbf{p}) < 0, k = 1, \ldots, n$ for all parameter values $\mathbf{p}$ by assumption (**A1**), the stability properties of the Jacobian (4) are not influenced by its diagonal entries. Therefore, we will not draw the loops in the digraph $D(J)$.

If $c_k = c_k(i_1, i_2, i_3, \ldots, i_{k-1}, i_k)$ is a cycle of order k of the digraph $D(J)$ we refer to the corresponding product

$$J[c_k] = J_{i_1 i_2}(\mathbf{p}) J_{i_2 i_3}(\mathbf{p}) \ldots J_{i_k i_1}(\mathbf{p}) \tag{13}$$

as *cycle weight*. If $J[c_k] > 0$, then c_k is referred to as a *positive cycle*, and if $J[c_k] < 0$, then c_k is referred to as a *negative cycle*. Similarly, $c_1(k)$ is referred to as a negative loop since $J_{kk}(\mathbf{p}) < 0$. If c_k is a positive cycle, then $J[c_k]$ contains an even number of negative weights $J_{kl}(\mathbf{p})$, and if c_k is a negative cycle, then $J[c_k]$ contains an odd number of negative weights $J_{kl}(\mathbf{p})$.

We say that a pair of cycles is *disjoint* if their node sets are disjoint. A set $g = \{c_1, c_2, \ldots, c_s\}$, consisting of pairwise disjoint cycles or loops c_j is called a *subfactor* of the digraph $D(J)$. If a subfactor g contains k vertices, then we say that it is of *order* k and it is denoted by g_k. We will use the notation $g_k(i_1, \ldots, i_k)$ for a subfactor g_k with a node set $I_k = \{i_1, \ldots, i_k\}$ and an arc set E_{I_k}, consisting of arcs between nodes from I_k. We will write shortly $g_k(I_k)$ for $g_k(i_1, \ldots, i_k)$. If $|g_k|$ is the number of cycles in g_k, then the *subfactor weight* is defined as

$$J[g_k] = (-1)^{|g_k|} \prod_{c \in g_k} J[c], \tag{14}$$

where $J[c]$ is the cycle weight of a cycle $c \in g_k$ defined in (13). If $J[g_k] < 0$, then g_k is referred to as a *negative subfactor*, and if $J[g_k] > 0$, then g_k is referred to as a *positive subfactor*. It follows by (14) that a positive subfactor contains an even number of positive cycles and similarly a negative subfactor contains an odd number of positive cycles. Since $a_0(\mathbf{p}) = 1$ in (5), we assume that a subfactor of order zero $g_0 = \emptyset$, where $\emptyset$ is the empty set, has weight $J[g_0] = 1$.

Lemma 2 *Any principal minor $M(-J(\mathbf{p}))(I_k)$ of the negative Jacobian (4), where $I_k = \{i_1, \ldots, i_k\} \subseteq \{1, \ldots, n\}$ can be represented in a graph-theoretic form as*

$$M(-J(\mathbf{p}))(I_k) = \sum_{g_k(I_k) \in D(J)} J[g_k(I_k)] = \sum_{g_k(I_k) \in D(J)} (-1)^{|g_k|} \prod_{c \in g_k(I_k)} J[c]. \tag{15}$$

For additional explanation and proofs of similar formulas see [3,4,28–30,40]. The next theorem follows by (6) and Lemma 2.

Theorem 3 *A coefficient $a_k(\mathbf{p})$ of the characteristic polynomial (5) can be represented in a graph-theoretic form as*

$$a_k(\mathbf{p}) = \sum_{g_k \in D(J)} J[g_k] = \sum_{g_k \in D(J)} (-1)^{|g_k|} \prod_{c \in g_k} J[c], \quad k = 1, \ldots, n, \tag{16}$$

where the sum is over all subfactors g_k of order k.

It follows by (16) that each non-zero summand in the expansion of $a_k(\mathbf{p})$ is in one-to-one correspondence with a subfactor $g_k \in D(J)$.

Next we obtain a graph-theoretic formula for the principal minor $h_k(\mathbf{p})$, defined in (12), using the graph-theoretic formula (16).

Corollary 2 *The graph-theoretic representation of $h_k(\mathbf{p})$, defined in (12), is*

$$h_k(\mathbf{p}) = \sum_{(g_{k-1}, g_k)} J[g_{k-1}]J[g_k] - \sum_{(g_{k-2}, g_{k+1})} J[g_{k-2}]J[g_{k+1}], \quad k = 2, \ldots, n-1 \tag{17}$$

where the first sum is over all pairs of subfactors (g_{k-1}, g_k) and the second sum is over all pairs of subfactors (g_{k-2}, g_{k+1}).

Proof By (12) and (16) it follows that

$$\begin{aligned} h_k(\mathbf{p}) &= a_{k-1}(\mathbf{p})a_k(\mathbf{p}) - a_{k-2}(\mathbf{p})a_{k+1}(\mathbf{p}) \\ &= \sum_{g_{k-1}\in D(J)} J[g_{k-1}] \sum_{g_k\in D(J)} J[g_k] - \sum_{g_{k-2}\in D(J)} J[g_{k-2}] \sum_{g_{k+1}\in D(J)} J[g_{k+1}] \\ &= \sum_{(g_{k-1},g_k)} J[g_{k-1}]J[g_k] - \sum_{(g_{k-2},g_{k+1})} J[g_{k-2}]J[g_{k+1}]. \end{aligned}$$

□

Each non-zero summand in the first sum of (17) corresponds uniquely to a pair of subfactors (g_{k-1}, g_k) and similarly, each non-zero summand in the second sum corresponds to a pair of subfactors (g_{k-2}, g_{k+1}). The idea of using the graph-theoretic representation (17) of the principal minor (12) is to find summands $J[g_{k-2}]J[g_{k+1}] > 0$ that cannot be cancelled by similar summands $J[g_{k-1}]J[g_k] > 0$ and can be made larger than any $J[g_{k-1}]J[g_k] > 0$. This way $h_k(\mathbf{p}) \geq 0$ can be made sufficiently small for some parameter values $\mathbf{p} \in \bar{S}$ which will allow us to use Theorem 2.

The *order* of a pair of subfactors (g_s, g_r) is defined as the sum of their orders, $s+r$. The node multiset of a pair of subfactors $(g_s(I_s), g_r(I_r))$ is the list of all their nodes $\{I_s, I_r\}$ including the repeated nodes. Similarly, the arc multiset of a pair of subfactors $(g_s(I_s), g_r(I_r))$ is the list of all their arcs $\{E_{I_s}, E_{I_r}\}$ including the repeated arcs. We say that a pair of subfactors (g_{k-1}, g_k) is *node identical* to a pair of subfactors (g_{k-2}, g_{k+1}) if they have the same node multisets, $\{I_{k-1}, I_k\} = \{I_{k-2}, I_{k+1}\}$. Similarly, a pair of subfactors (g_{k-1}, g_k) is *arc identical* to a pair of subfactors (g_{k-2}, g_{k+1}) if they have the same arc multisets, $\{E_{I_{k-1}}, E_{I_k}\} = \{E_{I_{k-2}}, E_{I_{k+1}}\}$. We say that a pair of subfactors (g_{k-1}, g_k) is *identical* to a pair of subfactors (g_{k-2}, g_{k+1}) if they are node identical and arc identical. Note that identical pairs of subfactors have the same multiset of cycles and loops.

For any pair of subfactors $(g_{k-2}(I_{k-2}), g_{k+1}(I_{k+1}))$ there exists a node identical pair $(g_{k-1}(I_{k-1}), g_k(I_k))$, because if the node set of g_{k+1} is $I_{k+1} = I_k \bigcup \{i_{k+1}\}$ where $i_{k+1} \notin I_{k-2}$, then the node set of g_k is I_k and the node set of g_{k-1} is $I_{k-1} = I_{k-2} \bigcup \{i_{k+1}\}$. On the other hand, there exist pairs $(g_{k-1}(I_{k-1}), g_k(I_k))$ that are not node identical to any pair $(g_{k-2}(I_{k-2}), g_{k+1}(I_{k+1}))$. If $I_{k-1} = I_{k-2} \bigcup \{i_{k-1}\} \subset I_k$, then the node set of g_{k-2} is I_{k-2} and the the node set $I_{k+1} = I_k \bigcup \{i_{k-1}\}$ of g_{k+1} must contain a repeated node, which is not allowed. If two pairs of the same order are not node identical, then they are not arc identical either, since their arc multisets are different.

There can exist pairs of subfactors (g_{k-2}, g_{k+1}) that are not arc identical to any pair of node identical subfactors (g_{k-1}, g_k). For example, if the pair (g_{k-2}, g_{k+1}) contains a cycle c_{k+1} of order $(k+1)$ which cannot be contained in a subfactor g_{k-1} or g_k, then the arc multiset of the pair (g_{k-2}, g_{k+1}) contains an arc which is not in the arc multiset of any node identical pair (g_{k-1}, g_k).

If (g_s, g_r) is a pair of subfactors, then its corresponding *weight* is defined as $J[g_s]J[g_r]$. A pair of positive (negative) subfactors (g_s, g_r) has positive weight $J[g_s]J[g_r] > 0$. Similarly, a pair of a positive (negative) subfactor g_s and a negative

(positive) subfactor g_r has a negative weight $J[g_r]J[g_s] < 0$. If two pairs of subfactors are identical, then they have the same weight. If a pair of subfactors (g_{k-2}, g_{k+1}) with a positive weight is not arc identical to any pair (g_{k-1}, g_k), then for some weighting $W(\mathbf{f})$ of $D(J)$ its weight $J[g_{k-2}]J[g_{k+1}] > 0$ can be chosen larger than the weight $J[g_{k-1}]J[g_k] > 0$ of all such pairs (g_{k-1}, g_k).

We say that a pair of subfactors (g_{k-2}, g_{k+1}), $k = 2, \ldots, n-1$ is *critical*, if it has positive weight, and if no pair of node identical subfactors (g_{k-1}, g_k) with positive weight is also arc identical to (g_{k-2}, g_{k+1}). Note that a critical pair of subfactors (g_{k-2}, g_{k+1}) is also not arc identical to pairs (g_{k-1}, g_k) that are not node identical to it.

Some examples of critical pairs of subfactors are: $(g_0, g_3) = (\emptyset, c_3)$, where c_3 is a negative cycle of order three and $\emptyset$ is the empty set; (g_1, g_4), where $g_1 = c_1(i)$ is a negative loop and $g_4 = c_4$ is a negative cycle of order four or $g_4 = \{c_1(i), c_3\}$ and c_3 is a negative cycle of order three; (g_2, g_5), where $g_2 = c_2$ is a negative cycle of order two, or $g_2 = \{c_1(i), c_1(j)\}, i \neq j$ consists of two negative loops and c_5 is a negative cycle of order five. Note that each of the critical pairs contains a cycle of order $k + 1 \geq 3$.

The weight $J[g_{k-2}]J[g_{k+1}] > 0$ of a critical pair (g_{k-2}, g_{k+1}) can be made larger for some choice of the weighting $W(\mathbf{f})$ by increasing the weight of the arcs that are not in the arc multiset of pairs (g_{k-1}, g_k) with a positive weight. Therefore, the weight $J[g_{k-2}]J[g_{k+1}] > 0$ of a critical pair (g_{k-2}, g_{k+1}) can be made larger than the weights $J[g_{k-1}]J[g_k] > 0$ of pairs of subfactors (g_{k-1}, g_k) in (17). Thus, $h_k(\mathbf{p})$ can be made smaller by making the weights of the critical pairs of subfactors the dominant negative summands in (17).

If the subfactors of a pair (g_{k-2}, g_{k+1}) consist of loops and cycles of order two, then there always exists an identical pair of subfactors (g_{k-1}, g_k). This can be proved similarly to Proposition 2 in [27]. Hence, a critical pair of subfactors (g_{k-2}, g_{k+1}) contains a cycle of order at least 3. Therefore, we will assume that the biochemical reaction network modeled by the ODE system (3) has $n \geq 3$ number of species. We note that the restriction on the number of species $n \geq 3$ applies to systems showing oscillations arising from a negative cycle and its generalizations studied here. Oscillations can arise from a positive cycle and more complex graph structures, such as critical subfactors [28], in which case the number of species n of a biochemical reaction network is restricted to $n \geq 2$.

In the next theorem we show that $h_k(\mathbf{p})$, defined in (17), can be made arbitrarily small for some weighting $W(f)$ of the digraph $D(J)$ and some parameter values $\mathbf{p} \in \bar{S}$, if $D(J)$ contains a critical pair of subfactors.

Theorem 4 *Let the biochemical reaction network associated with the ODE system* (3) *have* $n \geq 3$ *species. If the digraph* $D(J)$ *of the Jacobian* (4) *has a critical pair of subfactors* (g_{k-2}, g_{k+1}), $k \in \{2, \ldots, n-1\}$, *then* $h_k(\mathbf{p}) \geq 0$ *can be made arbitrarily small for some weighting* $W(\mathbf{f})$ *of the digraph* $D(J)$ *and some parameter values* $\mathbf{p} \in \bar{S}$.

Proof By assumption, $h_k(\mathbf{p})$, defined in (17), contains at least one positive summand $J[g_{k-1}]J[g_k]$, where the subfactors g_{k-1} and g_k consist of negative loops. Let $h_k(\mathbf{p})$ contain a summand $J[g_{k-2}]J[g_{k+1}] > 0$ corresponding to a critical pair of subfactors (g_{k-2}, g_{k+1}) which can be made dominant with respect to all summands

$J[g_{k-1}]J[g_k] > 0$ for some weighting $W(\mathbf{f})$. Then, we can choose parameters $\mathbf{p} \in \bar{S}$ such that the positive summands in $h_k(\mathbf{p}) \geq 0$ are small and the dominant summand $J[g_{k-2}]J[g_{k+1}] > 0$ is sufficiently large. Therefore, $h_k(\mathbf{p})$ can be made small for some parameters $\mathbf{p} \in \bar{S}$. □

The next corollary follows by Theorems 2, 4 and Corollary 1.

Corollary 3 *If $h_k(\mathbf{p}) \geq 0$, where $k \in \{2, \ldots, n-1\}$ can be made sufficiently small for some parameter values $\mathbf{p} \in \bar{S}$, then $H_{n-1}(\mathbf{p}_0) = 0$ for some $\mathbf{p}_0 \in \bar{S}$. If $a_n(\mathbf{p}_0) > 0$, $\det(M_{n-1,1}(\mathbf{p}_0)) \det M_{n-1,2}(\mathbf{p}_0)) > 0$, where $M_{n-1,i}(\mathbf{p})$, $i = 1, 2$ are submatrices of the Hurwitz matrix (8) and $\frac{\partial H_{n-1}}{\partial \mathbf{p}}(\mathbf{p}_0) \neq \mathbf{0}$, then a simple Hopf bifurcation exists.*

4 Example

In the 1960's F. Jacob and J. Monod introduced a negative feedback mechanism for the control of gene regulation in cellular pathways [17]. Later, Goodwin [13] proposed the first mathematical model for the same biochemical control mechanism. A four-variable variant of a compartmental model with diffusion and time delays, where the compartments are the nucleus and the cytoplasm of a cell, was created and analyzed in [23,24]. Here we will discuss the corresponding ODE model and find all critical pairs that can lead to oscillations.

We renumber the reactants and rename the kinetic parameters from Ref. [23] for convenience, but use the same order of equations. The concentrations of the mRNA and the repressor in the nucleus are denoted by u_1 and u_2, respectively. Similarly, the concentrations of the mRNA and the repressor in the cytoplasm are denoted by u_3 and u_4, respectively. Then, the ODE system is

$$\frac{du_1}{dt} = f(u_2) - u_1 + k_1(u_3 - u_1), \tag{18a}$$

$$\frac{du_2}{dt} = -k_2 u_2 + k_3(u_4 - u_2) \tag{18b}$$

$$\frac{du_3}{dt} = -u_3 + k_4(u_1 - u_3), \tag{18c}$$

$$\frac{du_4}{dt} = k_5 u_3 - k_6 u_4 + k_7(u_2 - u_4), \tag{18d}$$

where

$$f(u_2) = \frac{1}{1 + k u_2^h}$$

is an inhibiting Hill type function and $h > 0$ is a Hill coefficient. Therefore, we have

$$f'(u_2) = -\frac{k h u_2^{h-1}}{(1 + k u_2^h)^2} < 0.$$

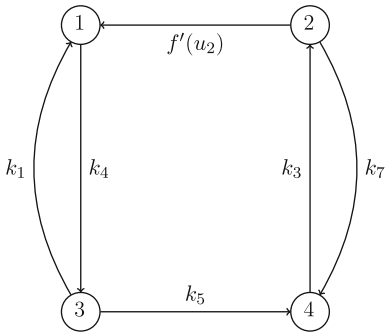

Fig. 1 Weighted digraph of the genetic model (18a)–(18d) with Jacobian (19)

Since the equation $f(u_2) = Cu_2$, where $C > 0$ is a constant, has a positive solution for some parameter values, it can be shown that there exists a positive equilibrium of (18a)–(18d).

The Jacobian of the right-hand side of (18a)–(18d) is

$$J = \begin{pmatrix} -1-k_1 & f'(u_2) & k_1 & 0 \\ 0 & -k_2-k_3 & 0 & k_3 \\ k_4 & 0 & -1-k_4 & 0 \\ 0 & k_7 & k_5 & -k_6-k_7 \end{pmatrix}, \tag{19}$$

and the digraph $D(J)$ of the model reaction is shown in Fig. 1.

Next we list all critical pairs of subfactors. Since a subfactor in a critical pair contains a cycle of order at least three, then a subfactor of order four should contain the negative cycle of order four $c_4(1,3,4,2) = c_4^-$. We show that $(g_1, g_4) = (c_1(i), c_4^-)$, $i = 1, 2, 3, 4$ where $c_1(i)$ is a negative loop, forms a critical pair of subfactors. For example, $(c_1(3), c_4^-)$ is a critical pair of subfactors with a node multiset $\{1, 2, 3, 3, 4\}$, because any pair of subfactors (g_2, g_3) with positive weight which is node identical to $(c_1(3), c_4^-)$ is not arc identical to it. The node identical pairs of subfactors to $(c_1(3), c_4^-)$ with positive weight are: $(g_2(1,3), g_3(2,3,4))$, $(g_2(2,3), g_3(1,3,4))$ and $(g_2(3,4), g_3(1,2,3))$, where each subfactor consists of negative loops only. The arc multiset of $(c_1(3), c_4^-)$ is $\{(3,3), (1,3), (3,4), (4,2), (2,1)\}$, where only the arc $(3,3)$ is in the multisets of its node identical pairs of subfactors. Therefore, $(c_1(3), c_4^-)$ is a critical pair of subfactors with weight $J[c_1(3)]J[c_4^-] = -(1+k_4)k_3k_4k_5f'(u_2) > 0$. Similarly, it can be shown that the pairs of subfactors $(c_1(1), c_4^-)$, $(c_1(2), c_4^-)$ and $(c_1(4), c_4^-)$ are critical.

Interesting and perhaps not surprising, is the fact that the arc multiset of each critical pair contains the arc $(2,1)$ with weight $f'(u_2)$. This, in particular, increases the possibility that a critical pair of subfactors (g_1, g_4) will correspond to a dominant negative summand in $h_3(\mathbf{p})$ given by (17). In fact, it can be confirmed using Maple that

$$h_3(\mathbf{p}) = (2 + k_6 + k_7 + k_4 + k_2 + k_3 + k_1)\, k_4k_3k_5 f'(u_2) + q(\mathbf{k}) \tag{20}$$

where $q(\mathbf{k}) > 0$ is a fifth degree polynomial in $\mathbf{k} = (k_1, k_2, k_3, k_4, k_6, k_7)$. If k_5 and $-f'(u_2)$ are chosen large enough, then the negative summands in (20) corresponding to critical pairs will dominate the positive summands in $q(\mathbf{k}) > 0$.

It follows by Theorem 4 that $h_3(\mathbf{p}) \geq 0$ can be made arbitrarily small for some $\mathbf{p} \in \bar{S}$ and for the weighting $W(\mathbf{f})$ of the digraph $D(J)$, where $\mathbf{f}$ is the right-hand side of (18a)–(18d). It follows by Corollary 3 that if $h_3(\mathbf{p}) \geq 0$, $\mathbf{p} \in \bar{S}$ is sufficiently small and the other algebraic conditions of the corollary are satisfied, then Hopf bifurcation and oscillations occur.

5 Discussion

The existence of a critical pair of subfactors (special subnetworks of mutually nonintersecting cycles) in the digraph of a biochemical reaction network is shown to be responsible for potential oscillations in a corresponding ODE model. The obtained graph-theoretic condition is novel, since it shows that oscillations can arise from a critical pair of subfactors where each subfactor contains an even number of positive cycles, rather than from a single negative cycle. For three-species biochemical networks the negative cycle condition for oscillations is a special case of the critical pair condition for oscillations, where one of the subfactors consists of the negative cycle and the other is the empty set. In larger biochemical networks, another special case of a critical pair of subfactors is a pair where both of the subfactors consist of either negative or positive cycles.

The existence of a critical pair of subfactors is only one of the conditions for oscillations arising from Hopf bifurcation. The rate functions giving the weighting of the digraph, as well as the parameter values and the equilibrium concentrations, will further determine if the ODE system will exhibit oscillations. Once a critical pair of subfactors has been identified, other algebraic conditions formulated in Corollary 3 need to be satisfied in order for oscillations to occur.

In this article we have discussed oscillations associated with a negative cycle, or as it is often referred to, with negative feedback. Oscillations arising from all possible generalizations of the negative cycle condition are still to be classified.

If multiple activations or inhibitions exist between species in a biochemical reaction network, then the network can be represented by a directed multigraph where more than one arc is allowed between any two nodes [28]. Graph-theoretic conditions generalizing the negative cycle condition for oscillations in the case of the multigraph will be studied elsewhere.

The graph-theoretic methods presented here are suitable for computerization and therefore can be applied to large biochemical reaction network models. The development of efficient algorithms for finding pairs of subfactors will be necessary.

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